

Abstracts*)

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Under the auspices of



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Editorial

Foreword

The Slovenian Association for Clinical Chemistry and Laboratory Medicine has been active in clinical chemistry for more than 50 years. Throughout this period it has supported continuing education of its members by organizing periodic training courses, professional meetings, as well as national and international symposia and congresses. This is the fifth time that the Association has organized the Slovenian Congress of Clinical Chemistry and Laboratory Medicine with International Participation. This year, the congress is joined with the 8th Scientific Symposium dedicated to Prof. Niko Jesenovec. Topics of this traditional symposium stress the importance of research in laboratory medicine. The congress of clinical chemists is held under the auspices of IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) and EFLM (European Federation of Clinical Chemistry and Laboratory Medicine). Moreover, the 5th Slovenian Congress of Laboratory Medicine Technicians takes place at the same time (abstracts are published in a separate abstract book).

At the congress, the Slovenian Association for Clinical Chemistry and Laboratory Medicine will confer awards to several clinical chemists in recognition of their outstanding work in the Association and for their contribution to the development of clinical chemistry and laboratory medicine.

The congress programme starts with the plenary lecture *Between Test Tube and the Bird of Paradise: Science in the Jungle and the Jungle in Science*, by Prof. Borut Telban, pharmacists and anthropologist, from the Institute of Anthropological and Spatial Studies at the Research Center of the Slovenian Academy of Sciences and Arts.

The programme continues with contributions from the following main topics: personalized laboratory medicine, implementation of new paradigms and technologies of laboratory medicine in the healthcare environment of the 21st century, laboratory diagnostics of autoimmune rheumatic diseases, and laboratory diagnostics of cardiovascular diseases. Poster session topics have not been limited to symposia topics. Educational workshops are also an important part of the programme.

We are confident that the scientific programme of this congress, including contributions by distinguished Slovene and foreign speakers, discussions and abstracts published in Clinical Chemistry and Laboratory Medicine, will greatly contribute to the further development of clinical chemistry and laboratory medicine in Slovenia.

Assist. Prof. Pika Meško Brguljan, EuSpLM
President of the Scientific Committee

Evgenija Homšak, MSc, EuSpLM
President of the Organizing Committee

Plenary lecture

PL-1

Between a test tube and the bird of paradise: science in jungle and jungle in science

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BACKGROUND: In 1986, after almost a year of traveling through South-East Asia and Australia, I finally arrived to Papua New Guinea as a pharmacist interested in a variety of healing practices. During first eighteen months I conducted an extensive research of medicinal plants in a remote area of Papua New Guinea. At the same time, I realized that for an in-depth understanding of people's lives, I would need a much better knowledge of their social structure, cultural background and local language. In 1990, after completing M.A. in botany, I returned to Papua New Guinea as a Ph.D. student of social and cultural anthropology at The Australian National University. I was adopted into the Bird of Paradise clan in Ambonwari village in the East Sepik Province and began my second long-term fieldwork slowly uncovering different aspects of people's lives. I've been returning to Ambonwari ever since. After thirty years of engagement as a pharmacist, botanist and anthropologist with different societies and cultures in Papua New Guinea, and subsequent affiliations with many universities around the world (in Port Moresby, Canberra, Manchester, Vienna, San Diego, Cairns, Marseille, St. Andrews) enabled me to develop a specific sensitivity for and perspective on different worlds and different realities.

METHODS: Long-term ethnographic fieldwork: participant observation, interviews and discussions, systematic study of cosmology, social organization, kinship, people's relation to their environment, culturally specific practices and rituals, local languages and poetics.

RESULTS: Many.

CONCLUSIONS: Studying different aspects of people's lives in Papua New Guinea for over thirty years brought me to a somehow simple though complex insight. In my view this insight is equally important for both sciences and humanities, and especially for cohabitation of different social and cultural realities in the globalizing world. It is not enough simply to look and observe but it is important to see, especially those things, which are for us invisible. And it is not enough simply to listen but it is important to hear, especially those things, which are for us inaudible. This requires a constant reflection upon and correction of our own understanding of the world.

Lectures

IL-1

Clinical Implementation of Pharmacogenetics: Do YOU have your DNA passport?

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Interindividual variation in drug metabolism is a factor affecting successful drug therapy. Adverse drug reactions are responsible for 5-7% of hospitalizations each year, and there is thus a need to personalize drug therapy. With the knowledge and tools available today, we can achieve this at reasonable costs. With over 5,000 articles per year currently being published on genomic markers to guide drug therapy, there is a huge potential. Several tests are readily accepted by the clinic, whereas others are not. Surprisingly, these acceptations may differ from country to country, but also from hospital to hospital. Laboratory Medicine can play a crucial and important role in providing this service for health care professionals and patients. Our experience in implementing pharmacogenetics in clinical diagnostics will be illustrated in this talk, based on our 10 year experience. This implementation strategy covers education, availability of testing, laboratory and clinical guidelines, quality, feedback from clinicians and patients, reporting, financial and ethical aspects, networking and interlaboratory collaborations. Current status in the Netherlands, as well as encountered and unexpected barriers will be addressed. Also the European initiatives like the European Pharmacogenetics Implementation Consortium (www.eu-pic.net) and the IFCC task Force Pharmacogenetics will be highlighted. At Erasmus MC, we provide since 2015 DNA passports, fitting in the trend of pre-emptive genotyping. With this passport, one can visit any pharmacy in the Netherlands to obtain medication adjusted on genomic profile for over 80 drugs. The question is, therefore: 'Do YOU have your DNA passport ready?'

IL-2

Personalised medicine in haematology

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The advent of modern sequencing technologies which allowed the identification of new genetic and epigenetic aberrations that drive tumorigenesis in haematological malignancies facilitated a paradigm shift that cancer therapeutics has undergone during the last two decades (1, 2). The traditional cytotoxic drugs have been replaced or upgraded by the specific targeting of cancer proteins. The forerunner of this approach is chronic myeloid leukaemia (CML) therapy using tyrosin kinase inhibitors (TKI) (3). New drug classes have been discovered and consequently other haematological malignancies are undergoing a treatment renaissance. This personalised therapeutic approach is, however, based on personalized laboratory medicine. Different algorithms which combine conventional as well as novel techniques assure a comprehensive insight into a specific genetic pattern for each individual patient. The established pattern is crucial for selection of the best available treatment, for exact prognostication, and a latter minimal residual disease (MRD) monitoring. The potential of personalised medicine approaches can be presented in different hematological malignancies. Close monitoring of the disease burden in CML with sensitive molecular methods helps clinician to early recognize TKI-resistant patients. On the basis of specific genetic aberrations we can define prognostic significance in majority of patients with acute leukemia and myelodysplastic syndromes. While specific treatment is available in a limited number of that patients, prognostic impact of genetics is crucial for decision for hematopoietic stem cell transplantation and for latter MRD monitoring. To ascertain patients with chronic lymphocytic leukemia and multiple myeloma which demand specific targeted therapy a stepwise diagnostic approach has been introduced. Integration of new comprehensive and demanding laboratory techniques into routine diagnostic workflow is challenging. Considering the overspill of emerging targeted therapies that rely on specific genetic profile the interpretative role of laboratory experts is becoming indispensable in precise hematology. Combining data from traditional and modern methodologies will pave the way towards personalized therapies (2).

References

1. Okosun J, Packham G, Fitzgibbon J. *Expert Opin Investig Drugs*. 2014;23:1321-32.
2. Nebbioso A, Benedetti R, Conte M, Iside C, Altucci L. *Expert Opin Ther Targets* 2015;19:1187-202.
3. Chessum N, Jones K, Pasqua E, Tucker M. *Prog Med Chem* 2015;54:1–63.

IL-3

Molecular genetics in solid tumor diagnostics

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BACKGROUND: Histology and localization of tumor are traditionally used to make treatment decisions in cancer patients. Lately, in the diagnostics as well as treatment of solid tumors the molecular tumor parameters became important. So called personal medicine uses the information on patient's and tumor's genotype or phenotype characteristics to prognosticate the outcome of disease, predict tumors sensitivity to anti-cancer drugs, and possible adverse effects. The information on individual's genotype can also help to evaluate individual's cancer risk, personalized prevention and monitoring plans for early cancer detection (1).

METHODS: Molecular markers that are used to guide the cancer treatment with targeted agents can be based either on protein or on genetic aberration. Broad spectrum of methods are used to detect the genetic aberrations including PCR, sequencing, FISH, as well novel high throughput genotyping techniques like next generation sequencing and microarray chips.

RESULTS: Tumor profiling is already a part of routine procedure in management of cancer patients. For example, the breast cancer patients with tumor having amplified HER2 gene can be treated with anti-HER2 monoclonal antibody; colorectal cancer patients with tumors bearing mutations in KRAS or NRAS genes, do not benefit from anti-EGFR therapy ect (1). Most targets of targeted anti-cancer therapy, used until today, are part of signaling pathways guiding cell division. Recently, the DNA repair mechanisms have emerged as new promising targets for anti-cancer therapy. The example of this is targeted therapy with PARP inhibitors, which are used in ovarian cancer patients with tumors bearing BRCA mutations (2). Currently tumor profiling is mostly focused on identification of genetic aberrations, but cancer can be associated also with variations in RNA expression or epigenetic changes.

CONCLUSIONS: The use of new high throughput technologies in tumor profiling gives an opportunity to simultaneously analyze larger number of targets. Yet, the processing of enormous amount of data brings also new challenges regarding data processing and interpretation.

References

1. Jürgensmeier JM, Eder JP, Herbst RS. *Clin Cancer Res* 2014 Sep 1;20(17):4425-35.
2. Abbotts R1, Thompson N1, Madhusudan S1. *Cancer Manag Res* 2014; 6: 77–92.

IL-4

System medicine and personalised laboratory medicine in bone diseases

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Personalised medicine is a medical model using new, 'omic' based approach to tailoring the right therapeutic strategy to the right patients at right time and/or to determine the predisposition to disease for deliver timely and targeted prevention. In postgenome era, the development of 'omic' advanced technologies is important because it offers the analysis thousands of chemically similar molecules of genome, transcriptome, proteome, metabolome, epigenome, microbiome. etc. in a selected biological system (tissue, cell). Such molecular profiling results in huge amount of data which are able to describe the cell or tissue or organ very precisely. In fact, all this data offer very 'personal' description of a biological compartment or actually an individual patient in whom molecular profiling was performed. Therefore personalized medicine represents a medical approach based on the 'omic'. On the other side, system medicine aims to contribute to predictive, preventative, personalized and participatory (P4) medicine by computational integration of routine and innovative clinical, biological and imaging data in formats that are accessible to health care professionals. Integrating data from 'omics' and medical records into biological models with the power of computer science, mathematics or engineering is showing the importance of systems medicine. Mathematical models integrating different types of data are already used in research but not yet in healthcare management. In our case two 'omic' technologies were used: the genome and transcriptome in osteoporotic women and bone samples. We found one thousand six hundred six genes differentially expressed in osteoporotic bone tissue, indicating increased demand for protein synthesis and decreased cell proliferation rate. Further biostatistical analysis of the microarrays data by gene set enrichment analysis suggested oxidative stress may have an important role in the pathogenesis of osteoporosis. Thus, secondly, we tested it by an in vitro experiment on human osteosarcoma cell line cells treated with hydrogen peroxide. Our results presented a novel list of genes and metabolic pathways that may be associated with the pathogenesis of osteoporosis. PTN, CXCL2, COL15A1, IBSP, ADRA2A, AOX1, MT1G, GSR and TXNRD1 could be candidate genes for further studies of the personalised medicine and genetic susceptibility to osteoporosis.

IL-5

Translational 4P Medicine in Inflammatory Autoimmune Diseases

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Translational medicine focuses on conveying information from the laboratory bench to the patient bedside. Giant cell arteritis (GCA) is an inflammatory systemic autoimmune disease affecting large and medium sized arteries (e.g. temporal artery) and branches of the aorta. Inflammation in these vessels may lead to occlusion of arterial lumen that could result in ischemia and severe clinical complications, such as permanent visual loss or cerebro-vascular insults. With 4P medicine (namely prevention, prediction, participation and personalization), we aim to determine earlier diagnosis, better prognosis, outcome and response to therapy in GCA. Although invasive and sometimes falsely negative, the temporal artery biopsy (TAB) is still the gold standard for diagnosis of GCA. However, no serological diagnostic markers are currently available. To prevent serious complications, prompt identification is required, since all patients undergo a therapeutic regime with large doses of glucocorticoids tapered over 6 or 12 months. In spite of this established therapy, patients can experience unpredicted relapses/symptom flare-ups, since there are no robust disease progression markers. We have data on a pristine cohort of patients where sera and TAB samples are available both prior to, and at 3, 6 and 12 months post-beginning of steroid therapy. We used multiplex microarrays in order to determine specific cytokines, chemokines, adhesion molecules and enzymes as potential diagnostic/prognostic markers and explored the levels of acute phase proteins (e.g. CRP, SAA, haptoglobin, fibrinogen, ferritin) in GCA. We tested early biomarkers in sera of GCA patients (e.g. IL-1 β , IL-6, IL-8, IL-10, IL17A, TNF α , TNFRI, IFN γ , VCAM-1, MMP-1, Resistin, CHI3L1, MARCO), comparing the prior-therapy time point with healthy sera and several matrix metalloproteinases with their inhibitors, in temporal artery tissue involved in disease pathology. These results provide an opportunity to identify novel critical biomarkers for GCA with a potential of shedding light on the mechanisms of pathogenesis.

IL-6

Epigenomics and micro RNA in personalised laboratory medicineBarbara Ostanek¹¹Department of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

All the somatic cells in the human body carry essentially the same genome, however there is great variability in cell and tissue phenotypes due to cell specific patterns of gene expression. Appropriate control of gene expression is therefore crucial for normal development and cellular function and dysregulation can result in various diseases. Epigenomics is the study of mitotically stable changes in gene expression not associated with changes in DNA sequence. The most important epigenetic mechanisms include posttranslational histone modifications, DNA methylation and noncoding RNAs. Micro RNAs (miRNAs) are short, noncoding RNAs, that regulate gene expression posttranscriptionally by inhibiting protein translation or by promoting mRNA degradation. It is estimated that that around 60% of mRNAs are targeted by at least one miRNA. miRNAs play an important role in tissue development and regulation of several cellular processes including differentiation, proliferation, metabolism and apoptosis. Moreover, they are involved in pathophysiology of various cancers, neurodegenerative, cardiovascular and other diseases. Regarding bone, miRNAs were shown to significantly affect the differentiation, proliferation and function of both osteoblasts and osteoclasts. Although few studies were performed so far, they have demonstrated the involvement of miRNAs in bone-related pathologies such as osteoporosis, osteosarcoma, bone metastases and osteoarthritis. Besides intracellular there are also extracellular miRNA present in plasma and other body fluids, which show remarkable stability. Their biological role has not been elucidated yet, most probably they represent cellular waste products but they may also be involved in intercellular communications. Intracellular as well as more easily accessible extracellular miRNAs are promising biomarkers for early disease diagnosis, classification, prognosis, selection and monitoring of treatment in several diseases. Moreover, they represent therapeutic targets. Namely, miRNAs that are upregulated in diseases may be reduced using anti-miRNAs, while miRNA deficiency can be overcome by exogenous synthetic miRNAs. miRNAs are already used as biomarkers in certain cancers and several miRNA based therapeutics are under development. It is expected that further association of miRNA profiles with particular diseases and standardization of miRNA analyses will contribute to their broader implementation into clinical practice and realisation of the promise of personalised medicine.

IL-7

Metabolomics: a tool in diagnostics and monitoring of inherited metabolic diseasesBarbka Repič Lampret¹, Andraž Šmon¹, Urh Grošelj¹, Mojca Žerjav Tanšek¹, Tadej Battelino^{1,2}¹University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia²Faculty of Medicine, University Ljubljana, Ljubljana, Slovenia

BACKGROUND: Inherited metabolic diseases (IMD) are rare diseases with a block in the metabolic pathway caused by a genetic defect of a specific enzyme, resulting in the increased concentrations of substrate and multiple metabolites of metabolic pathway. Result is metabolome characteristic for individual disease. Early detection of IMD is crucial to make a right and timely clinical decision, which can prevent serious consequences of the disease or even death. Later follow-up of patients is important for monitoring therapy. Tandem mass spectrometry (TMS) is a powerful tool for detection of diverse metabolic diseases with one analysis in a short time and is used for expanded screening in many countries. Last year a pilot study of expanded newborn screening for IMD using TMS started. 10,000 dried blood spots from newborns were analyzed retrospectively, as the filter paper cards from the existing screening programme were used.

METHODS: Techniques used most commonly for metabolites detection are gas chromatography-mass spectrometry for organic acids determination, ion exchange chromatography-post column derivatization for amino acids measurement and liquid chromatography-TMS for amino acids and acylcarnitine analysis.

RESULTS: 33 patients with amino and organic acidemias (phenylketonuria excluded) and 7 patients with disorders in fatty acid metabolism were diagnosed through selective screening till April 2016. During pilot study of expanded newborn screening for IMD, five newborns with IMD were identified so far. Those were newborns with markedly elevated metabolites, characteristic for glutaric acidemia type I in one patient, very long-chain acyl-CoA dehydrogenase deficiency in one patient and 3-methylcrotonyl-CoA carboxylase deficiency in three patients. Additionally 2 patients with phenylketonuria diagnosed in existing screening program were detected.

CONCLUSIONS: From conditions mentioned 1-2 cases were confirmed per year with selective screening. According to reported frequencies of IMD we would expect more identified cases of IMD in Slovenia. Results from the pilot study are confirming that, as we detected five newborns with IMD before clinical signs indicated disease. Based on the preliminary results around ten patients are expected to be confirmed per year, indicating that expanded screening should be implemented in Slovenian public health program.

IL-8

Challenges for laboratory medicine in the health care environment of the 21st century

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The health care is under constant development and pressure. It is important for laboratory medicine to be prepared for the challenges that will come and to try to influence its own future. In the near future laboratory medicine will be challenged along several lines:

1. Technological development: With all the new 'omics' that will generate new tests and new combination of tests, the laboratory must be prepared for new instruments and technologies. At the same time, through the development of e.g. nanotechnology, everything will get 'smaller'. This means that most laboratory tests can be performed on small movable devices that can be moved closer to the patients, closer to the clinicians. These devices should still be under the responsibility of the laboratory profession. How are we dealing with that?
2. The new MedTech Europe Code which put strict restrictions of the cooperation between industry and the laboratory/clinicians. It has been estimated that about 50% of participants on big conferences like Euromedlab has been supported directly by industry. How can we deal with this in the future?
3. Academic laboratory medicine: Probably the most important challenge for laboratory medicine is to preserve its role as knowledge centers in which the clinicians can find valuable discussion partners concerning the use and interpretation of laboratory tests. It is therefore important to underline that the laboratory is not a supermarket where hospital administrators only will try to cut costs and where clinicians 'shop' analyses.

IL-9

Harmonization of Laboratory Medicine in Slovenia

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BACKGROUND: Harmonization in laboratory medicine includes whole process of testing from the "pre-pre-analytical" phase through analysis to the "post-post-analytical" phase. Monitoring of the outcomes of harmonization activities is through surveillance by external quality assessment schemes (EQAS) that use commutable materials and auditing the whole phases.

METHODS AND RESULTS: Preanalytical phase: Harmonizing the pre-analytical phase requires use of standardized operating procedures for correct test selection, sample collection and handling. Each step of the preanalytical phase should be standardized, from test requesting, test ordering, patient preparation, patient identification, sampling, sample handling, storage and delivery to the laboratory. Standards for many of the preanalytical steps do not exist. Successful implementation of the evidence based standard is important because of the importance of compliance and cooperation has to be achieved through education of all involved stakeholders (medical doctors, nurses, laboratory technicians and patients, including ourselves). Analytical phase: According to the EU Directive IVD 98/79/ECDirective, manufacturers are obligated to perform extensive validation of methods and devices used for diagnostic purposes. According to ISO 15189, manufacturers have to provide method documentation with relevant technical characteristics. Laboratory professionals are obliged to verify the declared data and provide objective evidence for specification confirmation. Postanalytical phase: Use of harmonized reference limits and decision levels will reduce inaccurate clinical interpretation and unnecessary laboratory testing. In the post-analytical phase, harmonized procedures for the management of critical laboratory test results are required. We need harmonized critical value definition (alert thresholds of common biochemistry analytes) and communication with clinicians including harmonized interpretative comments of laboratory results.

CONCLUSIONS: Harmonization of laboratory medicine encompasses all efforts made in a search for differences in policies and procedures and minimizing these. Harmonization is improving patient service, safety, data comparability among laboratories and with time, savings and credibility and reliability of the clinical laboratories.

References

1. Aarsand AK, Sandberg S. How to achieve harmonisation of laboratory testing — the complete picture. Clin Chim Acta 2014;432:8–14.

IL-10

Calculations in laboratory medicine: the added value

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Clinical laboratories represent an area of healthcare that has always undergone major changes because of technological advances and external economic pressures. There are many contrasting observations about laboratory medicine, for example (i) there is too much testing vs insufficient testing; (ii) testing is expensive vs laboratories are expected to generate income; and (iii) test results have little impact on outcomes vs test results are crucial to clinical decision making. Test results may help make a diagnosis in symptomatic patients (diagnostic testing) or identify occult disease in asymptomatic patients (screening). However, test results may interfere with clinical decision making if the test poorly discriminates between patients with and without disease, if the result is inconsistent with the clinical picture, or if the test result is improperly integrated into the clinical context. Laboratory tests are imperfect and may mistakenly identify some healthy people as diseased or may mistakenly identify some affected people as disease-free. A test's ability to correctly identify patients with disease depends on how likely a person is to have a disease (prior probability) and on the test's intrinsic operating characteristics. Physicians are greatly confused by the amount of information and make many errors in the selection and interpretation of laboratory tests. In order to meet the needs, the role of the laboratory, actually the laboratory scientist is to improve laboratory tests derived from appropriate test request and utilisation. In routine laboratory work we perform tests and some of them serve as a basis for different calculation indexes. Those most used are in the case of haematologic count; next, from the field of biochemistry is blood gas analysis, anion gap, osmolality, lipid calculation... We could mention some additional calculations. In the field of cancer diagnostic we have more sophisticated calculations which are very helpful for better diagnosis, similar is in screening tests for Down syndrome where we use a combination of ultrasound and biochemical parameters for risk calculation. Laboratory Medicine, supported by computerised information and expert systems, will promote the use of this new knowledge in a timely and responsible manner, contributing to the provision of better care more economically.

IL-11

Implementation of trace elements reference intervals by ICP-MS

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For the interpretation of clinical laboratory data and a decision-making process it is essential that clinical laboratory uses reliable reference intervals. Establishing or verifying reference intervals established elsewhere is especially important when old and new methods differ greatly. Geographical location has large influence on concentration of trace elements in human body, so for a laboratory it is very important to establish reference intervals for its own population. In case of toxic trace elements values should be interpreted taking into account also the toxic values of particular elements, which are mostly poorly accessible. When introducing inductively coupled plasma mass spectrometry (ICP-MS) in our laboratory, we have established our own reference intervals according to the Clinical Laboratory Institute guidelines (1) on 192 blood donors (85 male, 107 female) aged from 18 to 65 years. After participant consent the blood was obtained according to the standardized specimen collection venipuncture procedures for trace elements and taking into account pre-analytical factors. All participants have filled in a detailed questionnaire with demographic and nutritional data, data about taking drugs, supplements and tobacco consumption. The trace elements were measured on Agilent 7700x ICP-MS in blood and plasma. Samples were prepared with 10 fold dilution in ammonium media solution with direct addition of internal standard. The interferences were removed by collision cell using helium as collision gas or without gas. All reagents were supra pure grade. The quality control was performed at the beginning, at the end and within the run. Analysis of obtained reference data was performed mainly by nonparametric method and in few cases with parametric method with outlier detection proposed by Tukey. As a result of this study, we now have our own reference intervals for 25 essential and toxic trace elements for adult population which is making us more comfortable in decision making.

References

1. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition. CLSI document C28-A3. PA: Clinical and Laboratory Standards Institute; 2008.

IL-12

Use of high performance liquid chromatography in the clinical laboratories

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High performance liquid chromatography (HPLC) is the most common of all chromatography techniques in the clinical laboratories and has been used since the 1970s for the separation and quantification of a many clinically relevant analytes. It offers high selectivity, sensitivity, reliability, but is also time-consuming. In recent years is often replaced by ultra HPLC, which is faster and give greater chromatographic resolution. Biological samples require sample pretreatment as an ion exchange solid phase extraction, which is mostly done manually or on-line in multiple-channel HPLC systems. Conventional detectors for HPLC are UV/VIS and electrochemical and more specific is mass spectrometry. In the clinical laboratory HPLC has been used for the analysis of drugs, vitamins, hormones, amino acids, carbohydrates, peptides, proteins and metabolites (1). In Clinical institute of clinical chemistry and biochemistry HPLC is performed for more than 25 years. We started with analysis of urinary catecholamines and metabolites (metanephrines, vanillin mandelic acid, homovanillic acid) and 5-hydroxyindole acetic acid and they remain the most performed tests. Catecholamines determination is primary of importance for the diagnosis and management of tumor diseases of the sympathoadrenal system, as pheochromocytoma. We routinely measure serum vitamin A and E concentrations. They belong to the fat-soluble class of vitamins and are determined in suspicion of hypovitaminosis and hypervitaminosis. Some years ago we developed method for determination of two antimycotic drugs voriconazole and posaconazole in serum with liquid chromatography coupled to mass spectrometry (2). We have been developing and validating other drugs in blood samples - antiepileptics (lamotrigine and oxcarbazepine and his metabolite monohydroxycarbamazepine) and immunosuppressives.

References

1. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. Missouri: Elsevier Saunders 2006:155-61.
2. Šter M, Skitek M. Liquid chromatography- mass spectrometry and applications of therapeutic drug monitoring. Biochemia Medica 2014;24(2):A1-A8.

IL-13

Autoimmunity – what is it and how do we measure it?

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BACKGROUND: The first definition of autoimmunity was introduced by Ehrlich and Morgenorth in 1900 as “horror autotoxicus”, meaning immediate death and so the reason for unfeasibility of existence of autoimmune diseases (ADs). ADs exist and are chronic conditions initiated by the loss of immunological tolerance to self-antigens. They have multi-factorial origin and diverse expression, which is described by term mosaic of autoimmunity. ADs are similar to each other's but not the same and the fact that they share several clinical signs and symptoms, pathophysiological mechanisms and genetic factors is called autoimmune tautology. Two another features of ADs make dealing with ADs a hard job: kaleidoscope of autoimmunity portrays the possible change from one disease to another in the same individual, while polyautoimmunity is defined as the presence of more than one autoimmune disease in a single patient. The latter represents the effect of single genotype on diverse phenotypes.

METHODS: In measuring of autoimmunity, there is the main problem in interpretations of the observed or measured features of ADs. They are diagnosed by anamnesis, clinical examination, imaging and laboratory methods, altogether involving genotypic and phenotypic approaches of observation and measurement of autoimmunity. Humoral autoimmune response (autoantibodies, cytokines) and cellular autoimmune response (cytotoxic and different subsets of regulatory lymphocytes) are measured by spectrum of laboratory methods, while high-through methods enable searching for modified self-antigens.

RESULTS: Impaired immune system (break down of normal tolerance) and altered autoantigens (phosphorylation, citrullination, deamination, hydrolysis,...) or even altered antibodies (oxidation) are in focus of laboratory measurements. Using molecular approaches it has become clear that many situation, which was determined as impaired immune system are indeed consequence of increased amount of modified self-antigens, which cannot be tolerated by immune system anymore. Humoral autoimmune reactions are far easier to demonstrate than cellular autoimmune response, but even than the distinction between “normality” and autoimmunity cannot be simply defined. Autoimmune potency, autoimmune responsiveness and autoimmune disease are three degrees of autoimmunity.

CONCLUSIONS: Considering mosaic of autoimmunity (different combinations of many factors produce several distinct clinical presentations) the measurement of autoimmunity and interpretation of the results is not an easy task and should be done by trained team.

IL-14

Posttranslational antigen modifications in pathophysiology and diagnostics of rheumatoid arthritisGer Pruijn¹¹Department of Biomolecular Chemistry, Radboud University, Nijmegen, Netherlands

Citrullination was the first post-translational modification that was shown to be important for the humoral immune response in rheumatoid arthritis (RA). The ground-breaking discovery that a majority of patients with RA have circulating antibodies to citrullinated proteins has led to significant advancements in clinical care and the understanding of disease mechanisms in RA. Autoantibodies to citrullinated proteins represent a very specific and sensitive serological marker for RA and play a prominent role in the classification of RA based on the criteria defined in 2010 by EULAR/ACR. More recently, also autoantibodies targeting carbamylated proteins and autoantibodies against proteins containing malondialdehydeacetaldehyde adducts (MAA) have been found to be associated with RA. Citrullination, carbamylation, and MAA are distinct post-translational modifications that are generated by distinct mechanisms and have very different cellular roles and consequences. Anti-citrullinated protein antibodies (ACPA), anti-carbamylated protein antibodies (anti-CarP) and probably also anti-MAA antibodies are produced during early stages of RA development, frequently years before the onset of clinical symptoms. The autoantibodies to post-translationally modified antigens contribute to pathophysiological processes underlying the generation and progression of RA and, therefore, provide opportunities for new therapies that may change the clinical course of RA. The elucidation of the molecular and cellular mechanisms underlying the initiation of the immune response to the modified antigens and their role in pathophysiological processes is complicated due to the heterogeneity of citrullinated and carbamylated proteins and the heterogeneity of the autoantibodies to these proteins. In this regard, it is interesting to note that the neutrophils, one of the most abundant cells in the inflamed joint of RA patients, have full capacity to generate all three post-translational modifications targeted by these antibodies. NETosis, programmed neutrophil death during inflammation, and the concomitant release of neutrophil compounds may lead to the excessive production of modified proteins in the extracellular space, which, in genetically susceptible individuals, may contribute to the initiation of the immune response, as well as persistent joint inflammation.

IL-15

The influence of selection of laboratory methods on the results of autoantibody testSaša Čučnik¹¹University Medical Centre Ljubljana, Dep. of Rheumatology, Immunology Laboratory, Ljubljana, Slovenia

Autoantibody analyses have a large impact on the diagnostic settings of autoimmune diseases and also appear as prognostic markers for therapy selection in already diagnosed patients. Determination of clinically important autoantibodies has changed dramatically over the last two decades. Modern autoantibody detection systems, such as enzyme linked immunoassays (ELISA), line immunoassays (LIA) and addressable laser bead immunoassay (ALBIA) such as Luminex have replaced the old antibody detection systems, such as counter immunoelectrophoresis (CIE) and double immunodiffusion (DID) (1). There are some distinct differences between the old and new generations of antibody tests. The old, slow and time-consuming techniques detected autoantibody with higher diagnostic specificity but lower diagnostic sensitivity (2), whereas the automated and faster modern immunoassay techniques (ELISA, LIA and ALBIA) had a higher diagnostic sensitivity at the expense of a decreasing diagnostic specificity (3). The older precipitation and agglutination techniques mainly used the native antigen in soluble form, while the coupling of the autoantigens to solid phase used in modern technologies with the theoretical risk of denaturation of the autoantigens and with the risk of hide of clinically important epitope. Modern techniques are more quantitative and therefore allowing better follow-up of autoantibody levels in already diagnosed patients in comparison with older technique which are qualitative or semiquantitative. The most important problem represent the fact that well known clinical associations between specific autoantibodies and specific autoimmune diseases originated from previous generation of older autoantibody determination techniques and are not or rarely regularly repeated with new tests.

References

1. Ronnelid J. The Choice of Laboratory Methodology Influences Autoantibody Test Results. *Frontiers in immunology*. 2015;6:392.
2. Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR. Mixed connective tissue disease--an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med*. 1972;52(2):148-59.
3. Orton SM, Peace-Brewer A, Schmitz JL, Freeman K, Miller WC, Folds JD. Practical evaluation of methods for detection and specificity of autoantibodies to extractable nuclear antigens. *Clinical and diagnostic laboratory immunology*. 2004;11(2):297-301.

IL-16

Animal Models: Tools for Assessing Aetiology and Therapy of Autoimmune Diseases

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In the 20th century, animal research studies have had a tremendous impact on better understanding of complex pathogenetic mechanisms of autoimmune diseases. Autoantigens identified in basic research lead to development of new diagnostics methods for specific autoimmune disease. In parallel, a wide assortment of effective drugs was brought to market based on pre-clinical drug safety studies performed in animals (1). Most of investigations today are done in rodent models. Three types of rodent models are used in basic and applied autoimmune research: spontaneous, induced, and genetically-engineered models (2). There has been a significant improvement in diagnostics and treatment of the disease where animal models are available: type I diabetes, systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis. The most commonly studied model of rheumatoid arthritis is collagen-induced arthritis mouse model (CIA) (3). Disease incidence of 80-100% is induced by immunization of genetically susceptible strains with complete Freund's adjuvant and type II collagen. Intradermal immunization results in moderate inflammatory reaction in 1-2 weeks, the onset of the disease is usually between 21-28 days. Pathogenesis of CIA involves T-cell and B-cell immune response, also visible deformation and swelling of joints (4). In recent experiments a novel compound, tuftsin-phosphorylcholine C (TPC), has been shown to successfully modulate immune response in CIA DBA/1 arthritis mice, by shifting Th1/Th2 balance towards development of Th2 effector response (5). Results of prophylactic and treatment experimental protocol confirmed that TPC inhibited production of TNF α , IL-17 and IL-1 β , and upregulated anti-inflammatory cytokine IL-10. A significant expansion of Treg and Breg population was confirmed by flow cytometry, thus providing a good potential for further drug development (5). The endpoints of the disease, evaluated in rodent models, are frequently similar to those in clinical research. However, obtained results need critical evaluation since there are important differences in immune response between animals and human. Whilst total replacement of animal studies with cell-based methods or molecular modelling remains the ultimate goal of the future, researchers should focus on animal welfare implementing the 3R's (Replacement, Reduction, Refinement) (6), when considering choice of the methods to be used.

References

1. AnimaResearch.info [Internet]. [Cited 18.5.2016] Available from: <http://www.animalresearch.info/en/medical-advances/>
2. Morel L. Mouse models of human autoimmune diseases: essential tools that require the proper controls. *PLoS Biol.* 2004;2(8):E241: 1061-64.
3. Bendele A. Animal models of rheumatoid arthritis. *J Musculoskelet Neuronal Interact.* 2001;1(4):377-85.
4. Brand DD, Latham KA, Rosloniec FE. Collagen-induced arthritis. *Nat Protoc.* 2007;1(5):1269-74.
5. Bashir T, Shovman O, Fridkin M, Volkov A, Barshack I, Blank M, Shoenfeld Y. Novel therapeutic compound tuftsin-phosphorylcholine attenuates collagen-induced arthritis. *Clin Exp Immunol.* 2016;184(1):19-28.
6. Guideline on regulatory acceptance of 3R (replacement, reduction, refinement) testing approaches. [Internet] 2014 [Cited 18.5.2016] Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/10/W_C500174977.pdf.

IL-17

Detection of Anti-phosphatidylserine/Prothrombin Antibodies and their Diagnostic Efficacy

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BACKGROUND: Antiprothrombin antibodies belong to a heterogeneous family of antiphospholipid antibodies. Their persistent presence is characteristic for patients with antiphospholipid syndrome (APS) who experience thrombosis and/or pregnancy complications. Laboratory markers of APS are Lupus anticoagulant (LA), anti-cardiolipin antibodies (aCL) and antibodies against β 2GPI (anti- β 2GPI). Antiprothrombin antibodies have not yet been included in the classification criteria for APS, however many studies confirmed their potential clinical value and pathogenic role. Our research group developed an in-house aPS/PT ELISA with increased analytical sensitivity which enabled determination of clinically relevant antiprothrombin antibodies (1-3).

METHODS: LA, aCL, anti- β 2GPI and antiprothrombin antibodies were measured in 254 patients with systemic autoimmune diseases: 131 APS, 47 systemic lupus erythematosus, 57 rheumatoid arthritis and 19 Sjögren's syndrome patients.

RESULTS: Positive levels of aPS/PT were determined in 101 patient sera (23 % IgG, 25 % IgM and 25 % IgA aPS/PT). IgG aPS/PT exhibited the highest percentage of LA activity (OR 18.4) compared to aCL (OR 4.1) and anti- β 2GPI (OR 2.7). IgG aPS/PT associated with arterial (p=0.016, OR 2.2) and venous thrombosis (p<0.001, OR 4.9) and presented the strongest independent risk factor for the presence of obstetric complications among all tested antiphospholipid antibodies (p<0.001, OR 6.5). Analyzing each of the three categories of pregnancy morbidity included in

the APS classification criteria, only aPS/PT antibodies were statistically significantly associated with all of them. In fact, they were the only aPL significantly associated with early recurrent abortions ($p < 0.026$, OR 5.3).

CONCLUSIONS: aPS/PT, in addition to LA, aCL and anti- β 2GPI antibodies show high specificity for APS and could serve as an additional marker in patients with clinical manifestations of APS. aPS/PT are associated with pregnancy morbidity and their measurement could improve the evaluation of patients with early recurrent abortions, undiscovered by other aPL tests.

References

1. Zigon P, Ambrozic A, Cucnik S, Kveder T, Rozman B, Bozic B. Clin Chem Lab Med. 2011; 49(6):1011-1018.
2. Zigon P, Cucnik S, Ambrozic A, Kveder T, Semrl SS, Rozman B, et al. Clinical and Developmental Immunology. 2013; ID 724592
3. Zigon P, Perdan Pirkmajer K, Tomsic M, Kveder T, Bozic B, Sodin Semrl S, et al. J Immunol Res. 2015; ID 975704.

IL-18

Prognosis and management of heart failure: more perspectives from biomarkers?

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Heart failure (HF) is a syndrome characterized by increasing prevalence, high morbidity, elevated hospital readmission rate and high mortality. The continuing improvement of diagnosis, prognosis, treatment and management of HF requires a better understanding of its different sub-phenotypes. The comprehensive understanding of neurohormonal activation, one of the hallmarks of HF, has allowed the identification of several biomarkers, such as natriuretic peptides, which are now playing an important role for the sub-phenotyping of HF and driving to a more personalized management. The monitoring of biomarkers contributing to cardiovascular remodeling such as soluble ST2, Fibroblast Growth Factor 23 and parathyroid hormone provides added value to the current biomarkers integrated to the standards of care and contribute to a more personalized management of HF patients as well as a decrease in morbidity and mortality. Furthermore, the clinical indications of these emerging biomarkers can also include the early evidence of pre-diastolic dysfunction, the prediction of adverse cardiovascular outcomes and the prognostication of HF with preserved ejection fraction as well as and HF with reduced ejection fraction. A better understanding of the role of these biomarkers of adverse cardiovascular remodeling in HF is also providing potential new therapeutic targets and/or companion assays to monitor the efficiency of the drugs used for the treatment of HF patients.

IL-19

Acute coronary syndrome: clinical - cardiologist point of view

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New onset of chest pain or pain at rest is typical for patients with suspected acute coronary syndromes (ACS) (1). ACS can encompass three entities. 1. Unstable angina - ACS patient with chest pain at rest or minimal exertion, ischemia and no signs of cardiomyocyte necrosis (1). In ACS with myocardial necrosis, the term 'acute myocardial infarction' (AMI) should be used (2). Two groups of patients with AMI are differentiated according to changes in the ECG: 2. ST-elevation myocardial infarction (STEMI)- persistent chest pain which lasted more than 20 min and ST-segment elevation or new (or presumed new) left bundle branch block: (3). The mainstay of treatment in these patients is immediate reperfusion by primary angioplasty (PCI) or fibrinolytic therapy (1). 3. Non-ST-elevation MI (NSTEMI) - acute chest pain but no persistent ST-segment elevation. Changes may include transient ST-segment elevation, persistent or transient ST-segment depression, T-wave inversion, flat T waves or pseudo-normalization of T waves or the ECG may be normal (1). Chest pain is characterized by a retrosternal sensation of pressure or heaviness ('angina') radiating to the arm, neck or jaw, which may be intermittent or persistent (1). Atypical presentations include epigastric pain, indigestion-like symptoms and isolated dyspnea. They are more often observed in the elderly, women and in patients with diabetes, renal disease or dementia (1). Diagnosis is obtained with a history of chest pain, ECG changes, biomarkers (hs-cTn), and echocardiography (1, 2, 4). Therapy STEMI Direct transport to the nearest facility for the primary PCI. Pain relief, anticoagulation and antiplatelet therapy should be provided (4). NSTEMI PCI immediately or at least in 72 hours according to initial risk stratification. If the risk is low, PCI is not mandatory (1).

References

1. Roffi M, Patrono C, Collet JP, et al. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. European Heart J 2016;37:1257-315
2. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Eur Heart J 2012;33:2551-67.

3. Montalescot G, Sechtem U, Achenbach S, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. *Eur Heart J* 2013;34:2949-3003.
4. Steg G, James SK, Atar D, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J* 2012;33:2569-619.

IL-20

High sensitive troponin for the management of acute coronary syndromes: The new ESC Guidelines

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Symptoms suggestive of an acute coronary syndrome (ACS), i.e. unstable angina or acute myocardial infarction (AMI), are one of the most common reasons for patients seeking acute care. However, the minority of the chest pain patients will ultimately be found to have ACS (typically less than 10% will have AMI), while the majority will have other conditions and often benign disorders. Rapid, but accurate, evaluation of such patients has major medical as well as economic importance. Failure to rapidly rule-in AMI may delay the initiation of effective and potentially life-saving treatments; and delays in ruling out AMI contribute to crowding of EDs. Biomarkers supplement, but never replace, clinical assessment and 12-lead ECG in the diagnosis, risk stratification and treatment of patients with suspected NSTEMI-ACS, while having a marginal role in ST-elevation AMI patients. The introduction of new generations of very sensitive cardiac troponin (cTn) assays has enabled the development of novel diagnostic algorithms for rapid rule-out and rule-in of AMI, some of which have been recommended in the latest European Society of Cardiology guidelines for non-ST-elevation ACS (1). The proposed algorithms are based on either a single cTn at ED presentation or on repeated measurements after one or two hours. Furthermore, the rapid algorithms are based on a new concept which has separate criteria for rule-in and rule-out of AMI, leaving a group of 'indeterminate' patients that need further testing for establishing the diagnosis. The diagnostic and prognostic properties of these algorithms will be reviewed and discussed during the presentation, as well as some important caveats (2,3). Furthermore, some general aspects of using very sensitive cTn assays ('high-sensitive' cTn assays) will also be discussed (4). Conclusions - Correctly applied these novel algorithms based on cTnI measurements with high-sensitive assays enable rapid and safe rule-in or rule-out of AMI in chest pain patients within two hours in approximately two thirds of chest pain patients with a negative predictive value in the rule-out group of >99% and a positive predictive value in the rule-in group of >70%.

References

1. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2016;37:267-315.
2. Mueller C, Giannitsis E, Möckel M, Huber K, Mair J, Plebani M, et al. Rapid Rule-out of Acute Myocardial Infarction - Novel Biomarker-based Strategies. *European Heart Journal: Acute Cardiovascular Care*. In press.
3. Möckel M, Giannitsis E, Mueller C, Huber K, Jaffe AS, Mair J, et al. Rule-in of acute myocardial infarction: Focus on troponin. In press.
4. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J*. 2012;33:2252-7.

IL-21

Heart failure: diagnosis and management

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Heart failure is a syndrome that is most commonly caused by coronary artery disease and/or arterial hypertension. Clinical presentation can be unspecific as many symptoms and signs are shared with other chronic disease. Patients therefore may be identified once heart failure is at full blown stage, and when management options are less optimal or even limited. The Heart Failure Association of the European Society of Cardiology has published guidelines for management of acute and chronic heart failure in 2016. In the diagnostic algorithm, natriuretic peptides have an important role; they primarily serve as a rule-out tool in patients presenting with non-acute onset of dyspnea or other symptoms/signs suggestive of heart failure. Diagnosis still needs to be confirmed, most frequently with cardiac imaging where echocardiography is preferred modality to be implemented in clinical practice. In this context, natriuretic peptides primarily play a role at the general practitioner level, and can be considered as a screening tool to identify patients in need of echocardiography. Once diagnosis has been confirmed and patient phenotype as per left ventricular ejection fraction is determined, management according to guidelines needs to be implemented. To

date, it is still under discussion whether natriuretic peptides can facilitate treatment decisions, e.g. regarding the therapy up-titration. Generally seen, natriuretic peptides are among main outcome predictors and a decrease over time is seen as a favorable signal. With results of the PARADIGM-HF trial, we have likely reached another turning point in the heart failure management. This, indeed, has relevant implications for patient follow-up; in the future, if the treatment is going to expand to other cardiovascular conditions (like arterial hypertension), diagnostic aspects will be affected as well. One of drug components that have been tested in PARADIGM-HF interferes significantly with natriuretic peptides: sacubitril, the neprilysin inhibitor, increases the brain natriuretic peptide levels whereas there is no additional effect on NT-pro brain natriuretic peptide. For clinical practice, this translates that the NT-pro brain natriuretic peptide is the tool of choice for evaluation of dyspnea. Research in this particular field is ongoing and years to come will guide the field in future.

IL-22

ST2- a new prognostic marker on the heart failure scene

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Heart Failure (HF) is one of the major disease of our era, with growing prevalence worldwide and is a major burden on hospital care costs. Patients with advanced HF have high rates of hospitalization and resource utilization, and similarly have a high risk for death. Therefore, disease prognosis and risk assessment for short-term events or death is of great importance. Soluble ST2 (sST2) is a new prognostic marker for HF patients who are at increased risk for cardiovascular events. ST2 (growth STimulation expressed gene 2) is a member of the interleukin-1 receptor family that is expressed as a transmembrane (ST2L) and soluble isoform (sST2). Plasma sST2 is expressed by fibroblasts in the heart and elevated in response to HF disease or injury, and it is a direct participant in the fibrosis or cardiac remodeling process. According to revised American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Guidelines, soluble ST2 (sST2) is a novel prognostic biomarker of HF risk assessment with growing importance in the prediction of cardiovascular and life-threatening events. The proposed cut-off value for risk stratification and prediction of life threatening cardiovascular events in HF patients is 35 ng/mL. sST2 concentrations are measured from serum/plasma samples using a manual ELISA method. HF and Chronic Kidney Disease (CKD) are conditions that very commonly coexist (as a cardiorenal syndrome) and result in a worse prognosis. Therefore it is important to recognise the life threatening cardiovascular events also among this group of patients. Several studies have confirmed that sST2 is independent of renal disease and that the serum concentrations of sST2 did not correlate with the stage of renal disease. In our recent study we also found that sST2 serum concentration is independent of hemodiafiltration treatment (HDF). Therefore, sST2 could be a potentially useful additional parameter, and promising marker for evaluating and monitoring patients undergoing HDF who are at increased risk for cardiovascular events, capable of stratifying and differentiating HF risk patients among End Stage Renal Disease patients.

IL-23

Cardiac biomarkers : measuring and manageing in Slovenia

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BACKGROUND: Cardiovascular diseases are the leading cause of mortality in developed countries, including Slovenia. Early identification of cardiovascular diseases depends on the selection of laboratory biomarkers and their detection methodology. We intended to provide an overview of cardiac biomarkers, detection methods and cutoff values utilized in Slovenian labs. In addition, we attempted to identify the level of quality and reliability of the provided results.

METHODS: The web survey was carried out in primary, hospital and urgent care based settings in order to collect the data on cardiac biomarkers measuring in the labs.

RESULTS: 60 interviewees responded to the web survey. 28 interviewees responded to the second set of questions. The most frequently determined tests per year are troponin I (more than 5000 tests in 5 labs), troponin T-hs (more than 5000 tests in 3 labs), NT-proBNP (1.000 to 5.000 tests in 7 labs) and myoglobin (100 to 500 tests in 7 labs). The labs are included into external quality control scheme, mainly to RIQAS (11 labs), followed by INSTAND (6 labs) and RfB (3 labs). Cutoff values of troponins for acute myocardial infarct diagnosis varied and are selected in cooperation with physicians. 27 interviewees responded to the point of care related set of survey questions. The most frequently determined near patient tests are troponin I, troponin T and NT-proBNP. The frequency of tests in these labs is lower than 100 tests per year. Five of these labs confirmed their inclusion into external quality control scheme.

CONCLUSIONS: The results of our study provide a valuable insight into cardiac biomarker testing in Slovenia. Our labs have the capability of measuring biomarkers, especially troponin I, troponin T, NT-pro BNP and myoglobin, which are considered as a standard for diagnosis, therapy and monitoring of cardiovascular diseases. Each test ratio depends on the type of lab and its capacity for different patient care settings. From the quality perspective, the testing is adequately performed in larger labs only, but this may not be the case in point of care labs.

Posters

P1

Evaluation of the diagnostic value of thymidine kinase 1 activity for early stages of breast cancer

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BACKGROUND: Cancer is a disease of abnormal proliferating cells. Serological methods could provide information of yet invisible tumors prior to detection by modern imaging, thus increasing the potential to early diagnosis in this patients. Prognostic and predictive factors are crucial for identifying patients at a higher risk of relapse and for selecting the most appropriate systemic treatment for individual patients. Several biomarkers have been evaluated for prognosis, diagnosis, and treatment monitoring in breast cancer and cancer antigen 15-3 (CA 15-3) is the most commonly used, but it has relatively low sensitivity and poor specificity for early stages of tumors. There is a need for new biomarkers that can fulfil the dual purpose of detecting the tumors at early stages of progression and monitoring treatment response. Thymidine kinase 1 (TK1) is a cell-proliferating marker that has been used for the prognosis and monitoring of treatment, follow-up and survival in patients with lymphoma and leukemia. It is an enzyme involved in nucleotide metabolism and is important for the supply of thymidine monophosphate for DNA synthesis. There are two types of thymidine kinase, cytoplasmic (TK1) and mitochondrial (TK2); however, the expression of the latter is not associated with cell proliferation. The aim of our study was to find the relationship between TK1 activity in serum with CA 15-3 and tumor stages in breast cancer patients.

METHODS: We analyzed sera from 77 breast cancer patients with known TNM classification along with sera from 115 healthy females for CA 15-3 in routine use and TK1 activity levels. Both, CA 15-3 and TK1 were determined by chemiluminiscence method but on different systems.

RESULTS: We calculated AUC values of ROC curves for CA 15-3 and TK1, separately according to stages of the tumor. The AUC values of marker CA 15-3 was 0,675 and 0,699, for stages T1 and T2, respectively. For TK1 activity, we got AUC values 0,883 and 897 for T1 and T2 stage, respectively.

CONCLUSIONS: Our results show that TK1 activity has better sensitivity and specificity for identification of breast cancer tissue in stage T1 and T2 in comparison to CA 15-3. We can conclude that TK1 activity may be useful as a marker for early detection of breast tumors.

P2

The level of infliximab as a biomarker of chronic inflammatory bowel disease treatment

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BACKGROUND: Inflammatory bowel disease (IBD) is an unpredictable rare disease that runs with acute relapses and mid remissions of various lengths. Its most common forms are ulcerative colitis (UC) and Crohn's disease (CD). There are used invasive methods of coloileoscopy and laboratory diagnostics to determine inflammatory activity and monitor treatment progress. The most established biomarker that has been used so far is known to be fecal calprotectin (FC). Measuring the concentration of infliximab (IFX) in the serum of patients with IBD receiving maintenance treatment with the biologic drug infliximab, is likely to be a new biochemical marker of inflammation activity.

METHODS: We enrolled 76 patients and measured FC (Calprest®) and IFX (ELISA – Leuven). We compared IFX according to their low (< 3,0 ng/l), intermediate (3,0 – 7,0 ng/l) and high (> 7,0 ng/l) values with FC (negative, limit, positive).

RESULTS: The average IFX and FC were 4,3 ng/l (IQR: 1,36 – 8,66 ng/l) and 145,50 µg/g (IQR: 48,55 – 441,80 µg/g), respectively. Average IFX seemed to be higher (5,55 ng/l) in UC compared to CD (3,00 ng/l), (p=0,029). The average FC values in UC samples is 831,00 µg/g vs 415,00 µg/g in CD samples. Comparison of FC with IFX at low, intermediate and high concentration has weak and negative correlation. Furthermore, IFX in relation to the FC (normal and limit results) at the cut-off level 6,45 ng/l can serve according to the AUC value (0,644) only as moderately strong test to determine inflammatory activity of patients with IBD.

CONCLUSIONS: The comparison and correlation of both parameters FC and the IFX nevertheless should be furthermore examined with the coloileoscopy, that is the most known method to determine the inflammation activity and monitoring treatment success, but it would rather prove to overload the patients with its invasive process.

P3

New mutations in FVIII A domain with discrepant mild haemophilia A phenotype

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BACKGROUND: Missense mutations are the main cause for mild haemophilia A (HA) phenotype. Some of them results in significant discrepancy between one-stage clotting assay (FVIII:C1) and chromogenic method (FVIII:CR) when FVIII activity is measured. Such mutations are located mainly within exons encoding C2 and A2 domains of FVIII. Missense mutations in A2 domain can cause instability of molecule leading to reduced FVIII:CR. The differences in FVIII levels may lead to an incorrect biochemical phenotype and diagnosis of haemophilia. Genetic background was determined in patients with discrepant FVIII activity in Slovenian patients with mild HA. Two different specific coagulation assays were used to determine potential discrepancy in biochemical phenotype.

METHODS: F8 gene mutations were determined with Sanger sequencing. FVIII activity tests were performed with one stage clotting assay (APTT-SP reagent and FVIII deficient plasma, both Instrumentation laboratory-IL, USA) on ACL TOP 500 instrument (IL, USA) and chromogenic assay (Electachrome Factor VIII, IL, USA) using spectrophotometric manual method (Multiskan EX, Thermolabsystem, Finland). FVIII antigen (FVIII:Ag) was determined with Asseracrom FVIII:Ag kit (Stago, France).

RESULTS: We found three novel missense variants clustered in A2 domain (p.Val670Phe, p.Tyr683His, p.Asp685Gly) and they were associated with fourfold FVIII activity discrepancy. Four patients with those variants had FVIII:Ag from 26 to 62 % however, they would be classified as having moderate form of HA using FVIII:CR method. All three genetic variants are located at the interface between A2 and A3 domain affecting the stability of FVIIIa heterotrimeric structure. Clinical data classified all our patients as mild haemophiliacs with bleeding episodes provoked only by surgical interventions or trauma.

CONCLUSIONS: Three novel genetic variants were described causing a discrepant biochemical phenotype of HA. The nature and position of variants are strongly associated with phenotype and contribute to the FVIII molecule instability. The endogenous synthesis and secretion of FVIII is less affected. One-stage and chromogenic method should be used in diagnostics of non-severe HA to avoid the misdiagnosis and to complement the genetic findings.

P4

Next generation sequencing for genetic testing of hypogonadotropic hypogonadism

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BACKGROUND: Congenital hypogonadotropic hypogonadism (HH) is a rare but clinically and genetically heterogeneous disease caused by pathogenic mutations in several genes leading to impaired production and secretion of gonadotropin hormones and consequently sex hormones. HH is characterized by an absence or delay of puberty and infertility. The association of HH with hyposmia or anosmia is defined as Kallmann syndrome. Molecular genetic testing of HH is important, as it can prompt the treatment. The aim was to identify causative variants in coding regions and exon/intron junctions of 24 genes related to idiopathic HH and Kallman syndrome, in patients with clinical presentation of HH, using next generation sequencing (NGS) and Sanger sequencing confirmation.

METHODS: Eleven subjects (ten males, one female) aged between 16 and 67 years with suspicion of congenital HH were included. Seven males had Kallmann syndrome, three males had normosmic HH. The female had suspicion of Kallmann syndrome. Three subjects had other congenital anomalies associated with HH (colour blindness, anomalies of heart, learning disabilities, long limbs, sensorineural deafness, bimanual synkinesis, hypertelorism). Whole blood genomic DNA was used for NGS analysis. The regions of interest were enriched using TruSight One library enrichment kit (Illumina, USA). NGS sequencing was performed using MiSeq desktop sequencer (Illumina, USA) coupled with MiSeq Reagent kit v3 (Illumina, USA). Further evaluation of variants with Variant Studio 2.2 software (Illumina, USA) was restricted to those located in 24 genes related to idiopathic HH and Kallman syndrome. The presence of candidate causative variant was confirmed with Sanger sequencing.

RESULTS: Seven causative variants in five genes (PROK2, GNRHR, PROKR2, FGFR1 and CHD7) were detected in six out of eleven patients. Among them, three variants namely PROK2 NM_001126128.1: c.171_172delTT (p.Ile57MetfsTer17), FGFR1 NM_023110.2: c.196T>C (p.Tyr66Arg) and CHD7 NM_017780.3: c.5759A>G (p.Tyr1920Cys) have not yet been described in HGMD professional, dbSNP or ExAc databases.

CONCLUSIONS: NGS enables fast and reliable identification of causal mutations in several genes related to HH simultaneously. Presented subject group with HH was genetically very diverse and the results expand the spectrum of mutations implicated in HH.

P5

Combined immunodeficiency in 11q terminal deletion syndromeTinka Hovnik¹, Alojz Ihan², Luca Lovrečič³, Štefan Blazina⁴¹Unit of Special Laboratory Diagnostics, Children's Hospital Ljubljana, University Medical Centre Ljubljana, Ljubljana, Slovenia²Institute of Microbiology and Clinical Immunology, Faculty of Medicine, Ljubljana, Slovenia³Clinical Institute of Medical Genetics, Division of Obstetrics and Gynecology, University Medical Centre Ljubljana, Ljubljana, Slovenia⁴Department of Allergy, Rheumatology and Clinical Immunology, Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

BACKGROUND: Terminal 11q deletion syndrome also known as Jacobsen syndrome (JS; OMIM#147791) is a rare genetic disorder that affects different body systems. Absence of evident T-cell defect and lacking immunological evaluation in some reported patients with JS has led to the perception that JS is predominantly antibody deficiency. We present a patient with confirmed JS in whom T-cell deficiency was observed in addition to antibody deficiency. The patient suffered from recurrent bacterial and prolonged viral infections of the respiratory system besides other classic features of the syndrome.

METHODS AND RESULTS: Standard and molecular cytogenetics studies on peripheral blood lymphocytes from patient and her parents showed 46,XX,del(11)(q23.3).ish del(11)(q23.3)(D11S1037)-dn. Using array CGH, deletion breakpoint was shown to be at the band 11q24.1 encompassing most of the crucial genes responsible for typical phenotype of JS. Immunologic workup was consistent with antibody deficiency. In addition to low IgM, IgG4 and B-cells, low recent thymic emigrants, helper and naive T-cells were also found.

CONCLUSIONS: Patients with JS have typical dysmorphic features, short stature and cognitive impairment (1). Special emphasis has been given to deciphering gene candidates for specific phenotypic features, mostly cognitive and behavioural characteristics, immune system disturbances and congenital heart anomalies. Six genes from common deleted region are related to immune system regulation and response – TIRAP, ETS1, FLI-1, NFRKB, THYN1, SNX19 and all are deleted in our patient as well (2). Antibody deficiency is common in patients with JS but there has been only a few reports on additional T-cell defects. The prevalence of combined immunodeficiency in patients with JS is not known, as most patients have not yet been evaluated for possible T-cell deficiency.

References

1. Favier, R., Akshoomoff, N., Mattson, S., & Grossfeld, P. Jacobsen syndrome: Advances in our knowledge of phenotype and genotype. *Am J Med Gen C. Semin Med Genet.* 2015; 169(3):239-50.
2. Seppänen M, Koillinen H, Mustjoki S, Tomi M, and Sullivan KE. Terminal deletion of 11q with significant late-onset combined immune deficiency. *J Clin Immunol* 2014; 34:114-8.

P6

Association of selected single nucleotide polymorphisms with the concentrations of tumor markers in patients with prostate cancerTeja Fabjan¹, Tamara Rumenović¹, Kristina Kumer¹, Tomaž Smrkolj², Gregor Hlebič³, Janja Marc⁴, Joško Osredkar¹¹Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia²Department of Urology, University Medical Centre Ljubljana, Ljubljana, Slovenia³Department of Urology, University Clinical Centre Maribor, Maribor, Slovenia⁴Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

BACKGROUND: It is known that prostate specific antigen (PSA) as a tumor marker is neither specific nor sensitive enough to distinguish benign prostatic hyperplasia (BPH) and prostate cancer (PC). Patients with benign hyperplasia are often unnecessarily leading to uncomfortable diagnostic procedures. The aim of our study was to determine the frequencies of selected polymorphisms in Slovenian population and to evaluate their impact on PSA, fPSA, proPSA, PHI, %fPSA and %proPSA in individuals with benign or malignant prostate disease.

METHODS: 119 patients with PSA between 2.7 and 10 µg/L were enrolled to the study. DNA was isolated from saliva samples and the amount and quality of isolated DNA was spectrophotometrically evaluated. Five single nucleotide polymorphisms associated rs80130819, rs76934034, rs115306967, rs4054823 and rs1447295 located in different genes were selected according to literature data. For determining the genotypes, we used the KASP genotyping method.

RESULTS: Saliva was proven as a suitable sample for the DNA isolation and could be applied to genetic testing in the future. Genotype frequencies of all the investigated polymorphisms followed the Hardy-Weinberg equilibrium and did not deviate from the other European populations. Regardless the genotypes, we found that prostate cancer patients had significantly higher PSA ($P = 0,003$), proPSA ($P = 0,003$), PHI ($P = 0,000$) and %proPSA ($P = 0,014$) levels. We proved that allele A of rs80130819 polymorphism is associated with higher values of proPSA ($P = 0,029$) in PC patients and that allele C causes higher fPSA ($P = 0,027$) values in BPH patients. The presence of allele T of rs76934034 was

associated significantly with higher levels of PHI ($P = 0,033$) and %proPSA ($P = 0,027$) in PC patients, but not in BHP. However in this patients with allele C of rs1447295 had higher proPSA values compared to other BPH patients ($P = 0,034$).

CONCLUSIONS: The polymorphisms rs80130819, rs76934034 and rs1447295 were associated with PSA parameters in both groups, but clinical relevance of polymorphisms rs115306967 in rs4054823 was not found in present study. Further studies on larger sample are necessary.

P7

Total antioxidant status and 8-isoprostane in follicular fluid

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BACKGROUND: About factors affecting fertility relatively little is known, but a growing body of literature hypothesize that environmental and lifestyle factors and oxidative stress (OS) may influence the timing and maintenance of a viable pregnancy. Free radicals can influence the oocytes, sperm, and embryos in their microenvironments, for example follicular fluid, hydrosalpingeal fluid, and peritoneal fluid. There is a complex interplay of cytokines, hormones, and other stressors in these microenvironments that cause cellular generation of free radicals; these molecules influence further the modulation of many transcription factors and gene expression. Total anti-oxidant status (TAS) is known to be reliable and sensitive indicator of the current antioxidant situation in the body at a given time. The 8-isoprostane is also a marker of oxidative stress and provides a unique opportunity to investigate lipid peroxidation in different human pathologies and risk factors such as obesity, gender, smoking, alcohol abuse, and cancer.

METHODS: Follicular fluid samples from women with polycystic ovary syndrome (PCOS), endometriosis and tubal factor infertility were obtained on the day of oocyte collection and were evaluated for OS bio-markers including TAS by colorimetric method and 8-Isoprostane (8-IP) levels by enzyme immunoassay.

RESULTS: Follicular fluid TAS values were significantly higher in women with endometriosis as compared to tubal factor infertility (1,0712 mmol/l vs 0,9328 mmol/l; $p < 0,0001$). There was no significant differences in follicular fluid concentrations of TAS between PCOS and tubal factor infertility group ($p = 0,4210$). Levels of follicular fluid 8-IP were slightly higher in test groups (85,3 ng/L in PCOS group and 91,065 ng/L in endometriosis group) vs tubal infertility group (69,2 ng/L), which we used as a control group, but the difference is not statistically significant.

CONCLUSIONS: Oxidative stress accompanied by reduced endogenous defences play a role in the pathogenesis of infertility and is reported to affect both natural and assisted fertility. This area is still poorly studied. Thus, identifying modifiable factors to decrease oxidative stress in the gynecologic environment may be an inexpensive and noninvasive therapy for increasing fertility. Further studies are needed.

P8

Comparison of two chromogenic assays for measuring anti-factor Xa activity of low-molecular-weight-heparins in human plasma

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BACKGROUND: Low-molecular-weight heparins (LMWHs) are widely used in the prevention and treatment of venous thromboembolism. Routine laboratory monitoring is not required, with the exceptions of patients with renal insufficiency and in patients at extremes of weight. For monitoring, chromogenic assays for anti-factor Xa, rather than aPTT, is recommended. The aim of our study was to compare two chromogenic methods for measuring anti-factor Xa activity of LMWHs in human plasma. They differ in adding exogenous antithrombin and dextran sulfate to human plasma in one (Siemens Berichrom Heparin reagent kit, Siemens, Germany) but not in the other assay (Stago STA Liquid ANTI-Xa reagent kit, Stago, France).

METHODS: 47 patients (median age 70, range 29 to 89 years; 12 women, 35 man) treated with LMWHs were included in the study. Anti-factor Xa activity in patients plasma (concentration range 0.1 to 1.39 kIU/L) was measured by Stago STA Liquid ANTI-Xa (Stago, France) and Siemens Berichrom Heparin (Siemens, Germany) reagent kit on the Compact Max Stago (Stago, France) and BCS-XP Siemens (Siemens, Germany) analyzer, respectively. Method comparison was performed using the Passing and Bablok regression analysis which include Cusum test for linearity.

RESULTS: The Passing-Bablok regression line equation was $Y = 0.038 + 0.81x$ with the 95% CI for intercept of -0.014 to 0.073 and for slope of 0.71 to 0.92 (Stago vs Siemens method). 95% CI for slope of 0.71 to 0.92 indicated that proportional difference between two methods exists. Cusum test for linearity indicated no significant deviation from linearity ($P=0.39$).

CONCLUSIONS: We successfully applied both methods for measuring anti-factor Xa activity of LMWHs in human plasma. The proportional difference between two methods is small and increases from lower to higher activity of LMWH in human plasma. The difference in the design of the assays could be a reason for the proportional difference observed between the two methods.

P9

Assessment of maternal serum inhibin A in normal, preeclamptic and intrauterine growth restricted pregnancies

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BACKGROUND: Inhibins are circulating dimeric glycoprotein hormones that are secreted by the granulosa cells of the ovary, sertoli cells of the testis and also the foetoplacental unit throughout pregnancy. Inhibin A is the predominant molecular form in maternal circulation after 4 weeks of gestation and is characterized by its ability to inhibit the secretion of follicle stimulating hormone. Concentrations initially peak at 8 weeks' gestation then steadily decrease up to 16 weeks. After remaining relatively constant during second trimester weeks 15–18, levels increase again during the third trimester to reach a peak concentration at 36 weeks. The possible clinical applications for the measurement of inhibin A could be in predicting miscarriage, Down's syndrome, preeclampsia, and fetal growth.

METHODS: The aim of the present study was to evaluate serum samples concentrations of inhibin A collected from healthy normotensive pregnant controls (n = 28), women with intrauterine growth restriction (n = 16) and women with preeclampsia with (n = 40) or without superimposed intrauterine growth restriction (n = 26) from 28 to 41 weeks gestation. Also to verify whether this marker is related to these pathological conditions. Blood samples were collected in plain tubes, centrifuged, and stored at –20°C until analyzed. Inhibin A concentrations were measured by automated sequential two-step immunoenzymatic assay (Beckman Coulter, Inc. Fullerton CA). The lower limit of detection and the highest calibrator value of the assay were 1 ng/L and 1500 ng/L.

RESULTS: Inhibin A levels were significantly higher in the intrauterine growth restriction group (1148 ± 415 ng/L, $p=0,0004$), preeclampsia group with (1267 ± 383 ng/L, $p<0,0001$) or without superimposed IUGR (1306 ± 336 ng/L, $p<0,0001$) than in the control group (660 ± 395 ng/L). The presence of IUGR did not significantly modify these concentrations.

CONCLUSIONS: Elevated inhibin A concentrations are strongly associated with preeclampsia and not with intrauterine growth restriction. Higher maternal serum concentrations of inhibin A in preeclampsia than in healthy pregnant controls could be helpful in the diagnosis of preeclampsia.

P10

Comparison of the performance of point-of-care testing total cholesterol, triglycerides and high density lipoprotein cholesterol measurements with laboratory methods

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BACKGROUND: Point-of-care testing (POCT) is used in management of many diseases, such as diabetes, hyperlipidaemia and cardiovascular disease. Point-of-care methods must be as accurate as traditional laboratory methods, so it is important that the users follow POCT guidelines.

METHODS: We compared POCT method (CardioChek®Plus; Polymer Technology Systems-PTS, Inc.) and reference laboratory method (ADVIA 1800® Clinical Chemistry System-SIEMENS) for the measurement of total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) in 25 EDTA venous blood samples obtained from subjects referred to our laboratory. Lipids were measured in whole blood on CardioChek®Plus and in plasma samples on ADVIA1800®. Measurements were done in duplicates. Statistical analysis was done with MedCalc® software (version 16.2.0.).

RESULTS: The linear regression results indicate a good correlation between the CardioChek®Plus POCT method and ADVIA1800® method for TC ($R^2 = 0.93$, $r = 0.97$ ($P < 0.0001$)), TG ($R^2 = 0.98$, $r = 0.99$ ($P < 0.0001$)) and HDL-C ($R^2 = 0.89$, $r = 0.94$ ($P = 0.0001$)). Despite a strong correlation the comparison showed statistically significant differences ($P < 0.05$) between methods in all three cases. The mean difference between the CardioChek®Plus and ADVIA1800® was calculated (TC = -7.3%, TG = -15.6%, HDL-C = -8.3%). According to the PTS protocol for a comparative study of the CardioChek®Plus analyser to a laboratory method the mean difference is expected to be $\pm 10\%$, $\pm 15\%$ and $\pm 12\%$ for TC, TG and HDL-C, respectively. Only the TG mean difference falls out of this range. The mean differences between methods show that the CardioChek®Plus provided significantly lower values as compared to the laboratory values (TC = 0.39 mmol/L, TG = -0.28 mmol/L, HDL-C = -0.10 mmol/L). The line of equality (= 0%), which indicates a systematic error, is outside of the 95% confidence interval in all three cases.

CONCLUSIONS: CardioChek®Plus analyser produced significantly lower values compared to those reported for the same patients' samples analyzed in laboratory. Whereas CardioChek®Plus might be useful for monitoring of metabolic disorders and cardiovascular risk factors, great care must be taken to ensure that results are not misinterpreted.

P11

Monitoring turnaround time

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BACKGROUND: Turnaround time (TAT) is an example of an indicator of the quality of a process in the laboratory. Doctors and other medical personnel often evaluate the quality of laboratories according to the speed of providing analysis results. TAT can have a direct impact on the time a doctor needs to diagnose.

METHODS: TAT indicator was measured for analysis of urea and creatinine of patients who need CT. TAT is measured from the time a patient is inscribed in LIS until the final validated results.

RESULTS: After validating analysis, we notify the RTG department by telephone about the results. Informing on the telephone was introduced in 2015 to eliminate the communication gap. These two parameters have been monitored in previous years but the RTG department did not have results in an hour. According to our records we had all the results validated in less than an hour. We found that private patients are not enlisted in the hospital information system and the RTG department personnel did not have an insight into the results. The RTG personnel therefore waited for the patient to physically bring the results to the department. Thus the sum of time from the patient being sent from the RTG department until his return with the laboratory results was more than an hour. In order to reduce the time, we studied all the phases of the analysis and improved the communication between the personnel in the pre-analysis phase by specifically marking these patterns. In 2015 we reduced the time of delivering results for the analysis of urea and creatinine by 6 minutes.

CONCLUSIONS: Monitoring allowed analysis of where in the procedure one can find a possibility for improvement and better communication. We found that our TAT for the analysis of urea and creatinine complies with the times set for "urgent" biochemical samples. In the laboratory the entire process is executed in the requisite time. Our evaluation is that the time is lost when delivering the biological sample from the department. The solution is pneumatic tube systems to send biological samples to the laboratory.

P12

Periodic fever with aphthous stomatitis, pharyngitis, and adenitis – clinical and genetic characteristics of 81 patients

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BACKGROUND: Periodic fever with aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is the most common autoinflammatory disorder in childhood with unknown etiology and genetic background, characterized by recurrent episodes of fever, sore throat, mouth sores and swelling of the glands in the neck. The aim of our study was clinical evaluation of pediatric PFAPA patients from a single tertiary care center and to determine, whether variants in AIM2, MEFV, NLRP3, and MVK genes are involved in PFAPA pathogenesis.

METHODS: We collected clinical and laboratory data of pediatric PFAPA patients, who were followed at the University Children's Hospital Ljubljana from 2008 to 2014. All 4 genes were PCR amplified and directly sequenced.

RESULTS: In the 7-year study period, 81 patients fulfilled criteria for PFAPA syndrome, 50 (63%) boys and 31 (37%) girls. Mean age at disease onset was 2.1 ± 1.5 years and mean duration of febrile episode was 4.2 days with interval between episodes 4 weeks. Adenitis, pharyngitis, and aphthae were present in 94%, 98%, and 56%, respectively. Family history of recurrent fevers in childhood was positive in 78%. Genetic analysis was performed in 62 patients. Nineteen variants were found in 17/62 (27%) patients, 4 different variants in NLRP3 gene in 13 patients and 6 different variants in MEFV gene in 5 patients. Two patients had 2 different variants. No variants of clinical significance were found in MVK and AIM2 genes.

CONCLUSIONS: PFAPA is classically considered as a sporadic and non inherited syndrome however extremely high rate of positive family history (78%) in our cohort clearly indicates genetic cause of the disease. Total of 17 patients (27.4%) carried variants in MEFV and NLRP3 gene that could play a role in susceptibility to autoinflammation. Our data suggest that PFAPA could be the result of multiple low-penetrant variants in different genes in combination with epigenetic and environmental factors leading to uniform clinical picture.

P13

Determination of blood lactate concentration during exerciseKristina Kumer¹, Teja Fabjan¹, Katja Pernek¹, Radoje Milić², Joško Osredkar¹¹Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia²Faculty of sport, University of Ljubljana, Ljubljana, Slovenia

BACKGROUND: The energy that is required for the entire process of muscle excitation and the creation of its forces is provided entirely by ATP which is produced in the cells aerobic or anaerobic. At moderate loads all the required energy is produced aerobically, because our body adjusts to the effort. When the strain exceeds the anaerobic threshold, part of the energy is produced by the anaerobic path resulting in the accumulation of H⁺ ions and lactate in the muscle cells. At any physical stress lactate concentration rises and it is basically a factor that defines the ability of an individual to a certain effort. At low intensity lactate originally not rise significantly above the level in the rest aerobic. With higher load we observe the increase in lactate levels. During further escalation of the burden athlete reaches the anaerobic threshold. At maximum load lactate continues to increase, and consequently all the more acidic environment resulting in failure of the many enzymes involved in the formation of energy. The athlete has to drastically reduce the speed or stop and the value of lactate is stabilized or declined if the athlete is in good physical shape.

METHODS: The aim of our study was to determine the suitability of lactate meter for the purpose of monitoring the concentration of lactate in the blood when performing exercise tests on a treadmill. In the investigation we included 12 athletes, aged 15 to 30 years. L-lactate was measured with the chip-shaped electrochemical sensing system. Interpretation of the test results was performed by Curve Expert 1.4.

RESULTS: The content of lactate as a function of the load increases in a characteristic curve. Its position and the shape, largely reflects the different physical fitness of the individual. The lower value of the lactate in the blood at the same load may mean that the formation of lactate is reduced, or in the other hand the consumption of the lactate is increased.

CONCLUSIONS: Measurements of aerobic and anaerobic capacity are routinely used in sports. The results are of great help to athletes and coaches.

P14

Preanalytical errors in arterial blood gas analysisLidija Gobec¹¹Department for Laboratory Medicine, General Hospital Celje, Celje, Slovenia

BACKGROUND: Blood gas testing is usually requested for patients with life threatening conditions. Arterial blood should be collected under anaerobic conditions, mixed immediately and promptly analysed. Plastic syringes have to be transported to the laboratory at room temperature and analysed within 30 minutes of collection. Because of the intense activities in the preanalytical phase, the preanalytical errors rate is high.

METHODS: Preanalytical errors were registered in laboratory information system through developed template at sample admission to the lab. Collective data on the preanalytical errors were extracted from the database and used for the error prevalence calculations.

RESULTS: In 2015 there were 11456 samples sent to Emergency lab for blood gas analysis. 878 preanalytical errors were recorded corresponding to the error rate of 7,6%. The most common preanalytical errors were clotted sample (24,8%), sample contamination with venous blood (22,9%), missing draw time (21,6%) and prolonged transportation time (15,3%). According to the established criteria 2,1% of all arterial blood samples were rejected as inappropriate. The main reason for the rejection was clotted sample. The overall rate of all preanalytical errors in the Department for laboratory medicine in year 2015 was 2,4%.

CONCLUSIONS: Due to low biological variability of blood gas parameters, blood gas testing requires special attention and compliance to the recommended procedures. Despite all standardised procedures blood gas analysis is more prone to preanalytical errors than other tests. Prevalence of preanalytical errors in blood gas analysis is several times higher than overall preanalytical errors rate in our lab. Because there is little data in literature on prevalence of preanalytical errors and there is no uniform methodology in approaches to evaluate the preanalytical phase the data is also difficult to compare. Preanalytical variations are often unknown to the clinicians interpreting the results. If the quality of sample is compromised it should be rejected. Results for samples with »acceptable« errors should be sent to the physician with a specific comment.

References

1. Šimundić AM. In: Guder WG, Narayanan S, editors. Pre-examination procedures in laboratory diagnostics. Berlin:De Gruyter, 2015:292-297.
2. Baird G. Preanalytical considerations in blood gas analysis. *Biochemica Medica* 2013;23(1):19-27.

P15

Incidence of thyroid antibodies in healthy subjectsAjda Biček¹, Adrijana Oblak¹, Katja Zaletel¹, Simona Gaberšček¹¹Department of Nuclear Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia

BACKGROUND: According to American survey, in 18% of thyroid disease-free population antibodies against thyroglobulin (aTG) and/or thyroid peroxidase (aTPO) can be detected (10,4% and 11,3%, respectively). There is no similar data for Slovenian population. The aim of our study was to determine the concentration of aTG and aTPO antibodies in healthy subjects (HS) and to establish the upper cut-off values of aTG and aTPO concentration in Slovenian population.

METHODS: The study included subjects who were examined by thyroid specialist for the first time between 2011 and 2014. A group of 2071 HS and 230 subjects with Hashimoto's thyroiditis (HT) were included in analysis. For all subjects serum levels of aTG, aTPO, thyrotropin (TSH), and thyroid hormones (freeT4, freeT3) were measured and thyroid ultrasound was performed. For HS, aTG and aTPO concentrations were below the cut-off value of 60 kU/L, as declared by the manufacturer. The lower limit of the aTG determination was in 2011 20 kU/L, while at the end of 2011 it was reduced by manufacturer to 15 kU/L. The lower limit of the aTPO determination was 28 kU/L.

RESULTS: In 20% of HS aTG concentration was above 20 kU/L and in 65.8% aTPO concentration was above 28 kU/L. To establish our own cut-off values of aTG and aTPO concentrations ROC curve analysis was done. For aTG concentration, a cut-off value of 53.7 kU/L returned 63.3% sensitivity and 99.6% specificity (AUC=0.817; 95%CI: 0.801-0.833), 97.3% positive predictive value (PPV) and 92.5% negative predictive value (NPV). Similar predictive values (94% PPV and 92% NPV) have already been described at the cut-off value of 44 kU/L, but on a much smaller sample. For aTPO concentration, a cut-off value of 57.2 kU/L returned 76.1% sensitivity and 97.6% specificity (AUC=0.910; 95%CI: 0.898-0.922), 87.6% PPV, and 94.9% NPV.

CONCLUSIONS: Diagnosis of HT is also based on elevated aTG and/or aTPO concentrations, therefore these cut-off values should be set appropriately. Cut-off values determined in our study population are lower than declared by manufacturer. This can explain a relatively high portion of HS with thyroid antibodies above the lower and below the upper routinely used cut-off value.

P16

Sample reception process in medical laboratory – implementation of quality control plan based on risk managementStaša Mikec¹, Nina Karakaš¹, Pika Meško Brguljan¹¹Clinical Chemistry Department, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

BACKGROUND: Laboratory has to be aware of different factors which could influence quality of laboratory test results. In Laboratory for Clinical Biochemistry and Hematology of Clinic Golnik the process of systematic error monitoring have been implemented. Based on most common errors, the need to assess the risk for their appearance was recognized. Determining the nature of errors, preanalytical phase has been shown to be most prone to errors which could have the biggest effect on the quality of test results and would present a risk of harm to patients. Among key processes, the first goal was to identify errors in process of sample reception, assess the risk and implement quality control plan with continuing monitoring.

METHODS: The risk management has been made in compliance with requirements of different regulatory, accreditation and certification bodies. Quality control plan has been performed according to CLSI EP 23-A guidelines, based on the three-factor model with defined assessment of the occurrence, severity, detection and calculated criticality of harm to patients (HFMEA). Mapping of sample reception process has been performed, potential hazards in the form of a fishbone diagram of errors have been identified. Spreadsheet with identification of actions to reduce errors and calculation the criticality for each identified hazard in sample reception process has been performed.

RESULTS: Identified hazards with estimated criticality >20 have been chosen as potential risk for patients. Based on risk assessment quality indicators (QI) for sample reception process have been chosen. Selected QI were a ligned with IFCC WG-LEPS model of quality indicators (mostly priority 1). Those QI are: samples not received, primary sample container received without identification label, misidentified samples (divided to errors made by laboratory and by client), unsuitable samples (insufficient volume, clotted sample), hemolysed samples, samples wrongly distributed while added in additional containers for further analysis and laboratory information system downtime episodes. In addition to those, one more QI (samples received without request in the LIS) with lower criticality, has been added due to frequency of its occurrence.

CONCLUSIONS: Selected quality indicators represent an effective way of detecting, controlling and preventing errors in the process of sample reception. Implemented quality control plan should be constantly monitored for effectiveness and modified as needed to maintain risk at clinically acceptable level in order to improve patient safety.

P17

Monitoring of *Helicobacter pylori* eradication therapy success with non-invasive diagnostic methodsPetra FINDERLE¹, Joško OSREDKAR¹¹Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia

BACKGROUND: *Helicobacter pylori* (HP) is a spiral, microaerophilic, Gram-negative bacteria with 2-7 polar sheathed flagella, measuring $0.3 \times 3-5 \mu\text{m}$. Is one of at least 28 known members of the *Helicobacter* family, which is exclusively adapted to the acidic environment of the stomach. HP infection has different impact to the individual's health regarding the immune response and the age of the patient, the environmental influences and virulence and strain of the bacteria. Therefore, HP infection can have a totally asymptomatic course or can lead to gastritis, ulceric lesions of stomach or duodenum, and to stomach cancer or MALT lymphoma in 20% of patients. It is highly important to early detect and treat HP infection. Invasive and non-invasive diagnostic methods are used to confirm and monitoring of treatment success. Usually, for HP eradication a combination of a proton pump inhibitor and two antibiotics are used, regarding the national guidelines.

METHODS: In our work we observed results of seven non-invasive diagnostic methods (Urea breath Test INFAI[®], semiautomatized stool HP antigen test LIAISON[®] DiaSorin, two quick stool HP antigen tests from DIMA[®] MKBio, and H&R[®], HP urease activity in saliva determination with dBEST One Step Saliva Urease, and determination of specific HP IgG and IgA in serum with ORION Diagnostica[®] test) for 38 patients with median age of 48 (16–78) years, taken in two different clinical visits. For 23 patients we had information about the prescribed treatment, however all patients were managed through the Gastroenterology clinic because of HP infection, so we assumed all patients were treated, accordingly. We statistically assessed all data with t-paired test for homogeneous variance and Wilcoxon test for non-homogeneous group results with probability value for hypothesis rejecting of 0,05.

RESULTS: Urea breath Test INFAI[®], and the three methods for stool antigen determination, Liaison[®] DiaSorin, and DIMA[®] MKBio, and H&R[®] showed a statistically significant difference of results before and after treatment, although the positive/negative results ratio of 0.63, 0.50, 0.75, 0.44, respectively. However, dBEST One Saliva Urease[®], and serum antibody IgG and IgA results gave no statistically significant difference between two clinical visits.

CONCLUSIONS: Treatment of HP infection is complex and depends on HP strain resistance on antibiotics and patient cooperation for following treatment regime. Our small study group gave perspective results for monitoring of eradication success for at least three observed non-invasive diagnostic methods.

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Diagnostic potential of CA-125 and HE4 tumor markers in endometrial cancerTamara KNIFIC¹, Tea LANIŠNIK RIŽNER¹, Joško OSREDKAR², Špela SMRKOJ³, Snježana FRKOVIĆ-GRAZIO⁴, Teja FABJAN²¹Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia²Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia³Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Ljubljana, Slovenia⁴Department of Pathology, University Medical Centre Ljubljana, Ljubljana, Slovenia

BACKGROUND: Endometrial cancer is the fifth most common malignancy among females worldwide and due to increased obesity and other risk factors related to endometrial cancer it is becoming a considerable health concern (1). Detection in early stages promises better survival rates though no effective screening for endometrial cancer exists. Surgery is needed to assess the stage of the disease and includes hysterectomy as part of the treatment. There is still no consensus regarding lymph node dissection as part of the staging procedure (2, 3). Imaging techniques lack sensitivity in detecting deep myometrial invasion and extrauterine disease thus showing the need for endometrial cancer specific biomarkers in order for preoperative stratification of patients into risk groups (3). The aim of our study was to determine serum levels of CA-125 and HE4 and evaluate their diagnostic value in Slovenian endometrial cancer patients.

METHODS: A total of 65 patients with endometrial cancer and 69 control patients were included in this retrospective study. Serum samples were analysed by means of electrochemiluminescent immunoassays specific for CA-125 and HE4 on cobas e411 immunoassay analyser. Serum levels were compared between two independent groups using the two-sided Wilcoxonov rank-sum test (Mann-Whitney test).

RESULTS: Median CA-125 (denoted in kU/L) and HE4 (denoted in pmol/L) serum concentrations for endometrial cancer patients were 19.03 kU/L and 81.59 pmol/L and for control patients 13.07 kU/L and 57.80 pmol/L, respectively. Comparison of serum levels for CA-125 and HE4 between patients with endometrial cancer and control patients showed a significant difference ($p < 0001$).

CONCLUSIONS: Our results support published studies in other populations (4) and thus confirm that CA-125 and HE4 could be used as diagnostic biomarkers for endometrial cancer. Further analysis is needed to evaluate their prognostic potential in terms of myometrial invasion, lymph node status, tumour grading, histology and FIGO stage.

References

1. Renehan AG, et al. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet*. 2008;371(9612):569-78.
2. Sonoda Y. Surgical treatment for apparent early stage endometrial cancer. *Obstet Gynecol Sci*. 2014;57(1):1-10.
3. Kalogera E, et al. Correlation of serum HE4 with tumor size and myometrial invasion in endometrial cancer. *Gynecol Oncol*. 2012;124(2):270-5.
4. Kemik P, et al. Diagnostic and prognostic values of preoperative serum levels of YKL-40, HE-4 and DKK-3 in endometrial cancer. *Gynecol Oncol*. 2016;140(1):64-9.

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Establishing the cut-off value for aldosterone-to-renin ratio for screening test in diagnostics of primary aldosteronism

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BACKGROUND: Aldosterone-to-renin ratio (ARR) has been recommended for screening of primary aldosteronism (PA) as a more sensitive indicator than either of these analyte tests alone or hypokalemia (1). In the past, measurements of both analytes in plasma based on radioimmunoassay (RIA) with determination of aldosterone concentration and activity of enzyme renin. Today, automated chemiluminescence immunoassays (CLIA) are available for determination of both analytes, but they differ from RIA in measuring renin concentration. This generates changes in calculation of ARR cut-off value. The ARR cut-off value in our laboratory for RIA methods is 1000 (pmol/L)/(μg/L/h), whereas manufacturer's recommended CLIA ARR cut-off value is 30.5 (pmol/L)/(mIU/L). Newer methods also change antibodies used in reagents for aldosterone determination from polyclonal to monoclonal. Consequently, the interlaboratory reproducibility of aldosterone measurement is poor, and methods are difficult to compare (2). Additionally, calculation of ARR depends on good reproducibility of aldosterone determination and sufficiently sensitive determination of renin (1). Our aim was to determine our own cut-off value for ARR obtained by CLIA on iSYS system by IDS.

METHODS: The calculation of ARR was performed using 49 samples of patients suspected of having PA. ROC curve was calculated using program MedCalc® Version 14.8.1. Results were compared to the RIA ARR using comparison of sensitivity and specificity of both ARR and false positive (FP) results.

RESULTS: New calculated ARR cut-off value using CLIA method was 24.7 (pmol/L)/(mIU/L) with 100% sensitivity and 91.4% specificity. RIA ARR for the same patients showed lower specificity, 80%. Out of 49 samples (14 positive, 35 negative; confirmation was made with saline infusion test) there were 8 FP samples using RIA methods and 3 FP using CLIA method. All CLIA method-positive samples were also RIA method-positive.

CONCLUSIONS: A change from RIA to CLIA gives less FP results. CLIA also allows calibration of renin against NIBSC Standard reference preparation and aldosterone against accredited IDMS method (2). Our results show the importance of establishing laboratory-own ARR cut-off value due to differences in aldosterone determination, and in the sense of good laboratory practice, which recommends that every laboratory should determine its own cut-off values.

References

1. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF Jr. Case Detection, Diagnosis, and Treatment of Patients with Primary Aldosteronism: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2016;101(5):1889-1916.
2. Manolopoulou J, Fischer E, Dietz A, Diederich s, Holmes D, Junnila R, Grimminger P, Reincke M, Morganti A, and Bidlingmeyer M. Clinical validation for the aldosterone-to-renin ratio and aldosterone suppression testing using simultaneous fully automated chemiluminescence immunoassays. *J Hypertens*. 2015;33:2500-2511.

P20

The usefulness of P-MFV parameter in evaluation of platelet volume

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BACKGROUND: Platelets have an essential physiological role in hemostasis. Because of their importance in the process of blood coagulation it is important to know the platelet count. However, platelet counts alone do not reveal the true pathological mechanism of thrombocytopenia. Modern analyzers also provide additional information about platelet morphology by presenting the sum of all impulses of the respective cell

population of a specific size. The majority of analyzers present the platelet volume distribution curve and determine Mean Platelet Volume (MPV). In patients with platelets production defects analyzers often do not determine MPV. Sysmex analyzers provide another service parameter - Platelet Mean Frequent Volume (P-MFV) even when distribution curve is not optimal. MFV represents the most frequently occurring value in the PLT hydrodynamic focusing channel. The aim of our study was to investigate whether the P-MFV parameter adds an information of platelet morphology in cases where MPV cannot be determined.

METHODS: Hematological parameters were measured in 64 patients on two different analyzers, XT 2000i (Sysmex) and Advia 2120 or Advia 120 (Siemens). The statistical comparison of platelet counts and platelet volume parameters were done.

RESULTS: 88% of our patients with no presentation of MPV on XT 2000i had thrombocytopenia ($<150 \times 10^9/L$). We found no significant difference and good correlation between measuring platelet count on both analyzers ($p=0,744$; $r=0,977$). The difference between MPV on Advia and P-MFV on XT 2000i was significant ($p=0,0004$), and the correlation between parameters was poor. With Bland-Altman analysis we found low bias between MPV and P-MFV $0,7 \pm 1,7$ fL (95% CI: -2,6; 4,1 fL). MPV on Advia is 0,7 fL higher than P-MFV determined on XT 2000i.

CONCLUSIONS: Patients who present a severe thrombocytopenia are diagnosed routinely in clinical practice in tertiary hospitals. Besides the platelet count information, MPV helps identify the origin of thrombocytopenia. In pathological samples some analyzers cannot measure MPV. According to our results P-MFV service parameter determined on Sysmex analyzer can add an information to platelet count but it can not substitute the MPV in thrombocytopenic patients. Regarding to our experience we recommend the microscopic review of platelet morphology.

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Case report: First confirmed case of human babesiosis in Slovenia

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BACKGROUND: Babesiosis is a malaria-like infectious disease caused by intra erythrocytic protozoan of the genus *Babesia*. Transmitted by ticks of the genus *Ixodes*, it affects a wide range of domestic and wild animals, occasionally also people. Babesiosis may also be transmitted via blood transfusion. It is usually an asymptomatic infection in healthy individuals. The disease most severely affects elderly, immunocompromised or asplenic patients.

AIM: The first confirmed case of human babesiosis in Slovenia would be presented.

PATIENT: A 55-years old asplenic female presented in the Ambulance of Infectious Diseases in General Hospital Murska Sobota, with a fever lasting for the last six days, complaints of pain in the neck and ears, headaches, increased urination, dark coloured urine and loss of weight. She did not have a history of a tick bite. Blood samples were submitted for hematologic and biochemical analysis. Microscopic examination of blood smear was not ordered.

RESULTS: Laboratory findings included thrombocytopenia ($75 \times 10^9/L$), elevated level of reactive lymphocytes (8.9%); aspartate aminotransferase ($1,29 \mu\text{kat/L}$); alanine aminotransferase ($1,21 \mu\text{kat/L}$); γ -glutamyl transferase ($1,30 \mu\text{kat/L}$); alkaline phosphatase ($1,97 \mu\text{kat/L}$); and C-reactive protein (60 mg/L). According to our guidelines we made a microscopic examination of a blood smear stained with May-Grünwald Giemsa and found unusual parasites in red blood cells (parasitemia level 1%). There were ring forms visible within erythrocytes and varied greatly in shape and size (round, oval, per-shaped, amoeboid). Ring forms were without any pigment, which is observed in *Plasmodium* species. In some erythrocytes we found paired per-shaped forms and tetrad formations ("Maltese cross"). Review of the literature and the comparative smear examination led us to the diagnosis of human babesiosis. Samples were then sent to the Institute of Microbiology and Immunology in Ljubljana, where babesiosis was confirmed with PCR.

CONCLUSIONS: Babesiosis is a very difficult disease to diagnose. Clinicians should suspect babesiosis in patients who have haemolytic anaemia, thrombocytopenia, fever, an influenza-like illness and a history of tick bites, or a transfusion with infected blood. Diagnosis depends on the degree of parasitemia and the expertise of the laboratory personnel, as it is usually made by a microscopic identification of the organism on a thin blood smear.

P22

Evaluation of the ELISA test for the determination of hepcidin concentration in serum of patients with anemia

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BACKGROUND: The aim of our study was to evaluate the ELISA test (Hepcidin-25 (bioactive) ELISA, EIA5258, DRG international GmbH, Germany) for hepcidin concentration determination in human serum.

METHODS: Peripheral blood from patients and healthy individuals was drawn between 8 am and 11 am. The assay was performed according to the manufacturers' package insert protocol. The reference values were determined in the group of 56 healthy individuals (HI; 29 women, 27 men; median age 29, range 20 to 60 years). 16 patients with iron deficiency anemia (IDA; 14 women, 2 men; median age 45, range 21 to 83 years) and 24 patients with anemia of chronic kidney disease associated anemia (CKD associated anemia; 10 women, 14 men, median age 63, range 45 to 86 years) were also included in the study. The repeatability was determined on the four samples within a single run with six replicates of each. The between series imprecision was determined on four samples in 3 runs with six replicates of each per run. Nonparametric Mann-Whitney test was used for comparisons between groups.

RESULTS: The reference interval of hepcidin concentration for our population of 1.34 to 11.20 nmol/L (Non-parametric percentile method) was thus calculated. The average coefficient of variation (CV%) of the hepcidin concentration (nmol/L) in the repeatability test was 3.24% (mean1=6.41; SD1=0.11, CV1= 1.77%; mean2=3.24; SD2=0.05, CV2=1.67%; mean3=2.72; SD3=0.11, CV3=4.01%; mean4=20.82; SD4=1.15, CV4=5.52%). The average CV% of the hepcidin concentration (nmol/L) in the between series imprecision test was 3.15% (mean1=6.46; SD1=0.17, CV1= 2.72%; mean2=3.35; SD2=0.08, CV2=2.35%; mean3=2.64; SD3=0.07, CV3=2.64%; mean4=21.16; SD4=1.03, CV4=4.89%). The correlation coefficient of linear regression was 0.984. The median value of hepcidin concentration was highest in the ACD, followed by the HI and IDA groups (median 26.47/4.68, $P < .001$ and 4.68/1.26, $P < .001$, respectively).

CONCLUSIONS: We successfully applied the method for the hepcidin concentration determination in human serum. The method showed very good repeatability and low between series imprecision. As was expected, the median value of hepcidin concentration was highest in the CKD associated anemia, followed by the HI and IDA groups. We concluded that the method could be used as a research parameter in the diagnosis and monitoring of iron disorders.

P23

Semi-quantitative result reporting and quantification of urinary protein excretion using a urine test strip

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BACKGROUND: Urinalysis is important for establishing early diagnosis, evaluating the disease severity, and monitoring clinical courses of various kidney and urinary tract disorders. These evaluations are typically performed by assessing both the physical and chemical characteristics of urine by using a urine test strip and analysing the urine sediment through manual microscopy. Urine test strip results are generally reported in categories (i.e., ordinal scaled), but automated strip readers are now available that can report semi-quantitative data (1). It is recommended that the range of quantitative concentrations corresponding to the assigned categories is always made available (e.g., for protein 1+ (0.2 ± 1.0 g/L)) (2). Proteinuria is an important manifestation of renal disease, and changes in the urinary protein excretion are valuable in monitoring the clinical course and predicting the outcome. Several authors have independently shown that the random urine protein/creatinine ratio (UP/UC) correlates well with timed protein excretion (3). Urine test strip Meditape II 10U (Sysmex) enables us to analyse concentrations of both the urinary protein and creatinine semi-quantitatively. The aim of this study is to investigate the performance of semi-quantitative urine test strip and whether the values of UP/UC correlate well with those obtained by quantitative method.

METHODS: Urine samples employed in this study derived from those sent to the laboratory for routine clinical analysis. All samples were analysed within 2 hours of collection by using UX-2000 (Sysmex). The urinary protein and creatinine concentrations were measured both semi-quantitatively by urine test strips and quantitatively by conventional method (Roche). The imprecision of semi-quantitative urine test strip was estimated by performing 20 consecutive measurements as a percentage of reproducibility of set-points (4). An interrater reliability analysis using the Kappa statistic was performed to determine consistency among semi-quantitative and quantitative results for urinary protein and UP/UC.

RESULTS: The results of imprecision analyses for haemoglobin and leukocytes (low and high set-points) revealed acceptable agreement of 100% (other data not shown). Kappa statistics revealed substantial agreement between methods, this being 0.74 (0.69, 0.79) for proteins and 0.65 (0.56, 0.73) for UP/UC.

CONCLUSIONS: Based on the results of imprecision analyses and comparison analyses for proteins we implemented semi-quantitative result reporting into routine practice. The initial evaluation revealed a substantial agreement for semi-quantitative UP/UC.

References

1. Penders J et al. Clinical Chemistry 2002;48(12):2236-2241.
2. European urinalysis guidelines. Scand J Clin Lab Invest Suppl 2000;231:1-86.
3. Kaneko K et al. Pediatric Nephrology 2005;20(6):834-836.
4. Haeckel R. J Clin Chem Clin Biochem 1985;23(8):473-492.

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Comparison of glucose Point-of-care measurement and laboratory values in General Hospital Murska SobotaMojca Bohar Topolovec¹, Greta Štrakl¹, Valerija Cvetko Weiss¹¹Department of Laboratory Diagnostics, General Hospital Murska Sobota, Murska Sobota, Slovenia

BACKGROUND: Point-of-care testing (POCT) refers to laboratory testing that occurs near to the patient. Obtained results are quick, reliable and require low sample volume. Occasionally glucose POCT results compared with the laboratory results differ; especially on different POCT devices and because analysis were performed by non-laboratory medical staff. The aim of the study was to compare different POCT glucometers for the measurement of glucose concentration in human plasma with laboratory values and external control results, to unify glucose POCT devices, their monitoring and reduce costs.

METHODS: 230 testings of 12 plasma samples (between 2009 and 2015) and 61 testings of 6 Slovenian external quality controls SNEQAS (between 2014 and 2015) with different glucose values, were included in the study. Plasma samples were tested on 11 different glucometers (HemoCue GLU 201+, Accu-check Active, Accu-check Go, Accu-check Aviva, Ascensia Contour, One Touch Ultra, One Touch Vita, One Touch Aviva, Glucometer Elite, Glucolab and Contour XT, from different manufacturers: HemoCue, Roche Diagnostics, Bayer, LifeScan, Infopia) and laboratory analytical system, Roche Diagnostics–Cobas 501, SNEQAS on HemoCue and Contour-XT, in General hospital Murska Sobota. Point-of-care testing glucose values for each POCT device were compared to the laboratory values, following ISO standard 15179:2013.

RESULTS: Point-of-care plasma glucose concentration range was between 0,6 and 26,7 mmol/L and between 1,8 and 16,3mmol/L on the laboratory system. Results of the study showed that only Contour-XT (3,9% of results deviate from the laboratory values) and HemoCue (9% of results) have similar accuracy acceptance criteria to ISO 15197:2013 ($<5,55 \text{ mmol/L} \pm 0,83 \text{ mmol/L}$ and $\geq 5,55 \text{ mmol/L} \pm 15\%$). Results of six external controls at HemoCue and Contour-XT showed that 100% of the measurement results were within the accuracy limits.

CONCLUSIONS: Most of the POCT methods are suitable to determine glucose concentration and some provide reliable results, but we also recommend checking with laboratory testing results at the hypoglycemic and hyperglycemic levels and education of non-laboratory medical staff. The results of the study shown that glucose values differ on different POCT devices. The use of one tested-type of glucometer with accurate results is reliable tool for glucose monitoring, due to the comparability of the results, easier handling and knowledge transfer between users, to facilitate monitoring by a laboratory as well as lower costs.

P25

Alpha-1-Antitrypsin Deficiency – The Laboratory DiagnosticsMladen Krsnik¹¹Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia

BACKGROUND: Alpha-1-antitrypsin deficiency (AATD) is a relatively common genetic condition, often undiagnosed. AATD has been identified in all populations, but is one of the most common inherited disorders among white persons. Similar rates are found among white persons worldwide, with an estimated 117 million carriers and 3,4 million affected individuals. Alpha-1-antitrypsin (A1AT) is an acute phase reactant. This means that it will be elevated in acute and chronic inflammatory conditions, infections. These temporary or chronic A1AT increases may cause levels to appear normal in people with mild to moderate AATD. Serum protein electrophoresis (SPE) is a test that evaluates many different proteins in the blood. Sometimes the test will detect a deficiency in A1AT unexpectedly, when it is done for a different purpose. In those cases, follow-up testing for A1AT may confirm a deficiency of the protein, even if there are no signs or symptoms of disease. Our aim was to verify the lower level of reference values of the screening methods for SPE (capillary electrophoresis, Capillarys 2, Sebia; alpha-1 globulin: 2,3 – 4,0 g/L), and for A1AT in serum (nephelometry, BN II, Siemens: 0,9 - 2,0 g/L).

METHODS: Between 2005 and 2015 we analyzed 1969 SPE and A1AT in serum simultaneously. At the same time 484 phenotype of A1AT were tested. For statistics were used only following phenotypes: MM (negative), and MZ (positive).

RESULTS: ROC curves showed AUC for SPE 0,739 (95% CI 0,667 to 0,803), and for A1AT in serum 0,953 (95% CI 0,909 to 0,979); p-value: $<0,0001$. Sensitivity and specificity for SPE, and A1AT in serum was 75,0 (60,4 – 86,4) and 65,3 (56,3 – 73,6) at 2,3 g/L, and 83,3 (69,8 – 92,5) and 94,4 (88,9 – 97,7) at 0,9 g/L, respectively.

CONCLUSIONS: Results showed that both lower level of reference values are appropriate for screening of AATD.

P26

Audit of capillary serum protein electrophoresis postexamination processesŠpela Sajko¹, Pika Meško Brguljan¹¹Clinical Chemistry Department, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

BACKGROUND: Serum protein electrophoresis (SPE) is a screening, diagnostic and monitoring method for serum protein quantification, focused on search of monoclonal immunoglobulin (M-protein, Mlg), hypogammaglobulinemias, poly- or oligoclonal patterns and dysproteinemias. Pathological SPE, especially with M-proteins, possible hide various plasma cell diseases including premalignant monoclonal gammopathy of undetermined significance (MGUS) and malignant multiple myeloma (MM). Analysis requires an experienced laboratory interpreter. Suspicious SPE results should be further analysed (M-protein typing with their κ or λ light chains) using more sensitive serum and urine immunofixation electrophoresis (IFE). In the last decade, alongside SPE and IFE, serum monoclonal immunoglobulin free light chains (sFLC) concentration analysis and κ/λ ratio are acknowledged in the international guidelines for the diagnosis, prognosis and monitoring of plasma cell dyscrasias and also in monitoring chronic renal failure because of the clonal free light chain nephrotoxic damage in kidney's interstitial (myeloma kidney).

METHODS: A three years retrospective analysis (2013-2015) of all SPE requests (7566) in our clinic, analysed on Minicap Sebia were examined. The first focus was analysis of our report's comments which accompany pathological SPE results (1-M-band present, 2-M-band possible present, 3-Hypogammaglobulinemia, 4-Polyclonal hypergammaglobulinemia, 5-Asymetric distribution/oligoclonal pattern of Ig subclasses) together with suggested further examinations (IFE or IFE and sFLC). On the next step we followed in what extent our clinic's physicians requested these further examinations, which were analysed in the referral laboratory (specialized Protein Laboratory) and finally their IFE/sFLC reports were examined.

RESULTS: SPE results revealed that comment 4 was the most frequent (18%) due to clinic's patient population, followed by comment 3 (14%) and 5 (9%). Comments 1(M-band present) and 2 (possible present) represents 4.3% and 3.4% among all analysed SPE. The referral laboratory analysed 294 requests. 33.4% and 20.5% samples were analysed due to comments 1 and 2 and 22% due to comment 3. There were 46% requests for IFE+sFLC examination as suggested and 53% orders for IFE only. 116 of all IFE requests (40%) were M-protein positive and in urine 62 requests (21%) were free light chain positive and 10 (9%) M-protein positive. Among 141 sFLC assays 15% have abnormal κ/λ ratio.

CONCLUSIONS: IFE with sFLC analysis is more sensitive than SPE results, but all three together are most efficient in screening and diagnosing of various B-cell diseases. Interpretative comments on laboratory reports are of great importance in the quality of patient diagnostic procedures.

P27

Can a simple cerebrospinal fluid analysis replace oligoclonal bands in the diagnosis of multiple sclerosis?Andreja Emeršič¹, Vanesa Anadolli¹, Timea Tomšič², Mladen Krsnik³¹Medical Faculty, University of Ljubljana, Ljubljana, Slovenia²Department of Neurology, University Medical Centre Ljubljana, Ljubljana, Slovenia³Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia

BACKGROUND: Evidence of intrathecal immunoglobulin G (IgG) production is highly suggestive for multiple sclerosis (MS). The current gold standard is electrophoretic appearance of cerebrospinal fluid (CSF) restricted oligoclonal bands (OCB), despite the method is technically demanding, enables only qualitative IgG determination and allows subjective interpretation (1,2). Quantification of CSF kappa free light chains (KFLC) could represent a rapid, automated and easier to standardize alternative (2–4). We therefore aimed to compare the diagnostic accuracy of an ultrasensitive OCB and nephelometric kappa FLC assay.

METHODS: KFLC concentrations were measured by nephelometry in matched serum and CSF samples of patients with MS (n = 80, 54 females) and non-inflammatory neurological diseases (n = 48, 26 females). KFLC indices and intrathecal KFLC fractions were calculated by previously defined formulae (1). Assessment of OCBs was performed by agarose isoelectric focusing and subsequent immunoblotting. Instead of the standard peroxidase method a more sensitive IgG immunodetection by alkaline phosphatase-labelled antibody was used.

RESULTS: CSF KFLC concentrations were significantly elevated in MS patients (median 1.7 mg/l, range 0.9 – 4.6 mg/l vs. control group median 0.1 mg/l, range 0.1 – 0.2 mg/l; $p < 0.0001$) as were CSF KFLC /total protein ratio (0.46 % vs. 0.03 %, $p < 0.0001$) and both measures of intrathecal KFLC production. ROC analysis revealed that CSF KFLC concentration alone provides a high degree of sensitivity and specificity in MS (AUC = 0.978). A cut-off of 0.3 mg/l resulted in 95.0 % sensitivity and 91.7 % specificity compared with the 90.0 % sensitivity and 100 % specificity of the OCB assay. Importantly, 4 out of 7 OCB negative MS patients had elevated CSF KFLC concentration when using this cut-off. Yet, 1 out of 4 MS patients who would be misclassified according to the CSF KFLC concentration returned a positive OCB test.

CONCLUSIONS: A simple quantification of CSF KFLC provided a sufficient differentiation between MS and control group patients. Implementation of KFLC determination as a screening tool for MS seems reasonable, however larger validation studies are needed to finally place it as a preferred or complementary diagnostic test for MS.

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