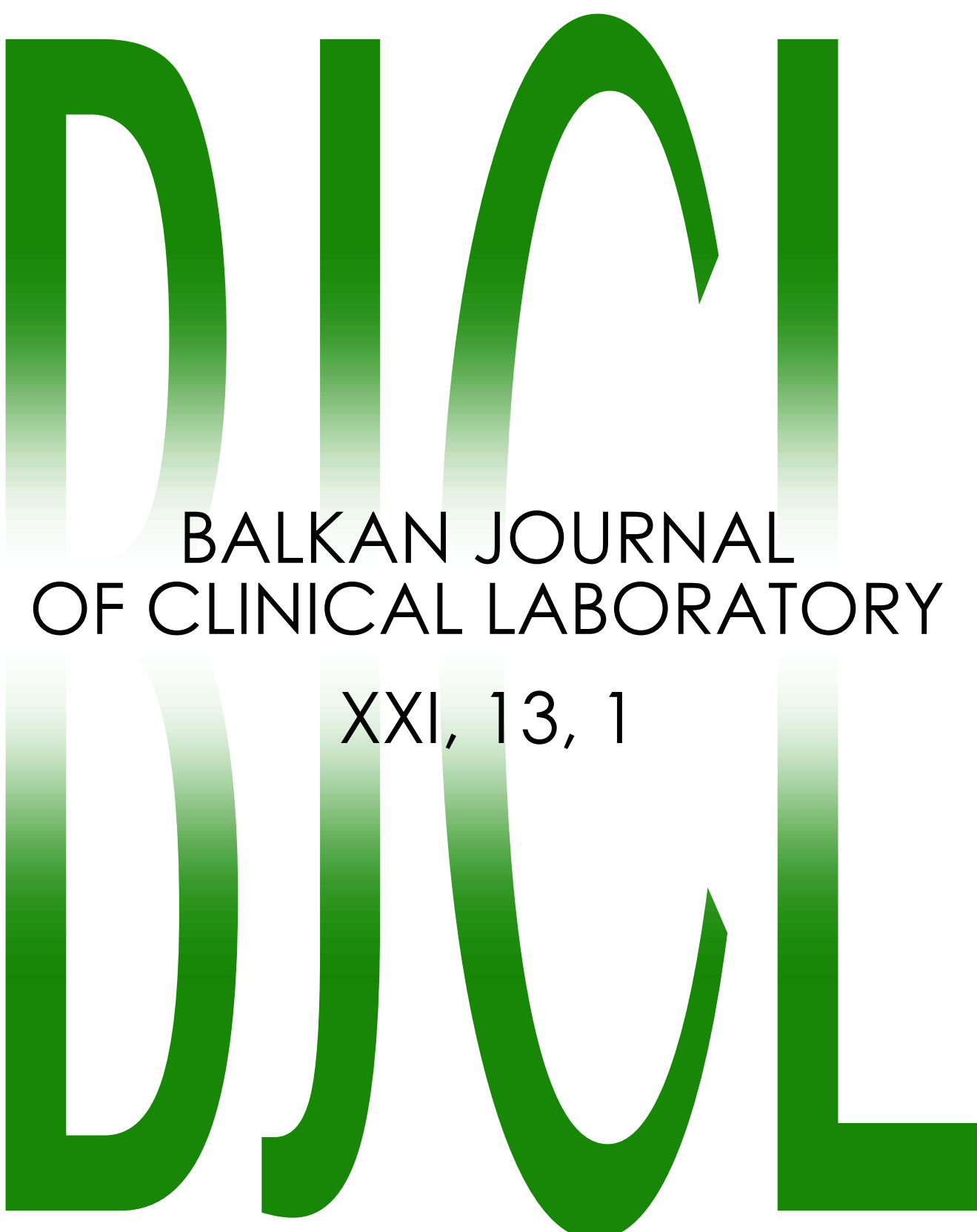




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*Opening Lecture***DIGITAL DARWIN - THE NEW RULES OF MANAGEMENT IN THE SOCIAL MEDIA ERA**

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Traditional media such as television, radio, newspapers and magazines are one-way push platforms, characterized by frequent unsolicited interruptions. In recent years, the media landscape has dramatically changed, with traditional media now supplemented by social media. New web technologies have made it easier for ordinary people to create and share their own multimedia content with a global audience. In order to earn and sustain customer trust, organizations must engage emotionally in authentic online conversations with their customers, and deliver experiences that is personalized and relevant. Social media is transforming the way we connect, learn and collaborate. At the same time, social media is disrupting corporate bureaucratic hierarchy, hinting a shift from the old command and control model to the new network model embracing openness, collaboration and relationship building.

The corporate world has clung too long to a model of management which was designed for the manufacturing revolution which depended on standardization, efficiency and control. Leaders today are faced with a world where unprecedented change is the norm, where once-innovative companies are struggling to stay alive, and century-old business models are being rendered irrelevant overnight. Industry after industry, the rules of doing business are rewritten - airlines, newspapers, magazines, TV stations, the recording industry, hardware companies, real estate brokers, travel agents, bookstores, insurance agents, art galleries.

Of the 25 biggest companies in the world in 1999, only eight of them made the same list in 2009. Have we reached a point in time when technology and society are evolving faster than the ability of many organizations to adapt? What is the future of business?

*Plenary lectures***ACHIVING RESULTS EQUIVALENCE IN THE HETEROGENEOUS MEASUREMENT: THE WAY FORWARD?**

Panteghini M.

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Measurement of heterogeneous analytes, such as glycoprotein hormones, cardiac and tumor markers, comprise an important part of the test menu of clinical laboratories. The clinical interpretation of results from different assays, however, is hampered because results often cannot directly be compared with each other. Several standardization activities are undertaken to improve this situation. Although challenging, standardization of heterogeneous biomarkers is not impossible indeed: additional tools have recently become available allowing for better understanding of the measurement processes and consequently for improvements in measurement result comparability. Establishing an appropriate and efficient traceability chain requires alertness with regard to critical parameters influencing the measurement results. For heterogeneous analytes in particular, the traceability model emphasizes the importance of the definition of the “measurand”. For complex substances the definition may not be so clear because of their potential intrinsic or acquired heterogeneity. Differences in selectivity of methods intended to measure the same quantity are common, as different methods employ different reagents for the same target analyte. Antibodies can be monoclonal or polyclonal, derived from different species (goat, mouse, chicken, etc.), and can be raised against different targets (e.g. recombinant protein, a mixture of isoforms, a single peptide). Proteins are typically quantified by measuring the amount of substance of a certain part of all isoforms or of particular isoforms (epitopes for immunoassays, peptides for mass spectrometry methods). So that, multimeric heterogeneity may affect different measurements procedures to a different extent (e.g. mass spectrometry: all polypeptides chains measured, also ‘non-native’ ones). One proposed way to circumvent the issue of analyte heterogeneity is to define the measurand as a unique, invariant part of the molecule that is common to all

components of the mixture present in blood. Methods used for the development of commercial assays should, without distinction, recognize this common part with a consequent increase in the homogeneity of assay reactivity. Practically, we should define a structural “common denominator” that eliminates the structural heterogeneity present in the intact analyte. Such “common denominators” could be specific amino acids of the protein analyte or peptides derived from it. Other possible pragmatic solutions can also be followed: in some cases, measurands can be defined by the measurement procedure itself. For instance, free T4 has been operationally defined as “thyroxine in the dialysate from equilibrium dialysis of serum prepared under defined conditions”. This has permitted the development of a reference measurement system for this hormone. For reference materials to be used in harmonization of heterogeneous analytes, it has been demonstrated that also small changes in the processing may have a pronounced impact on the measurement results and would consequently lead to a biased calibration. Hence a careful evaluation of the impact of different reference material formats and processing steps on the properties of the reference material and its suitability for calibration (such as commutability) is a crucial requirement. Only commutable materials can indeed be used for direct value assignment to manufacturers’ calibrators and trueness check of laboratory tests, having large importance to ensure an unbroken traceability chain. Moving from non-comparability to comparability of heterogeneous analyte measurements should be considered as an evolutionary process, whose progress parallels that of scientific knowledge on the analytical and pathophysiological aspects. Improvements due both to the advancements of knowledge and to consensus on reasonable compromises are possible with reasonable efforts and within acceptable timelines.

THE EUROPEAN DIRECTIVE ON PROFESSIONAL QUALIFICATIONS. COMMON TRAINING FRAMEWORKS

Zerah S.

Chair of EC4 foundation board, Chair of the Professional committee of L'EFLM/EC4, Vice president of CEPLIS (European Committee of Liberal Professions).

Background: The first Directive (2005/36/EC) on Recognition of Professional Qualifications had to be transposed by all Member States by 2007. After the revision period by the European Commission, the amended text proposed by the European Parliament, was finally adopted after trilogue negotiations with the council, in June 2013. The final “consolidated text” should be published soon, in the European Official Journal.

Methods: We will give a quick overview of the history to enable understanding where we are, after active participation and strong involvement in the numerous meetings and questionnaires, propositions of amendments to the final draft proposed by the European Commission to the European Parliament in December 2011. The Deadline for amendments was 15 October 2012.

We will present the main points of the revised directive: Professional cards, CPD, Partial access, rules on language skills, insisting on the “Common training principles”, replacing the common-platforms, back to harmonization instead of identifying differ-

ences for compensation measures. We will detail the level of education requirements for the safety of patients and the propositions that we have prepared.

We will present the two methods for recognizing qualifications, the automatic system for 7 “sectorial professions” and the “general system”.

Results and Conclusion: The European professional associations are taking the lead in devising “common training frameworks” for harmonization: our aim is the excellence of our profession in Europe. As soon as we managed to have 9 countries where professionals and Member States agree on the program of harmonization according to the “common training frameworks”, we will be able to make a proposition to the European Commission. When accepted, the next step will be the transposition and application of the Directive in all EU countries. We will then have the European recognition of our profession, “Specialist in Laboratory Medicine”, and the same actual system of free movement applicable to the 7 “sectorial” professions.

DEGENERATIVE DISEASES OF BRAIN AS PROTEIN DISEASES ("PROTEINOPATHIES")

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Many neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease (PD), Huntington's disease, and frontotemporal dementia, are proteinopathies that are associated with the aggregation and accumulation of misfolded proteins. While remarkable progress has been made in understanding the triggers of these conditions, several challenges have hampered the translation of preclinical therapies targeting pathways downstream of the initiating proteinopathies.

In healthy brain, these proteins are unstructured as a monomer, serving most likely as the physiological form. In a disease condition, the unstructured proteins experience a conformational change leading to small oligomers that eventually will aggregate into higher order structures. As proteinopathies are caused by the aggregation of disease-typical proteins with an ordered structure, active and passive immunization protocols have been used to expose model systems to therapeutic antibodies that bind to the aggregates thereby inhibiting the prolongation into higher ordered fibrils or dissolving the

existing fibrillar structure. A key step in the development of proteinopathies is structural change that triggers aggregation of proteins, which are intrinsically prone to form aggregates due to their physical and chemical properties. The lecture will focus PD, characterized by bradykinesia, rigidity, tremor at rest and postural instability, due to a degeneration of the nigrostriatal dopaminergic system, with neuronal loss and reactive gliosis in the substantia nigra found at autopsy. α -Synuclein (α -syn) is the most abundant protein found in Lewy bodies, a hallmark of Parkinson's disease (PD), and can aggregate to form toxic oligomers and fibrillar structures. Recent studies have shown that α -syn can be transmitted between neurons and can seed the formation of toxic aggregates in recipient neurons in a prion-like manner. In addition, it is known that Lewy body pathology may spread gradually and systematically from the peripheral or enteric nervous system or olfactory bulb to specific brain regions during progression of idiopathic PD. It is therefore conceivable that α -syn species could act as seeds that drive PD progression.

INTEGRATION OF CLINICAL AND LABORATORY GENETICS INTO SUCCESSFUL PROCREATION

In extenso

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Introduction

Procreation is an act of generating which result with a birth of offspring. Successful procreation should be defined as a process, starting before conception and closing with bringing a healthy offspring into the world. Vast majority of procreative processes result in healthy newborn, but there is also a momentous number of couples with problems in procreation, from infertility, through various adverse pregnancy outcomes to delivering a child with severe disease. In such cases more than couple, decided to have a child, should be involved in “creation” of successful procreation. The definition of successful procreation could be differently interpreted from reproductive, genetic, obstetrics, pedi-

atric, population, social or individual point of view. For parents — it is a “perfect” child with desirable gender. For ART specialist and obstetrician — it is a successful conception and pregnancy, uncomplicated delivery with a newborn in good. For clinical geneticist and pediatrician — it is a newborn without perinatal accident and no untreatable disorder which severely influences a life of child and his/her family. On the other hand, from population and social prospective “successful procreation” means a newborn-population with small rate of fetal/neonatal deaths, congenital anomalies and hereditary diseases, and with perfect gender balance.

Genomic burden influencing successful procreation

There is a broad spectrum of factors that are held responsible for unsuccessful procreation, manifesting as infertility, recurrent pregnancy loss, presence of fetal congenital anomalies, intrauterine growth restriction, unexplained fetal death or delivery of a child with severe disorder. The etiology of all those adverse outcomes still remains unclear in at least half of such events, although a strong common genomic impact is increasingly recognized. It is expected that 3-5% of couples from general population will have fertility or pregnancy problems related to genetic factors, and/or will have a child affected with a genetic disorder. Recent advances in medicine and medical genetics contributed to better understanding of the role that genome plays in health and disease. Despite the mission of the Human Genome Project, whose “true payoff will be the ability to better diagnose, treat, and prevent

disease” (Frensis Collins, the director of HGP), vast majority of genetic disorders are not yet treatable, and the McKusick's negative eugenic approach remains the best available management of genetic disorders.

Genetic risks should be evaluated from at least three perspectives:

1. Preconception – couples with infertility and recurrent pregnancy loss,
2. Prenatal – pregnancies with risk for chromosomal abnormalities, congenital anomalies, intrauterine growth restriction and unexplained sudden fetal death.
3. Postnatal – birth of a child with severe genetic disease

The most common group of genetic disorders is chromosomal aberrations, and they are the main cause of severe developmental abnormalities during pregnancy and infancy. This group of genetic disorders is present in 0.5% of newborns, and majority of them can be diagnosed by cytogenetic analyses. Couples and pregnancies in high risk for fetal chromosomal abnormalities are well recognized, due to development of new technologies in the fields of medical genetics, biochemistry and sonoembriology. Advanced maternal age, familial balanced chromosomal rearrangement carrier state and increased risk detected by screening tests for risk of fetal chromosomal abnormalities are the most common indications for prenatal diagnostics of these conditions.

There are more than 11,000 single gene diseases, with genetic diagnostic merely available for less than half of them, and even in those cases, there are numerous difficulties on the road to final and reliable genetic diagnosis. Dominant single gene diseases are evident in one of parents, or they appear as “de novo” genetic conditions, with no increased risk for further offspring. On the other hand, recessive single gene diseases can be present in carrier state and could be passed down quietly through many generations. Every human individual carries 14–18 recessive single gene mutation, which can cause a severe genetic disorder in homozygous state. There are more than 1000 recessive single gene disorders, which are detectable to genetic testing.

Diagnostics protocols for genetic diseases detection

Prenatal diagnosis of chromosomal abnormalities is performed using invasive procedures for more than 40 years, but with associated risk of fetal loss (~1%). In the past decades many efforts have been made towards the development of rapid and non-invasive prenatal diagnostic (NIPD) tests, and recently in combination with some single gene testing or whole genome analyses. New genetic technologies have quickly brought an increasing amount of information about the fetus into pregnancy care providing. Few years ago, karyotyping was the leading method for evaluation of chromosomal abnormalities risks, used predominately for the identification of relatively large genomic changes of 7–10 million base pairs or larger.

Given the fact that recessive gene's carriers predominantly do not have family history or symptoms of the disease, the only way to identify increased risk for recessive single gene diseases is carrier screening, but only for the disorders which appear with high frequency in certain population. Additionally, mutation detection rate varies, from ≤50% to ≤10%, and depends on number of mutation within gene candidate, and this fact is the most challenging issue in the field of genetic testing, especially in the process of preparing the design for genetic testing and genetic interpretation, and pretesting and post-testing counseling, about nature of disease, recurrent risk and possibilities for prevention. The selection for screening of carriers for recessive genes should be elected from a specific geographical and population area based on following criteria: carrier rate, detection rate, ethnic groups, body systems affected, physical damage, cognitive disturbance, quality of life, reduction in life expectancy and treatment availability.

There is also a wide spectrum of complex, polygenic diseases, which are predominantly responsible for isolated, non-syndrome congenital anomalies and with very limited possibilities for specific genetic analyses. The etiology of such epilog of dysmorphogenesis appears to be multifactorial, with strong genomic impact, but around 60% of those cases remain unexplained, despite constant research focus on the topic.

Several methods (FISH, QF-PCR, aCHG) have been introduced recently, aiming to perform rapid prenatal diagnosis and provide information, necessary for the further genetic counseling and pregnancy management for fetus with chromosomal abnormalities. Quantitative fluorescent PCR (QF-PCR), allows diagnosis within 1–2 days, but this diagnostics is limited to the most frequent autosomal (13, 18, 21) and gonosomal (X and Y) aneuploidies, accompanied by the risk for fetus/placenta discrepancy in 1–2% of cases, with majority of chromosomal abnormalities being of placental origin, while fetal abnormal karyotype is a rare occurrence. More recently, microarray technology has been introduced into prenatal evaluation. Routine aCGH analysis provides detection of chromosome abnormalities otherwise imperceptible by cytogenetic analysis and also iden-

Lecture Presentations

tifies abnormalities like chromosomal microdeletions and duplications. Array-CGH or Chromosomal Microarray Analysis (CMA) is increasingly used in prenatal diagnosis, including genetic testing prior implantation, following IVF procedures, using the single cell for genetic analyses (polar body, blastomere). The main applications of array are preimplantation genetic screening, preimplantation genetic diagnostics of fetal aneuploidies and complex chromosomal rearrangements and translocations, but also for single gene fetal disorders. Cost-effectiveness of these procedures is not sufficiently assessed and some negative consequences should be taken into consideration, particularly in the field of ethics, such as: ethical issue of detecting Mendelian and quantitative traits, the criteria to be used for embryo selection, the control mechanisms to be used for embryo selection?

Cell free fetal DNA (cffDNA) in maternal blood is recently used for non-invasive prenatal diagnosis (NIPD). The major clinical use of cffDNA genotyping has been for the NIPD of fetal RHD status and fetal sex in the pregnancies at risk of haemophilia or other X-linked disorders. This valuable source of fetal material is recently used as a screening for fetal chromosomal and/or some single gene disorders.

Most promising NIPD tests are: massively parallel sequencing (MPS) highly multiplexed targeted single-nucleotide polymorphism (SNP) amplification and sequencing and methylated DNA Immunoprecipitation (MeDIP). These techniques are predominantly focused on common fetal aneuploidies (13, 18, 21, X and Y). In the case of positive results, attained by NIPD, invasive prenatal diagnostics of entire karyotype or one of rapid PD is needed. Some of these techniques also detect parental origin of fetal chromosomes and uniparental disomy, and will allow detection of triploidy, microdeletions/microduplications, and haplotype reconstruction. NIPT should be considered as a first line test for aneuploidy screening in general pregnant population and could help lessen the unnecessary invasive PD and maternal anxiety. Despite all technological efforts, NIPD screening for fetal aneuploidies, using cffDNA has proven to be difficult, and it is commercially limited for 21, 18 and 13 chromosome aneuploidies. Before introduction of NIPT tests into routine diagnostic, more evidence is needed from broad perspective of issues, such as diagnostic accuracy, cost-effectiveness, timely, and equitable manner, also with additional physician and patient education.

Quality of genetic services – integration of knowledge, skills and ethic

Clinical guidelines are increasingly recommending the incorporation of new discoveries in genomic medicine into routine care, especially the transfer of genetic knowledge from research laboratories into clinical practice. Data derived from gene testing, genetic consultations, and genetic counseling in particular, represents a set of information apart from the rest of the patient's clinical record, and this data is regarded as "especially protected personal information" (i.e. genetic pedigree).

The importance of building a strong and coherent background for genetic counseling professionals was recognized in last decades, due to shortage of health professionals in the field of genetics, particularly in light of the rapid development of molecular genetics. Minimum education standards of and competence are needed to ensure patient safety. Europe-wide recognition is of tremendous importance to maintain the high-standards of clinical practice in human and medical genetics areas, as well as cross-border mobility and collaboration. The assessment of existing quality frameworks for genetic counseling in Europe, provided by ESHG Ge-

netic Services Quality Committee, has shown great variation ranged from no service (the situation in most countries) to well-organized genetic counseling on a national level (small number of countries). Furthermore, the majority of general health professionals have inadequate knowledge and skills in medical genetics and many are unaware of the technical, ethical, legal and psychosocial implications of genetic testing and importance of genetic counseling. The public is largely unaware about genetic risks and the possibilities of their prevention.

Despite a long history of prenatal testing, there are still no overall guidelines to ensure minimum standards of care for women and the upcoming children. With increasing use NIPD there is a concern that prenatal testing will become too "routines", and additionally misused for non-medical purposes. Multidisciplinary approach: professionals from clinical and medical genetics, fetal medicine and laboratory genetics should have an integrated approach to assure the best interest for family, future child and society.

The Increasing ability to evaluate fetal genome comes with significant responsibility for clinicians. Pretest and post-test counseling is mandatory and should be informative, non-directive and should prepare patients for the reproductive decisions they must make. The EuroGentest Expert Group provided guidelines for prenatal diagnosis which should be applicable to both invasive and non-invasive testing (EuroGentest website). The objective of prenatal diagnostic counseling is to enable families to make informed choices consistent with their needs and support them in dealing with the outcome. General principles include the need for appropriately trained genetic counselor, ensuring informed decision making and the availability of pre-test and post-test counseling. This type of service requires effective communication between clinical geneticist and family, sensitive counseling with interpreters if needed, and consideration of wider family implications. Counseling topics should include psychosocial and ethical issues as well as information about the condition, the available diagnostics and available options.

In accordance with GenDg protocols genetic diagnostics has to be performed in accordance with following recommendations:

1. Responsible doctor has to be qualified for human genetic counseling
2. Result has to be reported to responsible doctor, not directly to the patient
3. The only NIPT for fetal trisomies 21, 13, and 18 are available in Europe, which is in accordance with the IVD directive of the EU.

According to Council of Europe recommendation, professional genetic counseling prior to genetic testing is mandatory. CoE (Addit. Protocol & Rec (2010) 11) recommends “development and strengthening of genetic services to maximize the benefits of genetic application in health care for all patients... and provide adequate genetic counseling in an equitable manner.

Particular sensitivity should applied on the subject of the principles of procreative liberty and misuse of genetic prenatal diagnostics for non-medical purposes, such as prefertilization, preimplantation or prenatal gender selection. In assisted reproduction and prenatal diagnostics, professionals are actively involved in the “creation” of the embryo, and are considered to be co-responsible for the outcome of the procedure and the welfare of the potential children, future generations and society.

Conclusion

This lecture is designed to point out the necessity of integration of all medical/clinical genetic knowledge and skills, with a mission to provide optimal quality of clinical genetic service, in accordance with the recommendations of Council of Europe, GenDg protocols, EuroGentest and IVD directive of the EU, and to give some directions in which the countries

should develop guidelines for genetic service and quality and ethical control at the national levels. Open discussion could be provided for the ethical issues, criteria and cost effectiveness of genetic testing, and importance of providing appropriate and well controlled genetic service in the region.

FUTURE DIRECTIONS IN FIRST TRIMESTER ANEUPLOIDY SCREENING

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Down Syndrome screening over the past 20 years has developed from the measurement of second trimester maternal serum biochemical markers such as AFP, hCG, UE3 and Inhbin A, through to the early first trimester Combined test utilising the ultrasound measurement of Nuchal Translucency and maternal serum free beta-hCG and PAPP-A. Such changes have allowed detection rates and false positive rates to improve from the 30%/5% achievable using maternal age, through the 70%/5% achievable using the second trimester Quad test, to the 85–90%/5% achieved by the Combined test.

The focus of national screening programs in recent years has been to move to the Combined test, whilst at the same time aspiring to reduce the false positive rate and Invasive Testing rate to 2–3%. This lecture will outline these developments and suggest ways in which the Combined test can reach these new targets. New models of pregnancy care based on early first trimester screening for multiple conditions will also impact on future screening services and these will be briefly discussed. I will conclude with a view of how Fetal DNA technology can be incorporated into existing first trimester screening.

ULTRASOUND AND BIOCHEMISTRY SCREENING POSSIBILITIES AND LIMITATIONS

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Introduction: Every pregnant woman has risk that her fetus has some chromosomal defect (HD), especially after the age of 35.

Methods: In this article we want to show what are the chances of detecting the three most common HD in fetuses, trisomy 13, 18 and 21.

Discussion: The only 100% safe method of determining the karyotype of the fetus is invasive diagnostic testing. On the other hand screening methods are simple to perform and can be implemented in all pregnancies.

Currently being used Double test, Triple test and Quadriple test. Double or better say the Combined test is carried out between 11 and 13+6 in a way that the initial risk (maternity age and CRL) multip-

lied with likelihood ratios. Likelihood ratios are: free-BHCG and PAPP-A, NT and FHR. Accuracy is 90-95% with a false positive rate (FPR) of 3-5%. Triple test takes into account a priori risk, free-BHCG, AFP and uE3. In Quadriple test is used additionally Inhibin A. Their accuracy is up to 75%, with a FPR of 5%. Accuracy of Combined test increases with the so-called new ultrasound markers: the flow through the ductus venosus and tricuspid valve, nasal bone and facial angle. Accuracy is up to 96% FPR is 2.5%. Results can be improved by using a new algorithm that includes PIGF.

Conclusion: Screening for chromosomal defects in fetuses had its development route last 40 years. All this in order to raise the high level of the prenatal care.

SCREENING FOR PRE-ECLAMPSIA IN PREGNANCY: WHERE DO WE STAND TODAY?

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Preeclampsia is a serious pregnancy-related complication, characterized by elevated maternal blood pressure and proteinuria occurring after 20 weeks of gestation. Pre-eclampsia complicates approximately 2-5% of all pregnancies in developed countries, and remains a leading cause of maternal and perinatal morbidity and mortality.

Maternal complications include severe pre-eclampsia and eclampsia (eclamptic seizures, persistent maternal headache or visual disturbance, pulmonary edema, acute renal failure etc) and HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets). Fetal complications include abruptio placentae, fetal growth restriction, premature delivery and intrauterine fetal death.

While the underlying etiology of pre-eclampsia remains unclear, much has been learned over the past years regarding the pathological processes involved in the development of pre-eclampsia. Pre-eclampsia is a disease resulting from abnormal development of the placenta. It is generally accepted that is characterized by a cascade of events such as: impaired early trophoblast invasion, decreased placental perfusion, placental ischemia, oxidative stress, and consequentially impaired placental factors (dysbalance in angiogenic and anti-angiogenic factors) which are playing a key role in inducing systemic maternal endothelial dysfunction. The various clinical symptoms of pre-eclampsia appear to be the direct result of endothelial dysfunction.

The only effective therapy of pre-eclampsia is the removal of the placenta. After removal of the placenta, the pre-eclamptic symptoms vanish within days. Thus, early diagnosis and timely delivery are crucial for minimizing perinatal morbidity. Implementation of preventive measures such as the

prophylactic use of aspirin early in pregnancy may reduce the prevalence of preeclampsia in women considered at high risk and diminish mortality and morbidity in both the affected mother and her child.

Until recently, only maternal history and risk factors were available to identify women at higher risk with equivocal predictive values (low detection rate, high false positive rate). Nowadays, Doppler ultrasonography measurements (e.g. uterine artery pulsatility index) in the 2nd trimester are considered the "gold standard" in predicting pre-eclampsia while many studies propose that 1st trimester Doppler ultrasound could also be used. Several studies have also proposed the combination of Doppler ultrasound with serum biochemical markers and have led to more complex risk-prediction models based on multivariable algorithms. Almost all of these models evaluate biomarkers such as placental growth factor (PlGF), soluble Fms-like tyrosine kinase-1 (sFlt-1), vascular endothelial growth factor (VEGF), Asymmetric dimethylarginine (ADMA), pregnancy associated plasma protein-A (PAPP-A), Fb-hCG combined or not to biophysical markers such as uterine artery pulsatility index (PI). Some of these models present impressive detection rates of 90% for early-onset (<34 weeks) preeclampsia, at a false positive rate of 10%. Recently, human neutrophil gelatinase-associated lipocalin (NGAL) has been also proposed as a candidate biomarker for the prediction of preeclampsia.

Future directions of research in the field might be a) the selection of the most effective predictive model and b) the evaluation of this model (and possibly others) in many different populations and health-care environments in order to demonstrate its clinical utility.

ESEAP – THE GREEK PROFICIENCY TESTING SCHEME FOR CLINICAL LABORATORIES AND ITS CONTRIBUTION TO THE IMPROVEMENT OF LABORATORY TEST QUALITY

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ESEAP, Athens, Greece

External quality assessment schemes in Laboratory Medicine have been operating in most of the developed countries for several decades and constitute one of the basic tools for quality assurance of laboratory results. These schemes contribute to the evaluation of the overall quality level of the participants, as well as of the quality level of each individual laboratory, allowing the comparison of inter-laboratory performance and analytical methods. Moreover, they are educative as they provide valuable information to the participants in order to facilitate their self-assessment.

ESEAP is the only external quality assessment scheme in the field of Clinical Chemistry in Greece. It was established in 1994 at the Biochemical Department of “Evangelismos Hospital” in Athens after decision of the Greek Ministry of Health. In 2006, it became a “non for profit organization” and since then has been operating under the auspices of the Greek Society of Clinical Chemistry. In 2008 acquired the ISO 9001 certification and in 2012 it has been accredited according to the ISO/IEC 17043:2010 standard.

From 2007, a totally new, web enabled, software was implemented which allows participants to send their own results and obtain reports and information about their performance through the Internet. New software features permit further grouping of methods and analyzers and this results in the enhancement of the statistical evaluation of the results. From 2012 a “mobile version” for this application is offered for both the iOS (Apple- iPhone and iPad) and the Android platforms.

Actually, eight different schemes are operating in the fields of “classical” clinical chemistry, HbA_{1c} (in collaboration with the European Reference Laboratory for Glycohemoglobin), markers of cardiac damage and CRP, thyroid hormones, reproduction hormones and special proteins, therapeutic drugs monitoring (TDM), tumor markers and coagulation factors. For the next year is planned the introduc-

tion of an immunology scheme (antibodies for autoimmune diseases).

ESEAP operates through a monthly or bimonthly “distribution” to the participating laboratories of two lyophilized controls A and B of different concentrations sent at the beginning of the year, by courier, in isothermal packages along with freeze dice packs (gel packs). After analysis of these controls, participants submit data via the web application 6 or 12 times per year (depending on the scheme chosen) and receive reports and information on their performance through the Internet. Statistical evaluation is related to the method or the analyzer used and includes three different charts: distribution histograms, Levey-Jennings charts and Youden plots. Furthermore, at the end of each two-year cycle, or after 12 distributions for the monthly schemes, participants are assessed through an original scoring and ranking system, based on the normalized magnitude of deviation from the target value, allowing them to follow their individual performance across time and their position among all other laboratories participating in the cycle.

ESEAP has considerably contributed to the improvement of performance for the majority of the laboratories, as the mean CVs for all analytes showed a significant decrease from cycle 1 (1994–1996) to cycle 3 (1998–2000) of the program. The CV per cycle for each analyte was calculated as average between control A and control B mean CVs over the 12 distributions of the cycle (inter-laboratory CV). In this calculation we included only the 100 laboratories that participated continuously in all three cycles.

Originally about 100, the current number of participants has reached 350, including all public hospital laboratories, a growing number of diagnostic centers and private laboratories all over Greece, and 45 laboratories of the public and private sector in Cyprus.

The wide impact and acceptance of our schemes is due to the fact that, they operate in Greek lan-

guage, cover the most frequently ordered tests in Laboratory Medicine and, although there are very friendly and easy to use, they provide laboratories with an objective assessment of their own performance as well as in relation to that of other labora-

tories. Furthermore they provide information on the relative performance of the available methods and analyzers, identify factors associated with good and poor performance via the Youden plot and improve the inter-laboratory agreement.

PERFORMANCE OF TOTAL TESTING PROCESS AND PROCESS SIGMA CALCULATIONS IN A CLINICAL LABORATORY

In Extenso

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Errors in healthcare are of concern when they lead to adverse outcome for patients. Published data suggest that 24-30% of laboratory errors have an effect on patient care where molecular genetic testing can have harm rates of up to 100%. The results of the studies show that 62%, 15%, and 23% of laboratory errors come from pre-examination (pre-analytical), examination (analytical) and post-examination (post-analytical) processes of the total testing process (TTP), respectively (1). In this context, clinical laboratories should develop mechanisms to detect errors and improve quality in pre- and post-analytical phase as well as analytical phase. Accreditation bodies also require clear documentation of quality practices including total testing performance documentation.

There are different kinds of activities in all three phases, and they have different error-prone characteristics. The commonest causes of critical errors in TTP are being determined by several studies, and projects (2-4). Every laboratory can establish a system for collecting causes of errors in all processes, and a data management system for calculating performance measures (quality indicators-QIs) by using the information technology.

Process sigma is valuable measure for describing error rates in a process, hence performance of a process. Every laboratory should establish quality indicators to monitor and evaluate performance of the TTP (5). The QIs can be expressed on the Six Sigma scale. This talk will focus on the calculation of Sigma-metrics, and the documentation of the performance of the total testing process in the clinical laboratory in preparation for accreditation.

Six Sigma methodology

Six Sigma is a data-driven approach and methodology for eliminating defects in any process, and describes how is a process is performing.

In the industrial perspective Six Sigma implementation involves the following steps: 1) Recognize the problems. 2) **Define** the processes that contribute the problem. 3) **Measure** the capability of critical processes. 4) **Analyze** the data. 5) **Improve** the key characteristics. 6) **Control** the key process variables. 8) **Standardize** the methods for best performance. 7) **Integrate** the standard methods and processes with the product/service design steps (6,7).

Six Sigma metrics are indicators of process quality. The most common metrics are "Sigma level", "Defects Per Million Opportunities/Occurrences-DPMO" and "Yield" (6). "Sigma level" is an indicator of process centering, and process variation based on specified limits. "DPMO" is the number of critical defects that the process is estimated to generate per million opportunities (operations or steps or occurrences). "Yield" is the estimated percentage of defect-free items (probability of zero defects) produced by the process.

Lecture Presentations

Defects are the unacceptable levels of any measure of a process. Unacceptable levels are the values which are outside the specified levels as “target”, “goal” or “specification limit”.

The conditions under which a defect might occur are identified as “opportunities”. The probability that a defect will occur in a sample, also referred to as the “defective rate”, corresponds to a value called “Process Sigma” or “Sigma level”

A higher process sigma means a less defective process. The Six Sigma describes quantitatively by measuring of defects occurring per million outcomes of a process. To achieve Six Sigma, a process must not produce more than 3.4 defects per million opportunities (DPM). In The Six Sigma approach, the goal is Six Sigma, and Three Sigma is considered the minimum acceptable performance (7-9).

The process Sigma level also gives the opportunity of the cost savings (10-11). The cost of poor quality (COPQ) is directly connected to the number of defects per million occurrences, and proportional with the Process Sigma level. The COPQ is 25-40% and 15% when the Sigma level is 3 and 4, respectively; it will be smaller than 5% when the Sigma level is 6. This means the number of defects will be reduced from 60 000 to 3.4 per million opportunities.

The Six Sigma approach gives an overall view of a process. The elements of a process which might cause the defects can be examined according to its Sigma level by means of several quality tools such as the root-cause analysis, patient test results, etc.

“Sigma level” calculation

The Sigma metrics are calculated separately depending upon the nature of the data collected. If data is discrete as in the pre- and post-analytic processes, the outcome of the process is investigated and defects are counted, and that count is converted to Sigma level. If data is continuous such as in the analytic process, the variations and bias are measured and Sigma level is calculated.

For discrete data: 1) “Defects per opportunities (DPO)” is calculated with the Formula, $DPO = d/(n \times o)$ (d = total number of defects per receiving lot, n =number of components in the receiving lot, o =number of defect opportunities per component); 2) DPMO is estimated ($DPO \times 1\,000\,000$). 3) It is converted to Sigma level from the “Discrete Process Sigma Conversion Table”. Then, the probability that the opportunity/occurrence is not defective is found ($1-DPO$), and the likelihood that any specimen will contain zero defect is calculated as $(1-DPO)\%$ (12).

The Process Sigma level is also calculated directly with the Formula “NORMSNIV ($1- DPO$) + 1,5” in the MS Excel (12).

For continuous data: Sigma level = $(TEa\% - Bias\%) / CV\%$ (11). CV% and Bias% are obtained from the IQC and/or EQA programs, and the total allowable error (TEa) may be selected either biological variations or CLIA 88 analytical quality requirements (13); or can be converted from process capability index (C_{pk}) that can be calculated by means of the statistical package such a Minitab. (14). $C_{pk} < 1$ indicates that the process is off-centered, i.e, the overall process average is not at the target (6).

Pre-analytic process Sigma level calculation

Quality indicators for pre-analytic process have been defined (2,3,4,15). We determined fifteen quality indicators (*sample labeled improperly-barcode, wrong; no reading; order, duplicate; order, incorrect; sample, too much; sample, not received; sample, damaged; sample, hemolyzed index (total); sample, lipemic; sample, clotted; sample, inappropriate time; sample, wrong; sample, insufficient; cold chain, inappropriate; container, inappropriate*) for pre-analytic process.

We estimated the Sigma levels for all QIs and also the overall pre-analytic process sigma level by means of Microsoft Excel 10 Spreadsheet.

Example:**Overall pre-analytic process Sigma Level (January 2012):**

$n = 67\,758$, $d = 3\,761$; $DPO = 3\,761 / (67\,758 \times 15)$, $DPO = 0.0035$;

$DPMO = 0.0035 \times 1\,000\,000$, $DPMO = 35\,000$;

Sigma level = **4.2** (from the “Discrete Process Sigma Conversion Table”)

Probability that the opportunity is not defective = $1 - 0.0035 = 0.996$; “Yield” = 99.6%

Likelihood that any specimen will contain zero defect was calculated as $(0.996)^{15} = 94\%$.

The overall process Sigma level was also calculated directly with the Formula” NORMSNIV (1- DPO) + 1.5” in the MS Excel as **4.2**.

The QI that has the lowest Sigma level was found for hemolyzed samples with the Sigma value of 3.21.

The Sigma metrics are just the indicators of process quality, and give an overall aspect of the process. The detailed examinations of the process elements, process monitoring and control schemes may be required in order to sustain and improve the process performance.

Post-analytic process Sigma level calculation

The nature of data in the post-analytical process is discrete as in the pre-analytical process, and calculated in the same way with the same formula.

We have chosen the turnaround time (TAT) of troponin T (TnT) for post-analytical process. The TAT for TnT had been defined as 90 minutes at our hospital laboratory. The number of results delivered outside that specified time, and the total number of the TnT results were obtained from the Laboratory Information System (LIS).

Calculation Sigma level for TnT TAT in January 2012

$n = 1\,214$, $d = 131$, $DPO = 131 / (1\,214 \times 1)$, $DPO = 0.1079$;

$DPMO = 0.1079 \times 1\,000\,000 = 107\,900$; Sigma level = 2.74

Probability that the opportunity is not defective: $1 - 0.1079 = 0.892$; Yield = 89.2%;

Likelihood that any specimen will contain zero defect: $(0.892)^1 = 89.2\%$.

The Process Sigma level was also calculated directly with the Formula” NORMSNIV (1- DPO) + 1.5” in the MS Excel as **2.74**.

Lecture Presentations

Sigma levels calculation for analytic process

Table 1. Sigma levels calculated based on separate quality requirements, and converted from C_{pk} values.

Analyte	Unit	QCM	January 2012			
			Mean (Manuf.)	Sigma Levels		
				(Biol.)	from C_{pk}	(CLIA)
ALP	IU/L	PCC1	91	5.4	6	6.0
		PCC2	204	5.7	6	6.0
ALT	IU/L	PCC1	45	6.0	5.9	5.9
		PCC2	115	6.0	6	6.0
AST	IU/L	PCC1	47	5.0	5.6	6.0
		PCC2	133	2.7	4.2	3.9
Calcium	mg/dL	PCC1	8.8	0.9	0.8	4.7
		PCC2	13.4	0.8	0.9	3.1
Chloride	mmol/L	PCC1	81.7	0	0	1.6
		PCC2	104	0	0.2	3.6
Creatinin	mg/dL	PCC1	1.1	2.7	2	4.8
		PCC2	3.9	2.8	3.1	4.7
Glucose	mg/dL	PCC1	99	1.4	2.6	2.4
		PCC2	238	3.7	3.7	6.0
Potassium	mmol/L	PCC1	3.5	4.8	4.3	6.0
		PCC2	6.1	6.0	6.0	6.0
Sodium	mmol/L	PCC1	115	0.8	1.0	3.1
		PCC2	140	0.8	0.6	3.1
Urea	mg/dL	PCC1	39	3.8	6.0	4.9
		PCC2	145	2.8	6.0	3.5

QCM: Quality control material; PCC1: QCM Level 1; PCC2: QCM Level 2; Manuf.: Manufacturer, Biol.: biological variation; CLIA: Clinical Laboratory Improvement Amendments.

Table 2. Sigma levels for the measurement procedures of some analytes in six months.

Analyte	QCM	Mean	Sigma levels (Biological variation)					
			Jan	Feb	Mar	Apr	May	June
ALT (IU/L)	PCC1	45	6,0	4,9	6,0	6,0	6,0	6,0
	PCC2	115	6,0	6,0	6,0	6,0	6,0	6,0
Calcium (mg/dL)	PCC1	8.8	0,9 (4.7)*	0,0 (3.4)*	0,0 (3.5)*	0,9 (4.9)*	0,4 (3.2)*	0,3 (4.1)*
	PCC2	13.4	0,8 (3.1)*	0,1 (2.5)*	0,2 (2.5)*	0,3 (2.3)*	0,2 (1.7)*	0,0 (1.7)*
Glu. (mg/dL)	PCC1	99	1,4 (2.4)*	1,7 (2.9)*	4,0 (6.0)*	2,9 (6.0)*	4,3 (6.0)*	2,2 (4.0)*
	PCC2	238	3,7 (6.0)*	1,6 (2.7)*	4,7 (6.0)*	3,4 (5.0)*	6,0 (6.0)*	4,0 (6.0)*
Sodium (mmol/L)	PCC1	115	0,8 (3.1)*	0,0 (1.8)*	0,0 (1.7)*	0,0 (2.2)*	0,1 (2.5)*	0,2 (1.8)*
	PCC2	140	0,8 (3.1)*	0,0 (1.4)*	0,0 (1.1)*	0,5 (2.7)*	0,5 (2.2)*	0,0 (1.1)*

QCM: Quality control material; PCC1: QCM Level 1; PCC2: QCM Level 2; Manuf.: Manufacturer, Biol.: biological variation *The Sigma metrics based on the CLIA 88 quality requirements.

(Figure 1-A, B, C, and D).

When the statistical process control data and graphics are examined for the measurement procedures that have the sigma levels too low, it is seen that they were stable and in control. But the variations were high even if they were in the control limits.

Since an "Average of Normals (AoN)" algorithm can provide greater detection than available from QC using control materials where the laboratory tests' process capabilities are low (low sigma levels) (11), the patient test results were also assessed with by the AoN approach.

The performance of the analytic process depends upon the quality of measurement procedures. Although there are some discrete measures such as the number of failures in the EQA or PT test results, Number of IQC values that exceed the selected target (3,4,16), most of data for analytic process is continuous, and calculated for every analyte measurement procedure with the Formula, **Sigma-metric = (TEa% - Bias%) / CV%** (11). Bias%, and CV% are obtained from the IQC and/or EQA programs, and the total allowable error (TEa) either from biological variation data or CLIA Proficiency Testing Criteria (13); or can be converted from process capability index (C_{pk}) (11).

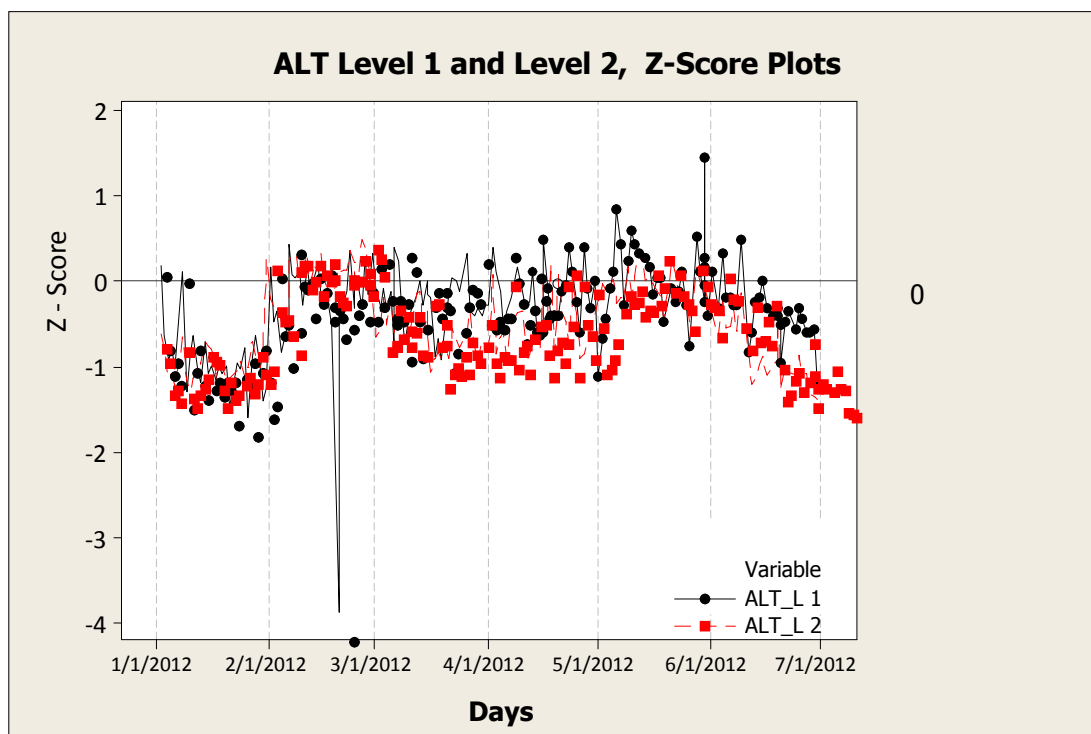
We have calculated the Sigma metrics in monthly basis for analytes requested frequently, and also three sigma levels for one measurement procedure were estimated separately based on biological variation, CLIA Criteria, and also converted from C_{pk} values (Table 1). As seen in the Table 1, the Sigma levels can be different according to the quality requirements specified.

Monthly Sigma-metrics for six months are given in the Table 2 as an example (the Sigma levels are calculated based on biological variations).

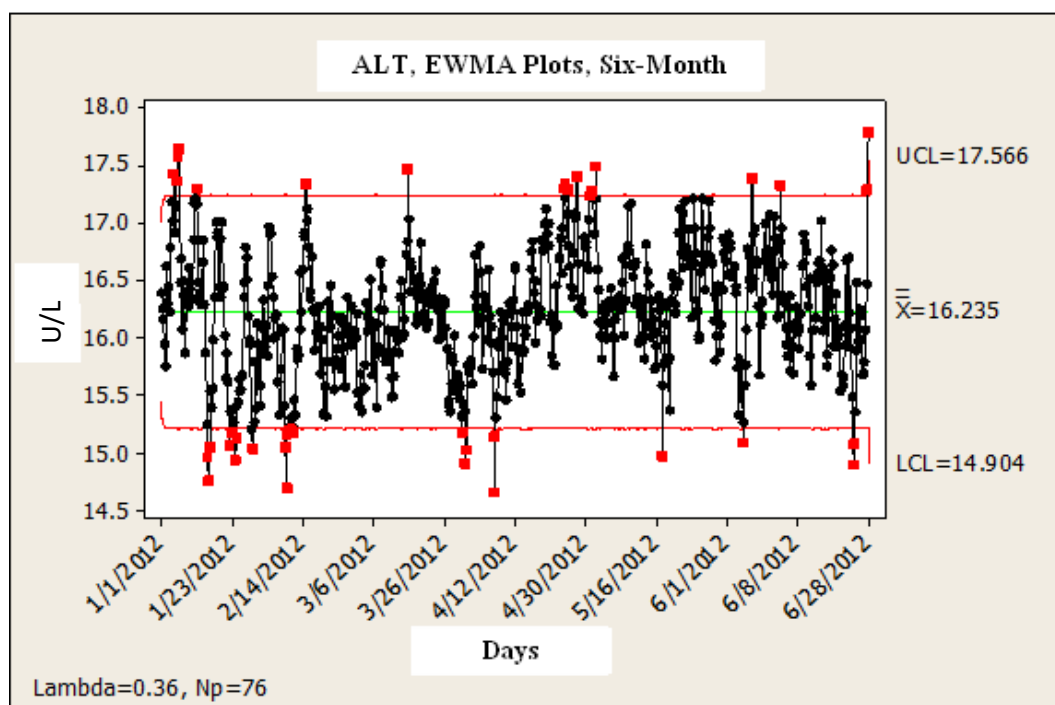
As seen in the Table 2, the sigma metrics based on biological variations for calcium, sodium are too low, and glucose has some low values. The sigma levels are more reasonable when they are calculated based on CLIA requirements with respect to the z-score plots, and the exponentially smoothed weighted averages (EWMA) plots created from patient test results

The patient test results for six months were obtained from the laboratory information system (LIS) and the exponentially smoothed weighted averages (EWMA) charts were created. The z-score graphics, the sigma levels, the EQA results were examined together with the EWMA Charts. Some examples can be seen in the Figure 1-A, B, C, and D. We have chosen one test (ALT) that has Sigma level above 6.0, one test (glucose) between 3.0-6.0, and two tests (calcium and sodium) below 3.0 from the Table 15.1 from the Book by Westgard (11). The acceptability criteria (ALT: 15%, glucose: 7%, calcium: 5%, sodium: 3%) used in that study were from the laboratory proficiency testing program they were involved, and they are different from the CLIA requirements.

As seen in the Figure 1-A, B, C, and D, when the Sigma levels are low values, the decision about the performance of the analytical process should not be made without examining the graphs of z-score, patient test results, and related control tools. It should be noted that the quality control materials may not be stable for some analytes.

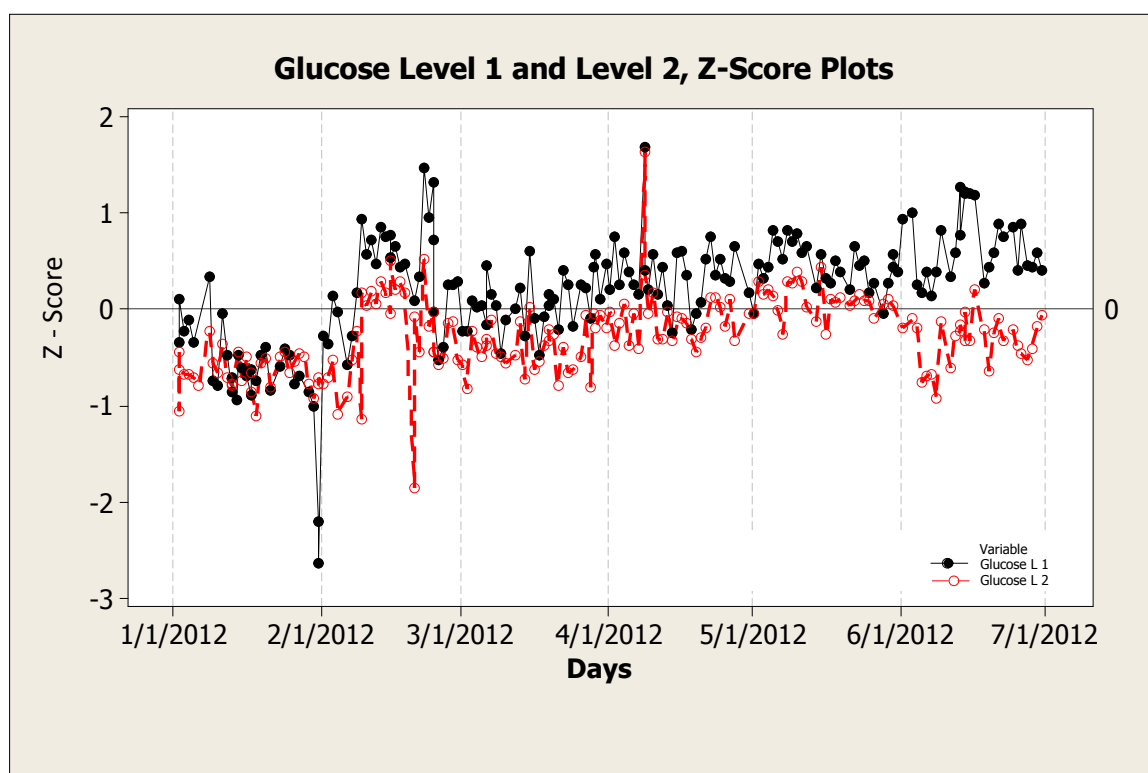


Months, 2012		Jan	Feb	March	Apr	May	June
Sigma	PCC1	8.4	4.9	12.9	13.4	8.8	11.7
Levels	PCC2	10.5	15.4	17.0	11.5	22.4	9.6

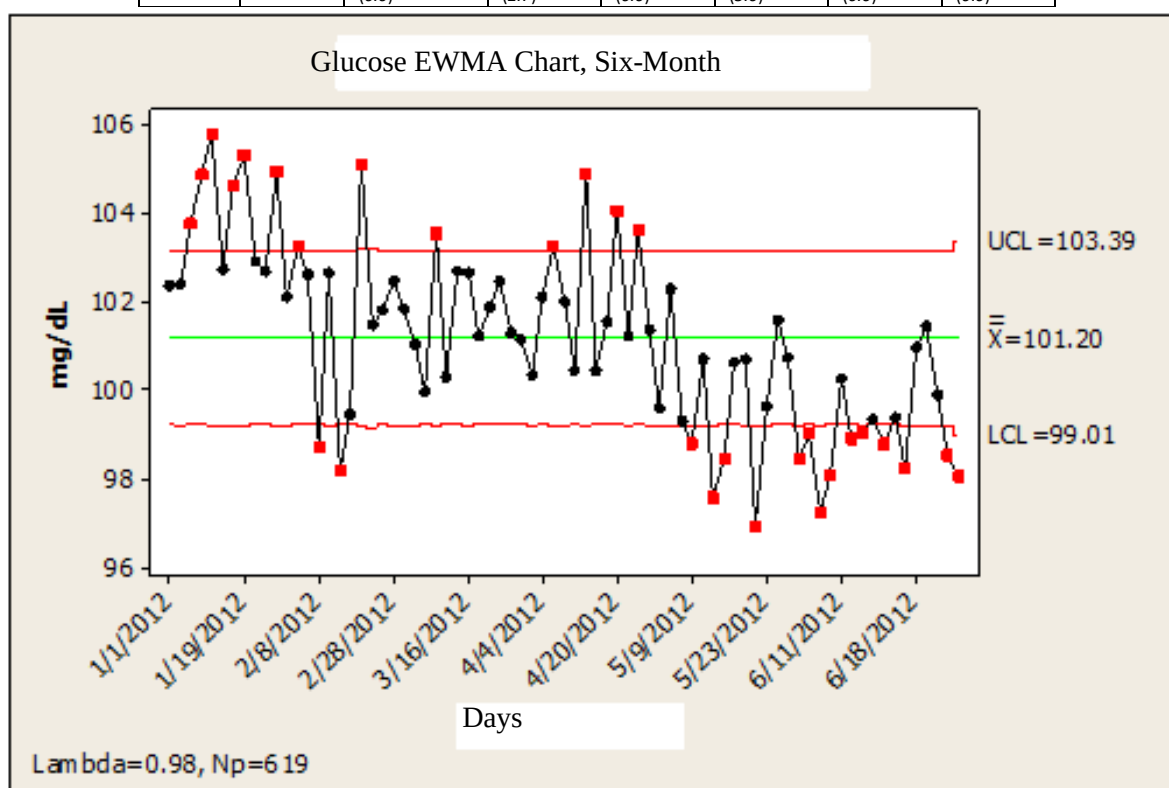


The AoN Control limits: 16 – 19 U/L; The AoN truncation limits: 3.2 – 32 U/L; Reference Interval: F: <33 U/L, M: <41 U/L.

Figure 1-A. Six-month Z-Score and EWMA Plots for ALT

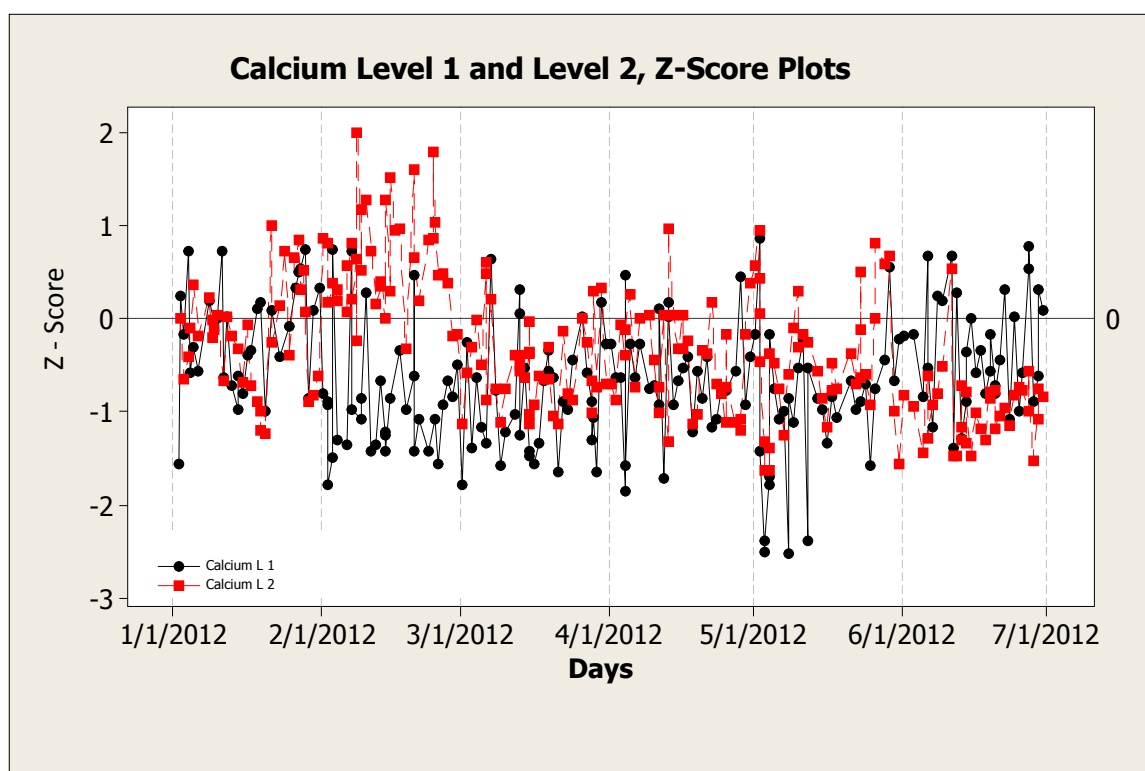


Months, 2012		Jan	Feb	March	Apr	May	June
Sigma Level	PCC1	1,4 (2.4)*	1,7 (2.9)*	4,0 (6.0)*	2,9 (6.0)*	4,3 (6.0)*	2,2 (4.0)*
	PCC2	3,7 (6.0)*	1,6 (2.7)*	4,7 (6.0)*	3,4 (5.0)*	6,0 (6.0)*	4,0 (6.0)*

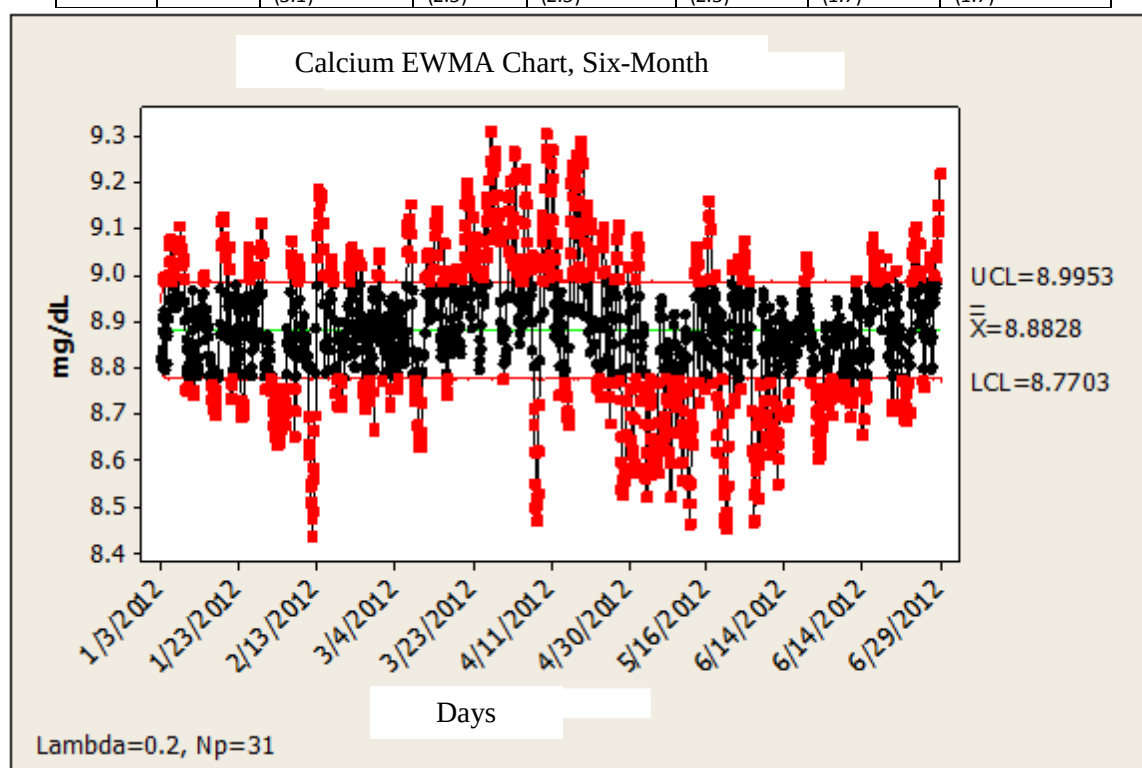


The AoN Control limits: 99 – 107 mg/dL; The AoN truncation limits: 63 – 144 mg/dL; Reference Interval: 74 – 106 mg/dL.

Figure 1-B. Six-month Z-Score and EWMA Plots for glucose

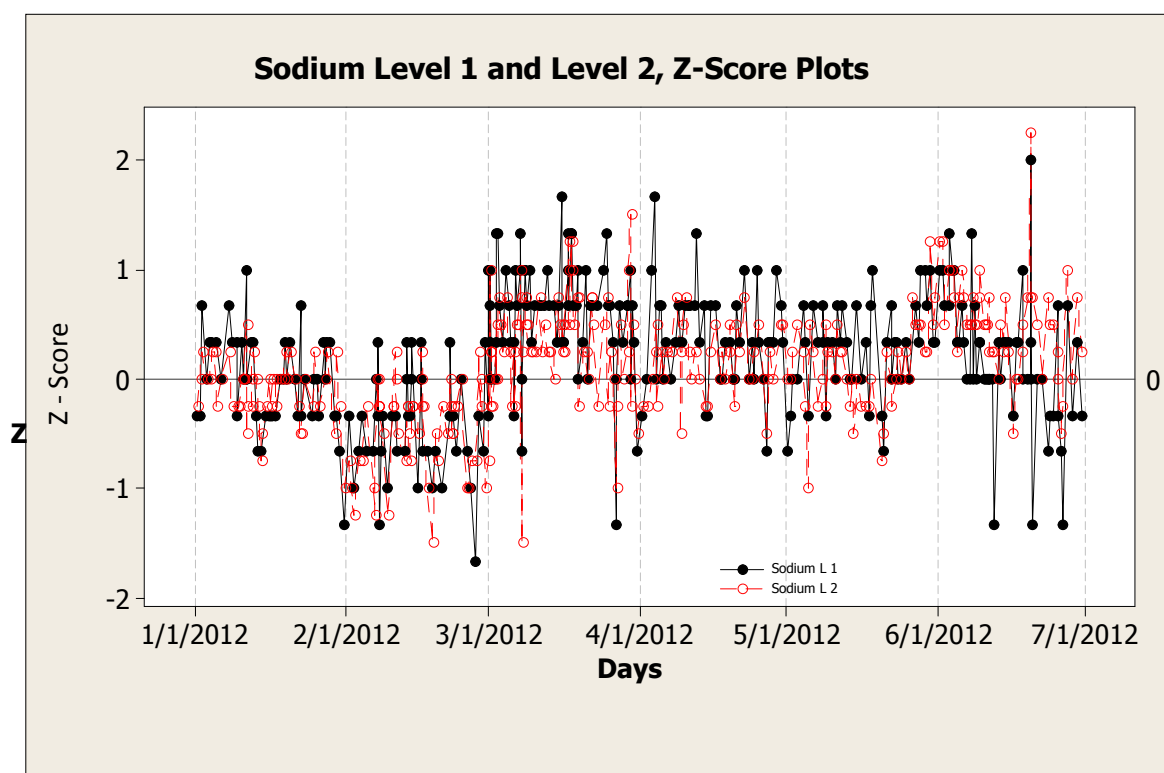


Months, 2012		Jan	Feb	March	Apr	May	June
Sigma Level	PCC1	0,9 (4.7)*	0,0 (3.4)	0,0 (3.5)	0,9 (4.9)	0,4 (3.2)	0,3 (4.1)
	PCC2	0,8 (3.1)*	0,1 (2.5)	0,2 (2.5)	0,3 (2.3)	0,2 (1.7)	0,0 (1.7)

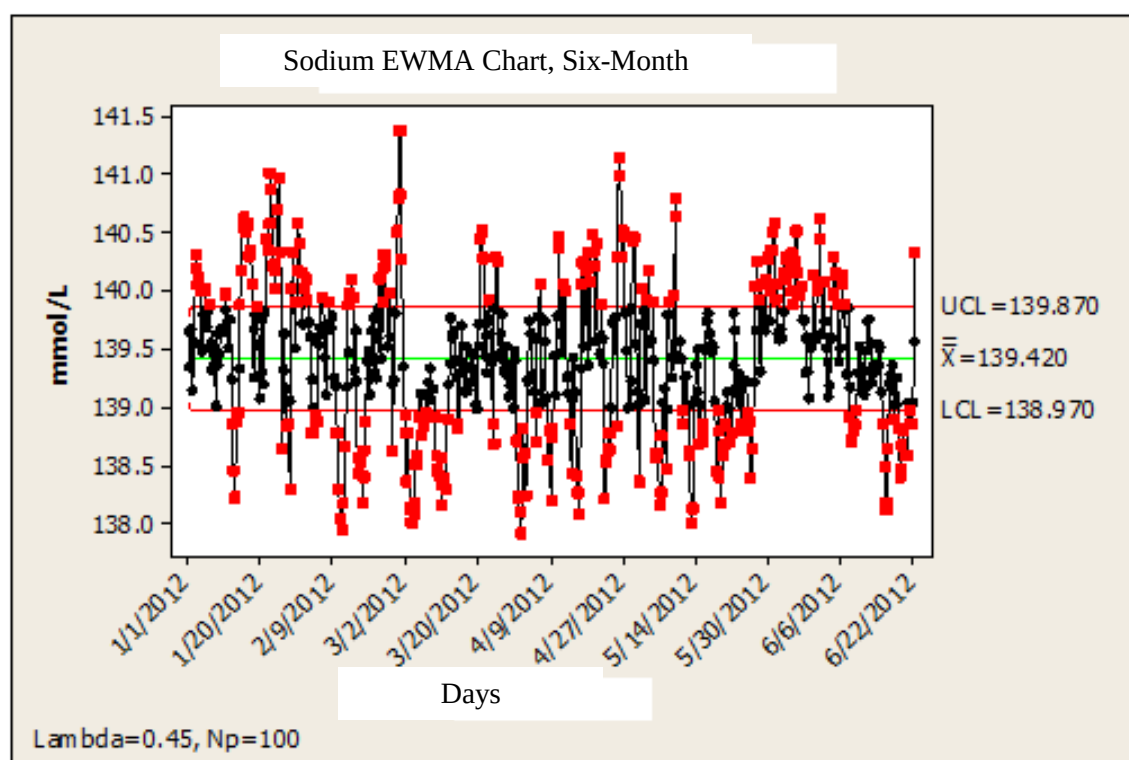


The AoN Control limits: 8.77 – 9.0 mg/dL; The AoN truncation limits: 7.4 – 10 mg/dL; Reference Interval: 8.6 – 10.2 mg/dL.

Figure 1-C. Six-month Z-Score and EWMA Plots for calcium



Months, 2012		Jan	Feb	March	Apr	May	June
Sigma Level	PCC1	0,8 (3.1)*	0,0 (1.8)*	0,0 (1.7)*	0,0 (2.2)*	0,1 (2.5)*	0,2 (1.8)*
	PCC2	0,8 (3.1)*	0,0 (1.4)*	0,0 (1.1)*	0,5 (2.7)*	0,5 (2.2)*	0,0 (1.1)*



The AoN Control limits: 139 – 140 mmol/L; The AoN truncation limits: 133 – 146 mmol/L; Reference Interval: 136- 145 mmol/L.

Figure 1-D. Six-month Z-Score and EWMA Plots for sodium.

Lecture Presentations

In conclusion, the medical (clinical) laboratories are essential parts of the health care organizations, and have responsibility to demonstrate the performance of total testing process beginning the pre-pre-examination phase to the post-post-examination phase by representing the defects come from all processes in the TTP as well as the examination phase for continuous improvement and the trueness and reliability of the test results provided by the laboratory. The ISO 15189 as an internationally accepted accreditation standard for medical (clinical) laboratories also requires clear and precise documentation demonstrating continuous quality improvement. There are several quality control tools for assessing the verification whether the quality requirements are fulfilled. However it may be needed more comprehensible way to explain the defects or nonconformities to the health care team including physicians, directors of hospitals, and/or the policy makers. The Six Sigma metrics seem to be the most comprehensible measures which provide the information that there are defects or variations that may cause harm to the patients and also create poor quality cost. It is a set of tools and techniques for process improvement by identifying and removing the causes of defects (errors) and minimizing the variability in process, and providing cost savings. In this context, we believe that QIs on the Sigma scale give invaluable decision support which elements should be prioritized for continuously improvement of total testing process performance. Every health care organization should establish a Six Sigma Team for laboratory performance in conjunction with the health care Six Sigma Team of the organization by taking advantages of information technology.

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PROGNOSTIC SIGNIFICANCE OF BIOMARKERS IN PATIENTS WITH ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION

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ST-elevation myocardial infarction (STEMI) continues to be a significant public health problem in industrialized and developing countries. Primary percutaneous coronary intervention (PCI) is the preferred reperfusion strategy for patients with STEMI. Despite the improvement in morbidity and mortality in these patients, groups at high risk of adverse clinical events remain. For these reasons, stratifying of this population is of great interest in order to select high-risk patients who can then benefit from a clinical follow-up and management. A plethora of traditional and novel biomarkers are being examined as prognostic markers. We will present our experience with the determination of prognostic value of different biomarkers in relation to in-hospital mortality, occurrence of major adverse coronary events including death, reinfarction, target

vessel revascularisation and stroke, and prediction of atrial fibrillation during 30 days and 1 year follow-up in STEMI patients treated by pPCI. Special emphasis will be placed on routinely available markers including troponin, B-type natriuretic peptide, C-reactive protein myeloperoxidase, and lipoprotein phospholipase A₂. Some studies indicate that several inflammatory biomarkers may predict adverse events. Our results point to the potential prognostic role of adhesion molecules and cytokines for short-term prognosis in STEMI patients. With expansion of the number and types of biomarkers available, the opportunity to improve risk stratification, and selection of therapy using these noninvasive, affordable tools continues to grow. But, the potential role of biomarkers in risk stratification should be further examined in large scale randomized studies.

HEART FAILURE AND GALECTIN-3

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Introduction: Galectins are a family of soluble β -galactoside-binding lectins that play many important regulatory roles in inflammation, immunologic response, and cancer. Galectin-3 has been the most widely-studied member of the Galectin protein family. One of the more interesting and well characterized effects of Galectin-3 is its role in the promotion of fibrosis. Fibrogenesis or scarring is a natural consequence of certain types of injury and inflammation. Galectin-3 is responsible for the activation of the fibroblasts—the cells responsible for fibrogenesis. This Galectin-3-dependent link between inflammation and fibrosis may have diagnostic and therapeutic implications. At the injury site, Galectin-3 is secreted into the extracellular space. This, in turn, activates resting fibroblasts into matrix-producing fibroblasts. Fibroblast activation is characterized by the increased expression of certain proteins

within and around the fibroblasts, including the extracellular type 1 collagen α -1 chain. Galectin-3 affects the synthesis of new matrix components and influences the degradation of extracellular matrix components through a set of tissue inhibitor metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs).

Clinical Utility: Galectin-3 levels higher than 17.8 ng/mL are indicative of Galectin-3 mediated heart failure. The presence of elevated Galectin-3 in chronic heart failure conveys two related messages:

- presence of Galectin-3-mediated fibrosis and adverse remodeling and
- increased risk for adverse outcomes.

According to the American Heart Association, heart failure affects an estimated 5.8 million Americans, with approximately 670,000 new cases occurring each year. According to the Study Group on Heart Failure Awareness and Perception in Europe (SHAPE), about 14 million people in Europe suffer from heart failure, and that number is expected to increase to 30 million by the year 2020. There is a pandemic among these chronic heart failure patients. 30–50% has Galectin-3 mediated heart failure, a sub-set of heart failure known to carry higher mortality and hospitalization rates.

Conclusion: According to some authors, Gal-3 can be a better prognostic marker for the development of cardiac disorders of the cardiac natriuretic peptides. Since the development of the BGM Galectin-3 blood test, levels of Galectin-3 have been measured in more than 23,000 samples from participants in a variety of heart failure-related studies. The Galectin-3 discovery opened a new door to understanding why some people develop heart failure. Understanding the myocyte and finding ways to improve contractility would hold the key to treatment of heart failure.

THE PREDICTIVE VALUE OF CIRCULATING LEVELS OF LIPID AND INFLAMMATORY MARKERS IN RESTENOSIS FOLLOWING PCI

In Extenso

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INTRODUCTION

Percutaneous coronary intervention (PCI) with stent implantation has revolutionized the management of coronary artery disease (CAD), enabling greater efficacy in prevention of acute occlusions [1]. The efficacy of PCI is limited by the need for repeat revascularization resulting from in-stent restenosis (ISR), as a long-term complication that occurs in 20-30% of patients 6 months after the procedure [2]. Stent implantation is inevitably associated with a vessel wall injury, resulting in activation of an inflammatory process in the vessel wall. There is an increasing body of evidence to suggest that inflammation plays a pivotal role linking early vascular injury to neointimal hyperplasia [3]. Secretion of pro-inflammatory cytokines by activated macrophages is a powerful stimulus for smooth muscle proliferation, which additionally induces the production of acute phase proteins, including C-reactive protein (CRP). [4, 5] Several studies have suggested that restenosis after percutaneous coronary intervention (PCA) is associated with increased circulating levels of the inflammatory markers such as C-reactive protein (CRP) before or after the procedure. [6,7] Similar relation was shown with serum levels of other inflammatory markers, such as adhesion molecules (ICAM-1, VCAM-1) [8].

The histological examination of restenotic tissue reveals intimal hyperplasia with a variable lipid component. This histological appearance suggests that similar cellular processes may be involved in atherogenesis and restenosis. Accumulation of oxidized low-density lipoproteins (OxLDLs) in atherosclerotic lesions from coronary[9] and carotid [10] arteries has been demonstrated, as well as elevation of circulating levels of OxLDLs in patients with unstable angina or acute myocardial infarction,[11-13]. However, to the best of our knowledge, the data on association between the level of circulating OxLDL and clinical outcome after coronary stenting are limited, even though some studies have suggested that elevated plasma concentration of lipoprotein(a) could be a risk factor for restenosis after PCA [14,15].

Lipoprotein a [Lp(a)] is a low-density lipoprotein (LDL)-like particle in which apolipoprotein (apo)B-100 is bound with a disulfide bridge to apo(a). [16] Lipoprotein a has been identified in the atherosclerotic plaque and its plaque levels correlate with its concentration in the plasma. [17] Even though the associations between elevated serum concentrations of Lp(a) and CAD, [18] myocardial infarction, [19] cerebrovascular disease, [15] and stenosis of coronary artery bypass vein grafts [20] have been established, the role of serum Lp(a) concentrations ISR after coronary angioplasty remains unclear.

The purpose of this study was to evaluate if plasma concentration of lipid and inflammatory markers could be associated with the development of restenosis in patients undergoing PCA, as well as ISR prevalence 6 months following successful implantation.

METHODS

Study group

Study group consisted of 81 patient with CAD who were successfully treated with either balloon coronary angioplasty or stent implantation. The study protocol was approved by Clinical Center of Montenegro Ethical Committee. 39 patients who had successful coronary stent implantation underwent follow-up angiography and series of biochemical analyses was performed prior to PTCA (baseline) and at follow-up, 6 months after the procedure. All patients fulfilled the following criteria: confirmed myocardial ischemia, coronary vessel constriction (defined reduction > 50 % vessel lumen diameter), no contraindications to the administration of antiplatelet agents, and agreement to undergo follow-up angiography. The clinical and angiographic exclusion criteria were: acute or chronic inflammatory disease, liver dysfunction, kidney dysfunction, treatment with steroids, pregnancy and presence of mental disorders. Written informed consent was obtained from all patients.

Angiographic Assessment

Coronary intervention and angiographic assessment were performed with a digital angiographic system (Siemens Axiom interventional cardiology systems), and quantitative coronary analysis (QCA) was performed on a digital angiographic workstation (Axiom Syngo artis zee family of C-arm system). Restenosis was defined as a percent diameter stenosis (%DS) >50% at the site of treated lesion in at least 2 orthogonal views.

Laboratory Tests

Blood samples were obtained after at least 10 h of fasting. Plasma concentrations of hs-CRP i ceruloplasmin were determined using immunochemical method at the Center for clinical laboratory diagnostics of Clinical Center of Montenegro. Plasma concentrations of Lp(a), oxLDL and ICAM-1, were dermined using an enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, USA) at the Laboratory for Research at the Faculty of Medicine, University of Montenegro. Conventional clinical risk factors for CAD were recorded as age, gender, current smoking habit (>5 cigarettes/day), hypertension (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or antihypertensive medication), diabetes mellitus (fasting blood glucose >126 mg/dl or hypoglycemic medications) and hyperlipidemia (TC >2,4 mmo/L, LDL-C >1,2mmol/L or cholesterol-lowering medications).

Statistical Analysis

Data are expressed as the mean $\pm$ SD. Student's t-test was used to compare differences between continuous variables. The significance of differences between the frequencies of incidence of risk factors was tested using the χ^2 -test. P values <0.05 were considered to be significant. All statistical calculations were done with IBM SPSS for Windows, trial version.

RESULTS

Baseline Characteristics

Of the 39 patients who underwent stent implantation, 30 (77%) were male and 6 (23%) were female and all were between the ages of 38 and 75 (56.0 ± 5.5 years). In-stent restenosis (ISR) occurred within 6 months of stent implantation in 6 patients (16.6%). In univariate analysis, age and gender did not differ between the groups with and without restenosis. No significant association between restenosis and hypercholesterolemia or smoking was observed (Table 1). However, there was a difference in hypertension, positive family history for CAD and previous angina. All patients took aspirin and antiplatelet agents.

Table 1 Baseline Characteristics of the Patients Stratified by Stent Implantation With Restenosis or No-Restenosis

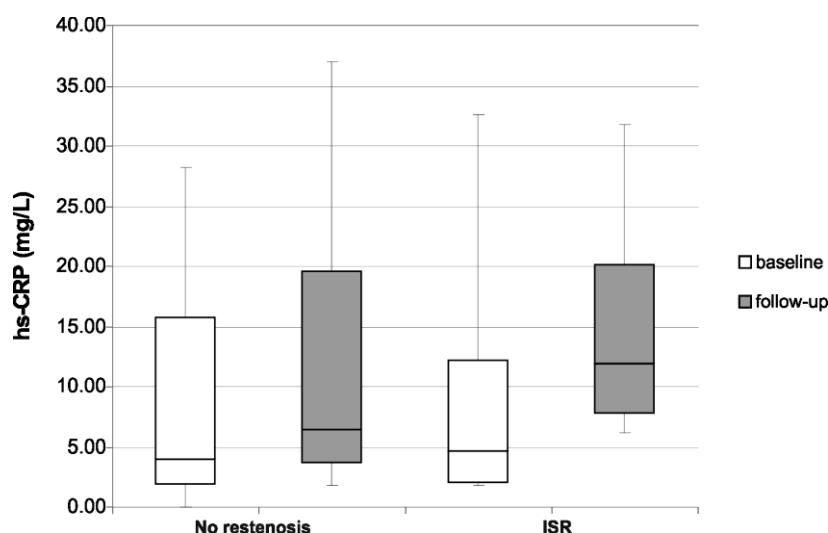
Risk factors	Restenosis (n=6)	No Restenosis (n=36)
Age, years	61.2 $\pm$ 7.3	60.2 $\pm$ 8.1
Males (%)	83.3	69.4
Smoking (%)	33.3	44.4
Hypercholesterolemia (%)	33.3	55.6
Hypertension (%)	66.7*	75.0
Family history for CAD (%)	33.3*	55.6
Previous angina (%)	50.0*	36.1

Values are mean or mean $\pm$ SD, * $p < 0.05$.

Association between restenosis and biochemical parameters

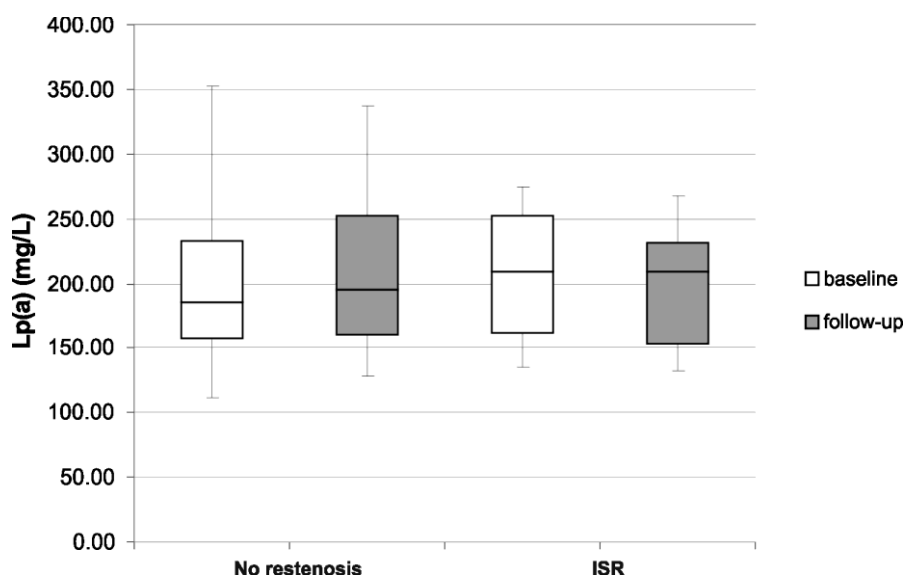
The study focused on the association between the biochemical risk factors and in-stent restenosis after coronary angioplasty. No significant difference was observed at baseline in any of the biochemical parameters investigated. However, after 6-month follow-up, evaluation of several markers of inflammation revealed increased serum concentration of CRP after 6-month follow-up in the samples of patients that developed restenosis (Figure 1).

Figure 1. Box plots of C-reactive protein (CRP) concentration at baseline and at 6-month follow up after percutaneous coronary intervention (PCI), according to no events or events defined as in-stent restenosis (ISR).



Since it has been shown that Lp(a) has a high degree of homology to plasminogen, thus inhibiting plasminogen activation [16], our hypothesis was that patients with elevated Lp(a) concentrations in blood may have a greater tendency to thrombosis after platelet activation initiated by the arterial injury of angioplasty. This may result in increased growth factor release and a propensity to coronary restenosis..= Our results have shown that, similar to CRP, the concentration of Lp(a) after 6-monthh follow-up was higher in patients that developed ISR (Figure 2).

Figure 2. Box plots of Lp(a) concentration at baseline and at 6-month follow up after percutaneous coronary intervention (PCI), according to no events or events defined as in-stent restenosis (ISR)



The difference in concentration of other investigated markers of inflammation, oxLDL, ICAM and TGF-beta was comparable between two groups of patients (without stenosis and with ISR). Our results failed to confirm change in oxLDL, ICAM or TGF-beta in group of patients with ISR, compared to patients without stenosis.

DISCUSSION

The aim of our research was an attempt to investigate if a connection can be established values of biochemical parameters of lipid status and inflammation development of in-stent restenosis. Multiple factors can contribute to in-stent restenosis, and the underlying mechanisms remain elusive. Vascular injury induced by angioplasty (PCA) may upregulate local cytokine expression leading to release of proinflammatory factors. The cytokines released from activated macrophages, smooth muscle cells, lymphocytes and from cells forming the vascular wall may stimulate neointimal formation [20, 21]

In our study, serum hs-CRP concentrations were significantly higher in patients who developed restenosis (Figure 1). It has been shown that elevated serum levels of inflammatory markers (CRP, IL-6) six months after PCI were associated with risk of in-stent restenosis [22]. It has also been shown that post-intervention rise of inflammatory markers reflects the inflammation response and with close correlation between raised post-intervention CRP levels and angiographic restenosis [23, 24]. This rise in CRP level may be an indicator of the extent of inflammation in atheromatous lesions, and such plaques could be prone to development of ISR.

Several studies have investigated the role of lipoproteins in the development of coronary restenosis after percutaneous coronary interventions with conflicting results. Lp(a) was independently associated with the presence of coronary atherosclerosis on angiography in the general population [11]. However, there are fewer data on the role of Lp(a) in patients with established CAD, implicating that that Lp(a) might be less predictive of vascular risk in patients with established vascular disease [25]. In our study, the level of Lp(a) was higher in patients that have developed ISR, but due to small number of patients, it has not reached the level of significance (Figure 2). Further studies are needed to help clarify how relevant Lp(a) concentration may be as a predictive factor for ISR.

Lecture Presentations

Oxidized LDL levels, autoantibodies against epitopes of oxLDL [26] and oxLDL:LDL ratio [27] have been independently associated with increased risk for coronary atheromatosis and ischemic heart disease, with increased levels of oxLDL [9] and MDA-LDL [12] being related to plaque instability. Other studies have failed to find a significant association between OxLDL and clinical presentation of the coronary artery disease or rate of complications in the first year after coronary stenting [28]. In our study, the values of oxLDL were slightly higher in the group with restenosis, but further study including larger number of patients with restenosis is required to assess whether this finding is relevant. It has been shown that oxidized LDL impairs endothelium relaxation [29] by inhibition of the expression of eNOS, reduces the responsiveness of smooth muscle cell to NO [30], induces the expression of adhesion [31] and inflammatory molecules [32]. All of the above contribute directly to dysfunction of the endothelium [33].

In this study, we evaluated if the levels of circulating adhesion molecule ICAM increase following the development of ISR. Our results failed to confirm change in ICAM in group of patients with ISR, compared to patients without stenosis. These results are in accordance with the previously published study that suggests a more dominant role for VCAM-1, but not for ICAM-1 in development of restenosis following coronary stent implantation. [34].

Unexpectedly, no positive correlation between the activity of the enzymes with antioxidative properties (catalase and glutathione peroxidase) and development of restenosis, neither with serum ceruloplasmin concentration before or after intervention and occurrence of restenosis (data not shown)

In conclusion, our results suggest that lipid and inflammatory markers may be considered for further investigation as possible markers of ISR. However, further studies with larger cohort of patient is required to to assess their influence on vascular disease.

Study Limitations

The sample size is relatively small, which may have distorted some important variables that only showed a trend towards statistical significance. Because the risk factors for restenosis after stent implantation may be multifactorial, further studies are required to confirm our preliminary data.

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INFLUENCE OF sTWEAK ON INTIMA-MEDIA THICKNESS IN RENAL PATIENTS

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Introduction: The cause of endothelial damage is complex and involves dysregulation of multiple pathways. One of those could be mediated by the TNF-like weak inducer of apoptosis (TWEAK). In the few studies, inverse correlation between soluble TWEAK (sTWEAK) level in circulation and endothelial damage of blood vessels was determined. We observed the influence of renal function on sTWEAK level in circulation and relationship between sTWEAK and intima media thickness (IMT) of carotid arteries.

Materials and Methods: This study included 93 hemodialysis (HD) patients, 42 patients with chronic kidney disease (CKD) and 50 apparently healthy subjects. IMT was determined by B-mode ultraso-

nography in all subjects. sTWEAK concentration was determined by enzyme-linked immunosorbent assay.

Results: sTWEAK values were significantly different between the three groups ($p=0.020$). To explore whether the association between sTWEAK and IMT were confounded by renal function, sTWEAK concentration in all groups were compared after adjusting for creatinine and urea values. After adjusting for renal parameters difference between groups was significant ($p=0.009$). Only in HD patients has been demonstrated inverse correlation between sTWEAK and IMT ($r=-0.225$, $p=0.043$). If we eliminated the influence of inflammation (after adjustment for hs-CRP) and renal function (after adjust-

ment for urea) sTWEAK negatively correlated with IMT, $\beta=-0.265$, $p=0.025$.

Conclusion: Renal function influenced on sTWEAK concentration in patients with CKD. Inverse correla-

tion between sTWEAK and IMT was a compensatory mechanism for prevention of sTWEAK adhesion to the blood vessels endo-thelium.

ASSOCIATION OF RETINOL-BINDING PROTEIN 4 WITH MARKERS OF KIDNEY FUNCTION IN TYPE 2 DIABETES MELLITUS PATIENTS

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Retinol-binding protein 4 (RBP4) is a novel adipokine that may be important in type 2 diabetes mellitus (T₂DM).

Aim: The aim of this cross-sectional design was to investigate the association between RBP4 and kidney and metabolic parameters in T₂DM patients with different stages of kidney damage.

Methods: A total of 106 T₂DM subjects, mean age 64.95±6.62 years were included in this study. Serum RBP4 levels were measured using particle enhanced immunonephelometric assays. Glomerular filtration rate was estimated using creatinine in the Modification of Diet in Renal Disease Study equation (eGFRMDRD).

Results: Serum RBP4 levels increased in parallel through the groups of diabetic subjects as the eGFR decreased ($p<0.001$). Also, serum RBP4 levels did not correlate with BMI, fasting glucose, and glycosylated hemoglobin. On the contrary, RBP4 correlated with age ($r=0.286$; $p=0.003$), gender ($r=0.196$;

$p=0.045$), kidney function markers - creatinine ($r=0.770$; $p<0.0001$), eGFR ($r=-0.745$; $p<0.0001$), urinary albumin excretion ($r=0.570$; $p<0.0001$), cystatin C ($r=0.726$; $p<0.0001$), and triglycerides ($r=0.282$; $p=0.004$). Moreover, after dividing subjects according to the amount of albuminuria, there was significant increase in RBP4 levels in macroalbuminuric as compared with microalbuminuric and normoalbuminuric T₂DM subjects (78.47 ± 20.37 vs. 56.67 ± 19.64 and 50.05 ± 14.31 respectively, p for trend <0.001). Between the last two groups we did not record significant difference in RBP4 levels ($p=0.198$).

In multiple linear regression analysis, creatinine, eGFR, cystatin C and triglycerides were significant independent predictors of higher RBP4 levels ($R^2=0.634$; $p<0.001$).

Conclusion: Serum RBP4 levels might be a useful biomarker of nephropathy in type 2 diabetic subjects.

BIOMARKERS OF DEMENTIA IN CEREBROSPINAL FLUID

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Biochemical markers for AD should reflect the central pathogenetic processes (i.e., the neuronal degeneration, the deposition of amyloid- β peptide in plaques, and the hyperphosphorylation of tau with subsequent formation of tangles). Numerous studies showed that low level of 42-amino acid form of amyloid-beta peptide ($A\beta_{1-42}$), and levels of tau protein (T-tau) and phosphorylated tau protein (P-tau) are possible biomarkers for these pathogenetic processes.

The aim of this study was to diagnostic potential of CSF biomarkers T-tau, P-tau and $A\beta_{1-42}$ as biochemical markers for AD.

Lumbar puncture was performed in 78 patients with probable AD and 52 age-and-sex-matched control subjects who were subjected to spinal anesthesia due to orthopedic surgery. There was no difference

in education level between the groups. The Innostest, ELISA sandwich test was used for measuring the levels of T-tau, P-tau and $A\beta_{1-42}$.

The results of the study show that mean levels of CSF T-tau and P-tau were significantly higher in the patients with AD ($p < 0.01$) compared to the control group, in contrast to significantly lower CSF $A\beta_{1-42}$ level in AD group ($p < 0.001$). A significant progressive decrease of $A\beta_{1-42}$, as well as significant progressive increase of T-tau and P-tau was found among AD subgroups (according to MMSE staging) and controls.

The obtained results confirm that CSF biomarkers can have a good diagnostic performance to identify AD also in the early phase of the disease and could be used in the routine clinical practice.

PERIPHERAL NERVOUS STIMULATION ENHANCES THE SELECTIVITY OF ELECTRO-CHEMICAL PROSTHESIS IN CASE OF THE COMPLETE SPINAL CORD TRANSECTION IN RAT MODEL

Raspopović S.

Switzerland

Recently, very promising method has been proposed as the treatment in the case of Spinal Cord Injuries. It consists in synergistic use of epidural electrical spinal stimulation and pharmacological treatment. Nevertheless some degree of motor recovery could be observed it is suffering some issues, among these, the lack of the selectivity during the walking. Established tool for muscular activation in case of missing supra-spinal input are

neural stimulating electrodes. The combined use of these with the treatment however was never tested. We tried to understand if it is synergistic with spinal stimulation and drug effect, and up to which extent is possible to augment the efficacy in the motor recovery. Preliminary results show the symbiotic effect and raise the hope that this could potentially be the way for the full walking ability recovery.

DETECTION OF C282Y/H63D COMPOUND HETEROZYGOTE MUTATION IN TWO PATIENTS WITH ALTERED LIVER FUNCTION AND IRON OVERLOAD: CASE PRESENTATIONS OF HEREDITARY HEMOCHROMATOSIS

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Introduction: Hereditary hemochromatosis (HH) is a genetic disorder of iron metabolism which leads to irregular iron absorption from the intestine and iron overload in patients diagnosed with the disease. The majority of patients with clinical iron overload are homozygous for the C282Y mutation in the HFE gene, however a minority of patients which express the disease show heterozygosity (C282Y/H63D) in the HFE gene.

Methods: In this study, we present two cases of compound heterozygosity (C282Y/H63D) in the HFE gene. C282Y and H63D mutations were evaluated in patients via 5' nuclease PCR analysis. Serum ferritin was measured by electro-chemiluminescence im-

munoassay while transferrin was determined via nephelometric method. Other biochemical parameters were measured by Roche Hitachi Cobas 8000 Modular Analyzer.

Results: Both patients, that were first degree relatives, presented with iron overload, elevated serum ferritin and transferrin saturation. C282Y/H63D compound heterozygote mutations were determined in both patients that were subsequently diagnosed with hereditary hemochromatosis.

Conclusion: In conclusion, we recommend screening of HFE mutations in all first degree relatives of patients that confer increased risk of iron overload.

Presentation:

IRON

- In the healthy human male adult, there is between 35 and 45 mg iron per kg body mass (2450-3150 mg of iron in a 70 kg male).
- Two-thirds of total body iron is incorporated into hemoglobin by erythroid cells.
- The remaining one-third is stored in the liver or as myoglobin in muscle or is found in reticuloendothelial macrophages.
- Only a small fraction (1%) circulates in the plasma bound to transferrin or is present in the cellular labile iron pool.

DISTRIBUTION OF IRON IN THE BODY

	Amount of Iron (mg)	Total body iron (%)
Hemoglobin	2000	67
Stored Iron	1000	27
Myoglobin	130	3.5
Other	91	2.5

IRON STORAGE IN THE LIVER

- The liver, or more specifically the hepatocyte, is the main site of iron deposition and storage.

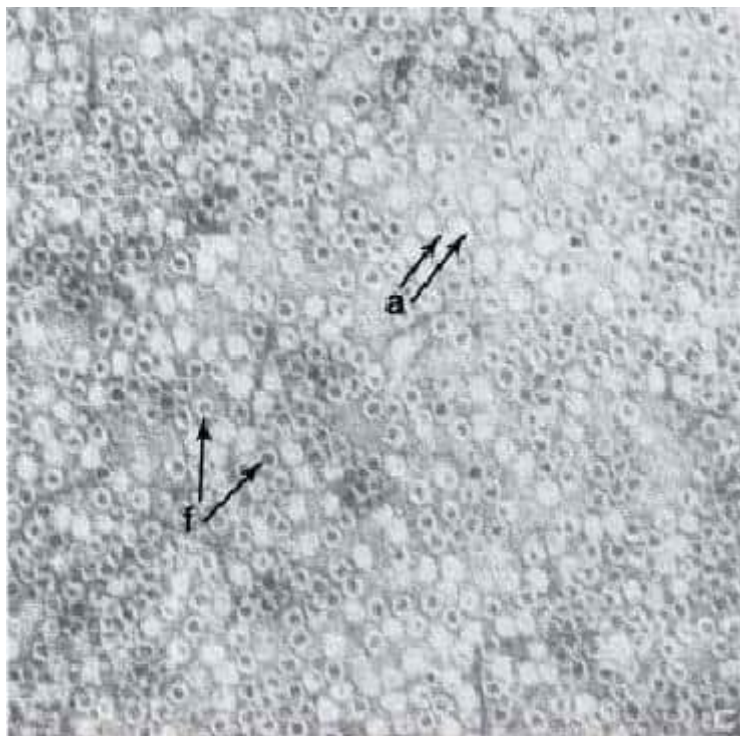
Lecture Presentations

- Stored iron in hepatocytes and macrophages can be mobilized to meet erythropoietic and cellular demands when body iron levels are low.
- Most of the iron in the liver is incorporated into ferritin or hemosiderin (approximately 80%), while 5% is associated with transferrin, 2% with heme, and the remainder is found in the labile or transit iron pools.

FERRITIN

- Ferritin synthesis is induced by high intracellular iron levels and repressed when iron is depleted.
- Ferritin is a water-soluble molecule that can be measured in plasma.
- Plasma ferritin concentration correlates with stored body iron.

ELECTRON MICROGRAPHS OF INTRACELLULAR FERRITIN



Source: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology*, 7th Edition: <http://www.accessmedicine.com>

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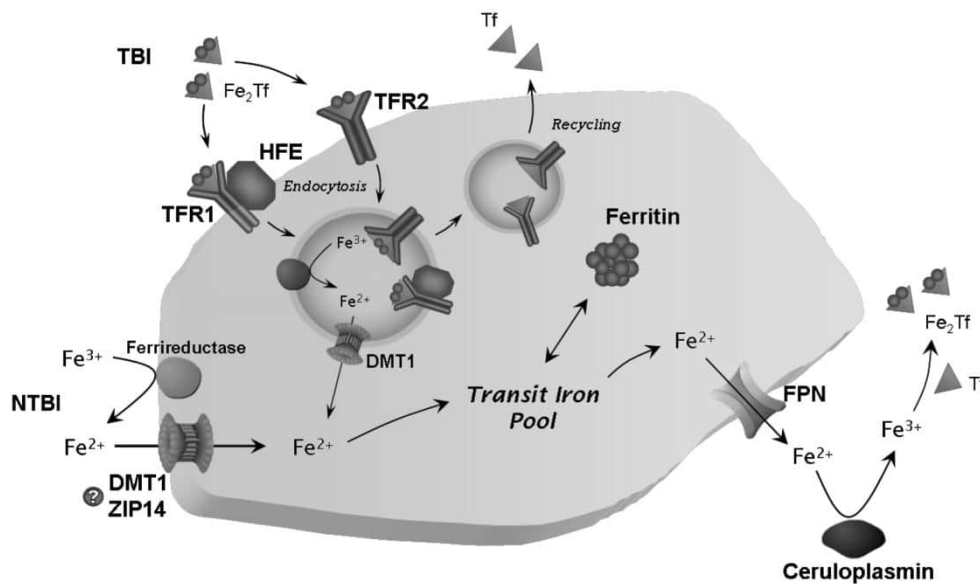
f= ferritin that houses atoms of iron

a= hollow sphere of ferritin protein.

HEMOSIDERIN

- Hemosiderin is a water-insoluble molecule present in lysosomes.
- Is thought to be a by-product of ferritin degradation through incomplete lysosomal processing.
- Is usually found in the spleen, bone marrow, and Kupffer cells.
- Both ferritin and hemosiderin are increased in Hereditary Hemochromatosis.

IRON TRANSPORT IN THE HEPATOCYTE



TBI, transferrin-bound iron; NTBI, non-transferrin-bound iron; HFE, hemochromatosis protein; Tf, transferrin; TFR1, transferrin receptor 1; DMT1, divalent metal transporter 1; FPN, ferroportin

ANALYTICAL METHODS USED TO MEASURE IRON AND RELATED ANALYTES

Serum iron (Fe^{+3}) concentration: refers to iron bound to serum transferrin.

- 1) Iron is released from transferrin by decreasing the pH of the serum.
- 2) Reduced from Fe^{+3} to Fe^{+2} .
- 3) Complexed with a chromogen. Such iron-chromogen complexes have an absorbance at certain wavelengths, which is proportional to iron concentration.

Iron Binding Capacity (=unsaturated iron binding capacity):

Normally only about one third of the iron binding sites of transferrin are occupied by Fe^{+3} , serum transferrin has considerable reserve iron binding capacity.

Determination of unsaturated iron binding capacity (UIBC):

- 1) Addition of known amount of Fe^{+2} to serum to bind all sites on transferrin.
- 2) Excess Fe^{+2} is reacted with a chromogen to form a color complex which is measured photometrically.
- 3) The difference between the amount of Fe^{+2} added and the amount of Fe^{+2} measured represents the UIBC.

The total iron binding capacity (TIBC): is determined by adding the serum iron value to the UIBC value.

Transferrin saturation (%): $(\text{serum } \text{Fe}^{+3} / \text{TIBC}) \times 100$

Total serum ferritin: Ferritin concentration correlates with stored iron amount.

WHAT IS HEMOCHROMATOSIS?

- Hemochromatosis is a disease in which too much iron builds up in your body (iron overload).
- In hemochromatosis, iron can build up in most of your body's organs, but especially in the liver, heart, and pancreas.
- Hemochromatosis may cause liver failure, liver cancer, cirrhosis, arrhythmia, heart failure and diabetes.

OVERVIEW

- The two types of hemochromatosis are primary and secondary.
- Primary hemochromatosis is caused by a defect in the genes that control how much iron you absorb from food.
- Secondary hemochromatosis usually is the result of another disease or condition that causes iron overload.

HEREDITARY HEMOCHROMATOSIS (HH)

- Common genetic disorder of iron metabolism which leads to inappropriate iron absorption from the intestine and iron overload in susceptible individuals.
- Most common among those of Northern European ancestry.
- Inherited in an autosomal recessive pattern, which means both copies of the gene have mutations.
- Most often, the parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but do not show signs and symptoms of the condition.

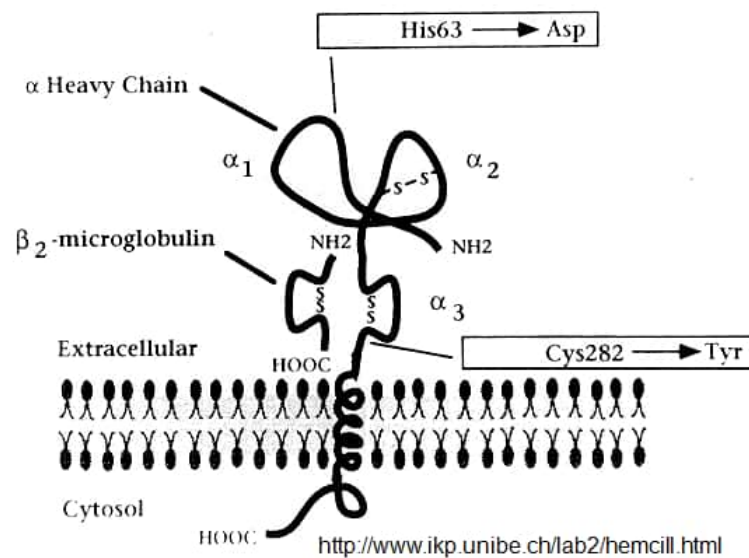
HFE GENE

- One of the better characterized genes responsible for hereditary hemochromatosis is HFE on chromosome 6 which codes for a protein that participates in the regulation of iron absorption.

CLASSIFICATION OF HEREDITARY HEMOCHROMATOSIS

- HFE related HH (account for ~90% of the cases):
 - C282Y homozygote: the cysteine residue at position 282 is replaced with a tyrosine amino acid
 - C282Y/H63D compound heterozygote: cysteine residue at position 282 is replaced with a tyrosine amino acid / histidine residue at position 63 is replaced with an aspartic acid
- Non-HFE related HH:
 - Juvenile Haemochromatosis (Hemojuvelin or Heparin related)
 - Transferrin receptor-2 related HH
 - Ferroportin related HH

HFE PROTEIN



- HFE interacts with transferrin and thereby regulates iron absorption.
- The mutation C282Y leads to inability to react with the transferrin receptor: iron is absorbed regardless of the iron status of the organism.

GENETICS OF HH

- In Northern Europe, Homozygous C282Y genotype is reported in <83% of HH patients.
- C282Y/H63D compound heterozygote genotype is reported in <3% of HH patients.
- The prevalence of the homozygous C282Y genotype is much lower in the Mediterranean region and southern Europe.

PHENOTYPIC SCREENING

- The appropriate interpretation of transferrin saturation and serum ferritin results is essential in the diagnosis of iron overload.
- Fasting transferrin saturation (the ratio of serum iron to total iron binding capacity) is the most sensitive initial phenotypic screening test
- Transferrin saturation value of ≥45% will detect almost all affected patients.
- Unsaturated iron binding capacity has also been used and is a valid alternative to transferrin saturation.
- Serum ferritin reflects body iron stores and generally rises later in the progression of iron overload.
- Other causes of hyperferritinaemia that warrant consideration include alcohol abuse, metabolic syndrome, inflammatory states and acute or chronic hepatitis.
- In the absence of these conditions, serum ferritin is a good marker of the degree of iron overload.
- The negative predictive value of a normal transferrin saturation and serum ferritin is 97%. In this situation, no further testing is recommended.

HFE GENE MUTATION TESTING

- Only those who are homozygous for C282Y and compound heterozygotes have the potential to develop significant iron overload related to HFE gene mutations.

Lecture Presentations

- Testing for HFE gene mutations is generally indicated in those with an iron overload phenotype and those with a family history of HFE-related HH.

GENOTYPING PROTOCOL

- Blood samples are collected in EDTA tubes and genomic DNA is extracted from venous blood.
- Isolated DNA can be tested for C282Y and H63D mutations with wild-type and mutant type master mixes.
- This system has been specifically adapted for 5' nuclease PCR mutation analyses.
- The test system is designed for use with sequence specific primers and probes.
- Each master-mix also contains an internal control targeting glucose-6-phosphate dehydrogenase .
- The fluorescent signal generated by the cleaved reporter dye is monitored by real-time PCR detection system.

LIVER BIOPSY

- Liver biopsy no longer has a primary role in the diagnosis of HH.
- The usefulness of liver biopsy in these patients is as a prognostic indicator to establish or exclude the presence of cirrhosis.
- Liver biopsy is generally recommended as a tool to determine severity of liver disease, particularly when other causes (such as alcohol or viral hepatitis) may be implicated and when *HFE gene mutation testing is negative*.
- Those for whom a liver biopsy has been recommended include those:
 - aged >40 years,
 - serum ferritin >1000 µg/L
 - those with clinical evidence of liver disease, including elevated transaminase levels

CASE PRESENTATION OF HH

- 15 year old male patient initially visits the doctor with his brother who is diagnosed with hepatitis B.
- During check-up, elevated transaminase levels are seen in his blood tests.
- Further blood tests reveal increased serum iron levels.
- Primary management is initiated via removal of excess iron through flebotomy.
- Future visits to the doctor and repeated blood testing shows the existence of high iron levels.
- He is referred to a pediatric gastroenterology clinic for further evaluation.

PAST MEDICAL HISTORY

- Prenatal evaluation is normal.
- Born at 40 weeks
- Was operated for inguinal hernia at 2.5 years.
- Has bronchial asthma (not taking any medication for the past 3 years)

FAMILY ANAMNESIS

- His mother and father are third-degree relatives (cousins).
- Mother is 48 years old, hypothyroid and Hepatitis B Carrier.

- Father is 52 years old and Hepatitis B Carrier.
- Sister and twin brother are Hepatitis B Carriers.

PHYSICAL EXAMINATION

- Common condition is good.
- Consciousness is clear.
- Body Weight is 32 kg, height is 143 cm (<3 growth percentile)
- Examination of the respiratory system, cardiovascular system, oral cavity, stomach, liver (no sign of hepatosplenomegaly) and urinary system is normal.

LABORATORY EXAMINATION

- Serum ferritin was measured by electro-chemiluminescence immunoassay while transferrin was determined via nephelometric method. Other biochemical parameters were measured by Roche Hitachi Cobas 8000 Modular Analyzer.

Test	Result	Reference Intervals	
Albumin	4.55 g/L	3.4	4.8
ALT	172 U/L	0	41
AST	116 U/L	0	38
GGT	14 U/L	8	61
Total Bilirubin	0.47 mg/dl	0.1	1.1
Direct Bilirubin	0.05	0	0.3
Total Creatine Kinase(CK) Activity	356 U/L	38	174
CK-MB Activity	29 U/L	0	24

Test	Result	Reference Intervals	
Serum Iron	246 µg/dL	59	158
Ferritin	1272 ng/ml	30	400
Transferrin	282 mg/dl	200	360
Total Iron Binding Capacity	255 µg/dL	228	428
Alpha-1 Antitrypsin	102 mg/dL	90	200

Test	Result	Reference Intervals
HAV IGM	NEGATIF	
HBS AG	NEGATIF	
HBS AB	1000 IU/L	
HBC IGM	NEGATIF	
HBC AB TOTAL	NEGATIF	
HBE AG	NEGATIF	
HBE AB	NEGATIF	
HCV AB	NEGATIF	
DELTA AB	NEGATIF	
HIV 1+2 AB (CORE +ENV)	NEGATIF	
HBV DNA (KANTITATIF)	NEGATIF	

Lecture Presentations

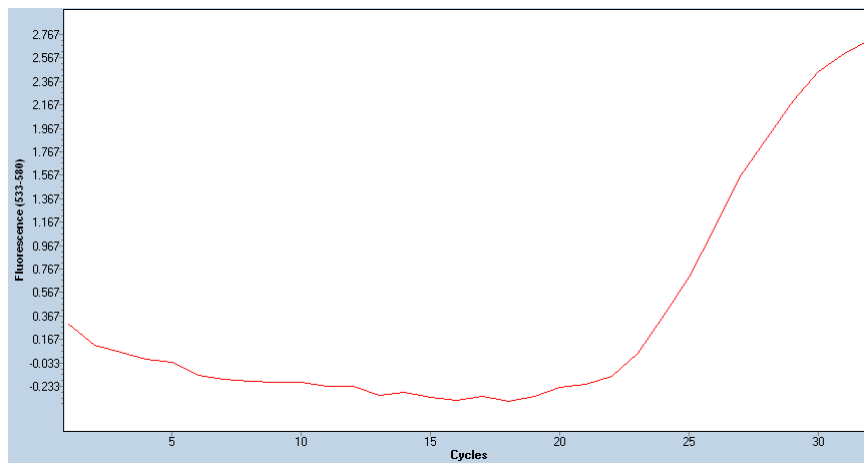
TRANSFERRIN SATURATION

- Transferrin saturation (%): $(\text{serum Fe}^{+3} / \text{TIBC}) \times 100$
- Transferrin saturation value of $\geq 45\%$ is important in the diagnosis of HH.
- $\text{TS} = (246 \mu\text{g/dL} / 255 \mu\text{g/dL}) \times 100$
- $\text{TS} = 96$

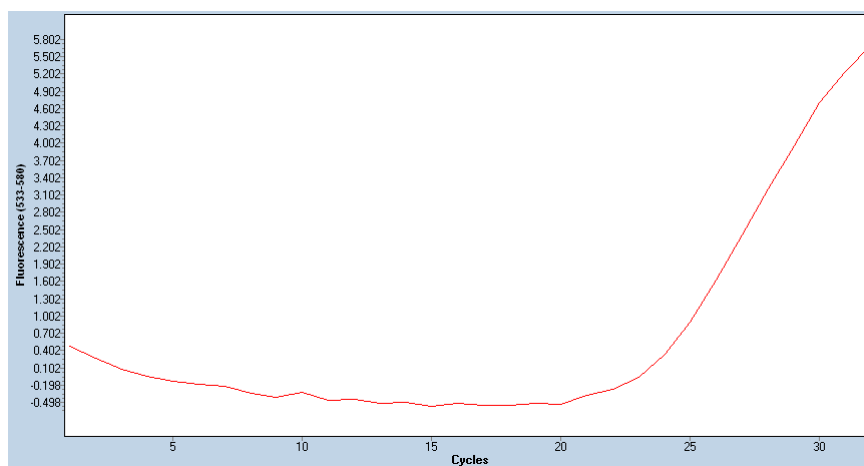
HFE GENE MUTATION TESTING

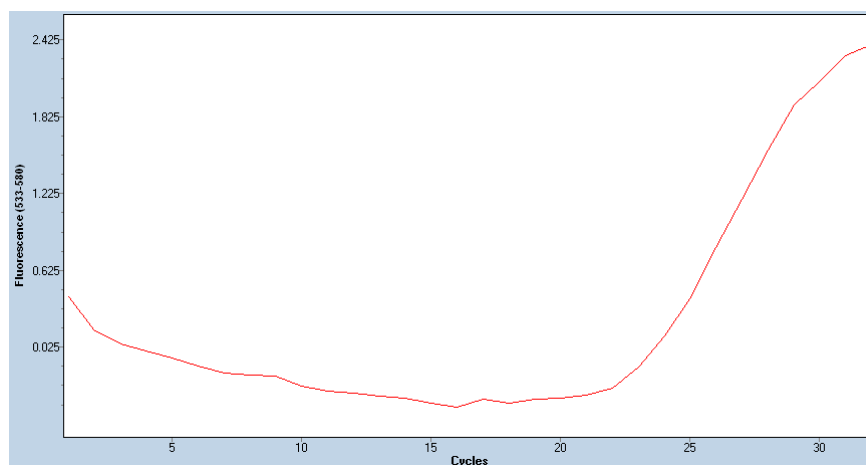
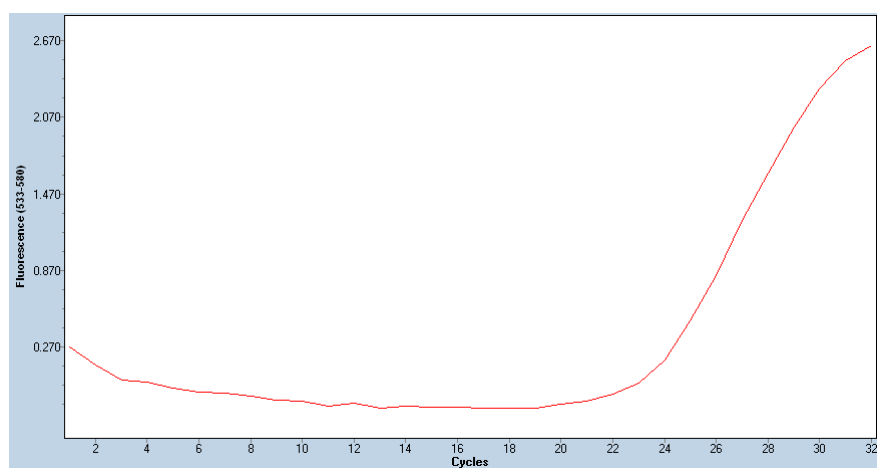
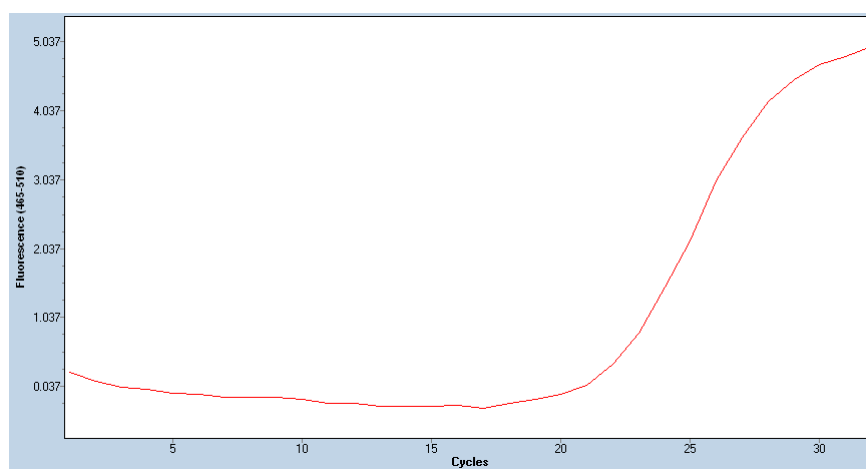
- Isolated DNA was tested for C282Y and H63D mutations with wild-type and mutant type master mixes.
- The analysis system has been specifically adapted for 5' nuclease real-time PCR mutation analyses.
- Each master-mix also contained an internal control targeting glucose-6-phosphate dehydrogenase.

H63D NORMAL INTERNAL CONTROL



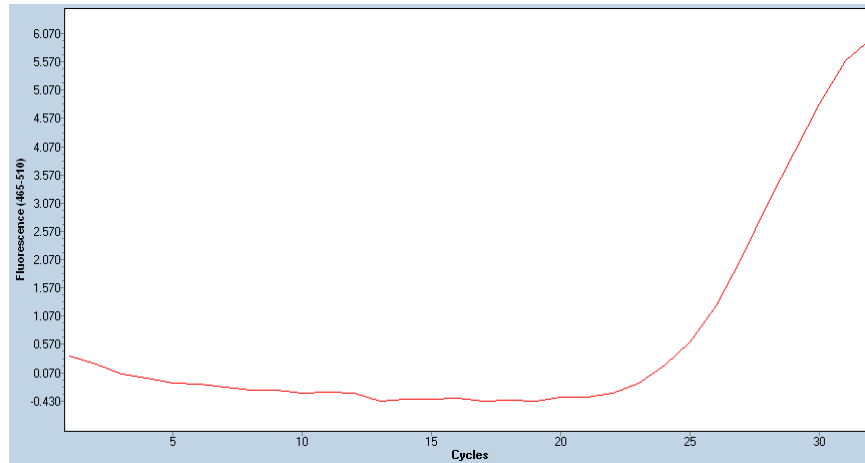
H63D MUTANT INTERNAL CONTROL



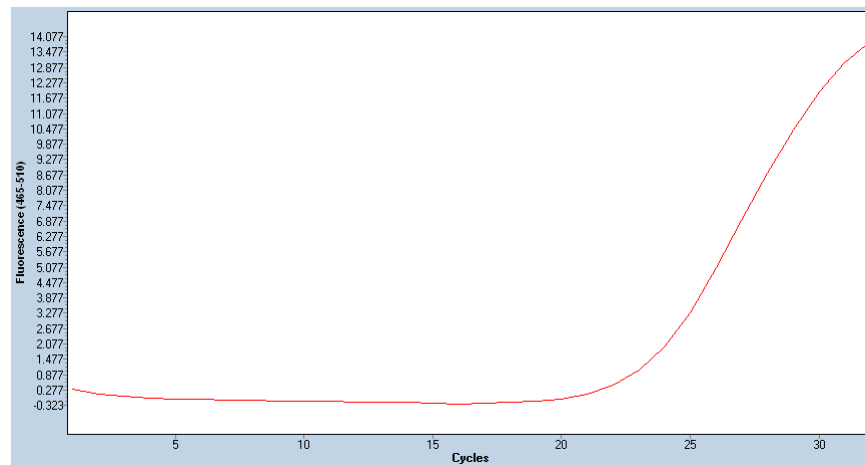
C282Y NORMAL INTERNAL CONTROL**C282Y MUTANT INTERNAL CONTROL****H63D NORMAL PCR RESULT**

Lecture Presentations

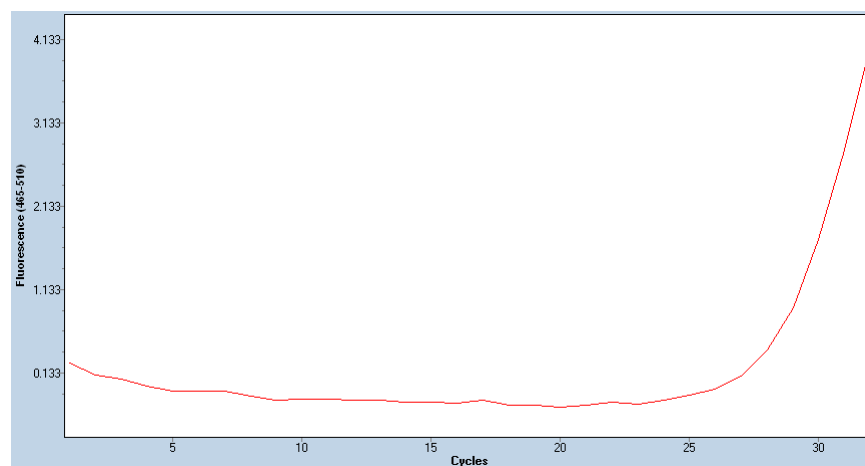
H63D MUTANT PCR RESULT



C282Y NORMAL PCR RESULT



C282Y MUTANT PCR RESULT



HFE C282Y/H63D compound heterozygote

Diagnosis: Primary hereditary hemochromatosis

SUMMARY AND CONCLUSION

- In this study, we reported a case of compound heterozygosity (C282Y/H63D) in the HFE gene.
- Screening of HFE mutations in all patients and first degree relatives that have increased serum ferritin and transferrin saturation will enable the diagnosis of HH.

ASSOCIATION OF CELIAC DISEASE WITH OTHER AUTOIMMUNE DISORDERS

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Background: It is well known that Type I Diabetes Mellitus (DM) is associated with Celiac disease (CD), Hashimoto thyroiditis (HT) and some other autoimmune disorders.

Intensive treatment of type I DM in children and young adults, with multiple daily injection of insulin, started in Kosovo, in the year 2007. All new patients with DM have been screened for other autoimmune disorders since then. The aim of the study was to investigate association and the prevalence of CD and HT in children and young adults with type I DM in Kosovo.

Aim of the study was to analyze association of celiac disease with other autoimmune disorders; children and young adults with type I diabetes, autoimmune thyroiditis and other endocrine diseases in Kosovo.

Methods: The study group was a clinical based cohort of children and adolescents with type I DM, who were being treated for childhood diabetes in Referent Centre, in Kosovo. Autoantibodies against the pancreatic islet, islet cell antibodies (ICA), antibodies against insulin, GADA, and IA-2 as markers of the autoimmune process were tested in all patients with type I diabetes. Screening for CD was performed by using serologic markers: antigliadin (AGA) IgG/IgA, tissue transglutaminase (tTG) IgG/IgA and Immunoglobulin IgA. Antibody positive subjects were selected for small bowel biopsy. The

screening for Hashimoto thyroiditis was performed by using serologic markers free thyroxine (FT4), thyroid stimulating hormone (TSH), anti-thyroperoxidase antibodies (anti-TPO) and anti-thyroglobulin antibodies (anti-Tg).

Results: There were 94 new cases with type I DM aged 0-18 years, 49 females and 46 males during a five year period. Biopsy confirmed that Celiac disease was present in 7 children with Type I DM, giving the prevalence rate of 7.1% (7/94). There were 5 females and 2 males out of the total number. Five children were younger than 5 years. Hashimoto thyroiditis was present in 5 subjects with type I DM, and Graves Disease in 1 subject, giving the prevalence of 6.3% (6/94). There were 3 males and 3 females out of the total number, and all of them were older than 9 years. 1 subject had CD in young age and two years later she develops type I DM and Hashimoto Thyroiditis.

Conclusions: The risk of having Celiac disease and type I diabetes is higher in children who are younger than five years, and particularly in female subjects. Autoimmune thyroiditis is present mostly in children who are older than 9 years and in young adults. The screening of children at onset of diabetes and children with CD helps in identifying other linked autoimmune disorders, and follow up is warranted.

THE EUROPEAN REGISTER OF LABORATORY MEDICINE SPECIALISTS EC4 www.ec4register.eu

In Extenso

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Due to their very complex and changing, old and recent history, the European countries make a divers area, with many differences in their political, social and cultural systems. In 1957, the Treaty of European Economic Community, proposed to create a common market of goods, workers and services within the EEC's member states. From 6 countries, who initially signed the Treaty in 1957, we have now, in 2013, 28 countries who are members of EU common market.

The EU Constitution guarantees all citizens equal rights within EU and the free movement of citizens and professionals in the EU countries. In order to create an equal European market for medical services, the health care professionals should be harmonize in what is their education and training, so as to give the same level of services to all the European citizens. Established medical specialties have names that everybody understands and knows. It describes who they are and what they do: e.g. gynecologist, radiologist, cardiologist but even there the education and training is not the same in all European countries. For the professionals who work in clinical laboratories, due to different individual historic, geographical, political and economic conditions, each nation diverged on its own option to supply well educated and proficient specialized professional to fulfill their citizen's needs. Consequently laboratory specialists have different backgrounds: MDs, Pharmacists, Scientists and the name of the profession is different in all the countries. Why is that? Because, historically, the profession didn't result from a medical super specialization, but from analytical chemistry experts who offered to the physicians some of their theoretical and

technical expertise in order to help them biologically define the diseases (that was why, initially, the name of the professional society was International Federation of Clinical Chemistry).

As a result each European country has its own system of training the laboratory specialists and different names for the professionals who work in clinical laboratories such as biologist, chemist, biochemist, microbiologist, medical doctor in clinical laboratory. Each specialist has a different level of academic studies and a different level of training, some are monovalent, some are polyvalent, others are subspecialized in one field, but in principle they all practice the same profession.

That is why the European Society of Laboratory Medicine (EFLM), whose mission is to represent the Laboratory Medicine at European level, has decided to work for the harmonization of the specialty in Europe. Together with UEMS (European Union of Medical specialists) during 2010–2011, they developed a study in 27 European countries in order to establish a clear description of the current situation of the laboratories organisation and the practices and responsibilities of laboratory professionals, using as method a questionnaire with questions related to the profession that was sent to representatives of both organizations.

The main questions were: number of labs, numbers of laboratory specialists, fields of interest, training standards, the recognition of the profession. The questionnaires were sent to 27 countries, with 497 million inhabitants and they got response from 24 countries (89%), that sum up to 490 million inhabitants (98.5% of the European population).

Results

- The total number of laboratory specialists is 36100 (100%), physicians: 14790 (41%), pharmacists: 11310 (31%), scientists: 10000 (28%).
- The number of laboratory specialists/million inhabitants is very much different from one country to another:

Ireland 5/million inhabitants, UK 10/million inhabitants, Netherlands 15/million inhabitants, Germany 25/million inhabitants, Denmark 25/million inhabitants, Sweden 25/million inhabitants, Poland 30/million inhabitants, Slovenia 35/million inhabitants, Austria 35/million inhabitants, Hungary 40/million inhabitants, Bulgaria 50/million inhabitants, Spain 50/million inhabitants, Finland 50/million inhabitants, Czech rep. 60/million inhabitants, Lithuania 75/million inhabitants, Slovakia 80/million inhabitants, Belgium 80/million inhabitants, Italy 100/million inhabitants, Romania 100/million inhabitants, Estonia 110/million inhabitants, Portugal 160/million inhabitants, France 180/million inhabitants, Cyprus 200/million inhabitants, Greece 350/million inhabitants.

- In most of the countries (13/24) 64%, have general/polyvalent laboratories (biochemistry + hematology + microbiology) so 88% of EU laboratory specialists work in "polyvalent" laboratories.
- The fields covered by laboratory medicine are Biochemistry 100% (24/24) Endocrinology: 96% (23/24), Hematology: 92% (22/24), Microbiology: 58% (14/24), Transfusion: 42% (10/24). The duration of the training for the laboratory specialists differs from one country to another. For the Scientists it is 2 years in 1/18 countries (6%) 3 years in 1/18 (6%), 4 years in 5/18 (28%), 5 years in 9/18 (50%), more than 5 years in 2/18

Conclusions

- The Laboratory Medicine is a postgraduate training based on a university education with an average period of 9 years (between 6–12 years).
- The academic education of Laboratory professionals is variable : between 3–6 years (with an average of 5 years)

(11%) and there is no organized training for the scientists who work in medical laboratories in Austria, Denmark, Estonia, Romania and training only in other countries for Cyprus.

- The duration of the specialist training for the Physicians is 4 years in 5/22 (23%), 5 years in 10/22 (45%), 6 years in 4/22 (18%), more than 6 years in 3/22 (14%). There are 2 countries with a particular situation: the Netherlands where there is no medical specialist training for the physicians since 2001 and Cyprus where there is no organized training in the country (only abroad). The recommended standards for the training of the specialists in Laboratory Medicine are also different: 16/23 countries (70%) use the EC4 European Syllabus for Post-graduate training in Clinical Chemistry and Laboratory Medicine: version 3 – 2005, and 4 countries: Hungary, Finland, Portugal and Slovakia use the Blue Book-UEMS. The restrictions for laboratory professionals other than MD's exists in many countries; for Scientists in 10/22 countries (45%) and for Pharmacists in 4/15 countries (27%).
- In some countries only MD's can be head of laboratory (Bulgaria, Portugal) in other countries the laboratory is reimbursed only if it is under the responsibility of an MD (Germany, Sweden) in Hungary in large laboratories the head has to be an MD. and in Greece only the MD's can own private laboratories

- The post graduate training is 4 years (between 0.5–6 years)
- Clinical Chemistry is monovalent in 9 countries and polyvalent in 22 countries.
- The training standard used in most of the countries is the EC4 Syllabus (70%)
- **Harmonisation of the profession is needed**

What will be the benefits of the harmonization?

- It will facilitate the movement of professionals from one country to another and will lead to automatic recognition or, at least, clear previous knowledge about the extra needs in education and training in order to work in another country.
- It will lead to faster and more transparent decisions regarding the complementary education
- It will allow trainee interchange between training centers from different countries
- It will improve the training programs – we will all learn from each other
- It will reunite all the laboratory professionals under the same voice and we will have more power in defending our professional interests

Lecture Presentations

What can we do for harmonizing the profession?

- Find a name for the profession
- Define a common standard for education and training
- Define the methods of trainee evaluation

Having all these in mind, the European Federation of Laboratory Medicine, representing the interests of the laboratory medicine professionals, has decided to create a Register of Specialists in Laboratory medicine that will be the core of this harmonization process and will define the required criteria for becoming a recognized European specialist in laboratory medicine. The Register EC4 (European Communities Confederation of Clinical Chemistry and Laboratory medicine) was opened in January 1999 for the professionals of the EU countries, members of the National Societies who are part of the EFLM and who met the agreed education and training requirements according to the EC4 Syllabus. The registration is entirely voluntary and the number of registrations have grown constantly, now, in 2013, comprising of about 3000 registrants from 22 EU countries. The professionals have to make the registration via the National Society or Register of laboratory medicine specialists, who have to demonstrate the equivalence of standards between their training programs and the EC4 Standards.

Why is the Register important? Primarily for patient safety, offering an equal European high standard of education and training for the laboratory professionals in order to harmonize the professional competences across Europe.

Why is the Register important for the profession? Because it is an important quality benchmark, offering a list of highly qualified professionals, that promotes the interests of laboratory professionals and the understanding of the profession as a medical specialty in front of other specialties, patients and EU political bodies.

Why is the Register important for the professionals? Because it speaks in one voice and in the interest of laboratory professionals, fights for a common name and the recognition of a high level of professional competence in order to make possible the free movement of laboratory professionals within EU countries.

The importance of finding a name for our profession has been discussed for years. The name should be clear, acceptable to all, including those who work in different sub specialties within the profession, should be short and easy to remember and will define the expertise and skills of laboratory professionals.

The discussions started in 2004 in Prague at the FESCC-UEMS meeting and since, at all national and European meetings, professionals were asked about the most suitable name for the profession. In October 2010 at the 1st EFLM-UEMS joint conference in Lisbon, the name that was proposed was **Specialist in Laboratory Medicine (Eusplm)** and was voted by 28 of the 39 professional national societies (72%). The number of academic and training years suitable for becoming Eusplm was also defined by taking the average number of years from all the EU countries -10 years, with 5 years academic studies and 5 years training (with at least 2 years polyvalent training and 3 years monovalent training).

How can you become member of the Register? If you are from an EU country and you are member of the National Society of Laboratory Medicine in your country or you are in the National Register of Laboratory Specialists, you can contact your EC4 National representative via www.ec4register.eu and you will get the necessary indications. The application is online and the website offers you more details about the Register: you can find the EC4 standards, the Code of Conduct that you have to agree with, a list of the members, by country and also up to date news concerning the profession.

After the National Representative validate your application, the application will be sent to the Board of EC4 Register and after they validate the application you will receive the certificate with the title of Eusplm and you can consider yourself part of the big family of highly qualified European laboratory specialists.

PATIENT SAFETY AND RATIONAL LABORATORY DIAGNOSTIC

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In the race to improve the health, laboratory diagnostic takes the central place. It is widely known that 60% of information that doctors use to set the diagnosis are the information obtained in laboratories. Lot of researches have the aim to improve the diagnostic system, that is, to find the possibility, for more complex analyses that give us more information about the patient's state.

It frequently occurs that big and unjustified demands, not for specific parameters but for "all and everything", are sent to laboratory and from results obtained the conclusions are made. In some, less clear situations, this way can help, but it can not become the diagnostic model. Many physicians

beginners beside the necessary often ask for numerous unnecessary parameters, probably led by the idea "not to miss something". However, physicians with more experience and knowledge require smaller number of parameters but they require specific parameters, in other words, those parameters that will give them useful information. The question is: Is the patient's health safe with a small number of parameters?

Health security may be obtained with relatively small number of parameters which are well selected. When you ask for parameters, there should be, clearly defined question and the parameters should give you the answer.

GLYCATED HAEMOGLOBIN: NEW ASPECTS AND COMPARISON OF METHODS

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Introduction: HbA_{1c} is the gold standard of diabetic survey. It is a longer-term average of estimating blood glucose levels than the current reading of the classic glucose measurements. HbA_{1c} can be measured by various methods, including high-pressure-liquid-chromatography, capillary electrophoresis and immunoassays. In this study, we analyze human whole blood samples using four different methodology assays, in an effort to illustrate the correlation between their results.

Materials and methods: One hundred fresh human whole blood samples were measured in four analyzers that employ different methodologies: BIORAD Variant II HPLC, SEBIA Capillarys 2 electrophoresis and two immunoassays, ABBOTT Architect i1000 and RANDOX RX series method applied on BECKMAN COULTER AU2700. The results were compared both in pairs and in correlation with

HPLC, which was chosen as an internal reference method.

Results: The RANDOX method gave the highest values, as expected from its performance in the EQA schemes. The correlation to the BIORAD HPLC method was acceptable ($R^2=0.903$). The results of the BIORAD HPLC and the SEBIA Capillarys 2 electrophoresis methods were similar, with remarkable correlation ($R^2=0.969$). The lower values were taken by the ABBOTT method with good correlation to the HPLC method ($R^2=0.920$). Poor correlation finally was obtained between the RANDOX and ABBOTT methods ($R^2=0.831$).

Conclusions: The four methods tested in this study, were proved reliable to be employed by a routine biochemical laboratory. The best concordance was found between BIORAD Variant II HPLC and the SEBIA Capillarys 2 electrophoresis methods. In addi-

tion, the fact that they measure all the protein fractions of the haemoglobin molecule helps to a better

appraisal of the results.

NEONATAL THYROTROPIN SCREENING AS AN INDICATOR OF IODINE DEFICIENCY IN POPULATION

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Introduction: Iodine deficiency is one of major public health concerns throughout the world. Iodine deficiency is becoming more common even in some developed countries, previously characterized with good supplementation, especially in female population in child-bearing age and among pregnant women. In conditions of iodine deficiency, the frequency distribution of neonatal thyroid-stimulating hormone (TSH) is shifted towards elevated values. Elevated serum TSH in the neonate indicates insufficient supply of thyroid hormones to the developing brain, and therefore constitutes the only indicator that allows prediction of brain damage, which is the main complication of iodine deficiency. World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF) and the International Council for Control of Iodine Deficiency Disorders (ICCIDD) have suggested that when a sensitive assay is used on samples collected 3–4 days after birth, a <3% frequency of TSH concentrations in neonatal period >5 mIU/L indicates iodine sufficiency in a population.

The aims of the study were to estimate the incidence of neonatal hypothyroidism and assessment of iodine deficiency in Montenegro by determination whether TSH concentrations in a large sample of neonates meet WHO, UNICEF and ICCIDD criteria for an iodine-replete population.

Material and methods: Retrospective analysis of the results of 24515 neonatal heel-prick blood tests for TSH obtained as part of a routine newborn screening program was conducted. Participants were anonymous samples of neonates born in 13 Montenegrin birthplaces in three consecutive years. The measurement of blood TSH was per-

formed by DELFIA method. TSH value equal to 10 mIU/L was considered cut off point for recall for testing. SPSS statistical software was used for descriptive statistic, Pearson's correlation coefficient (r) to measure the association of TSH distributions and χ^2 tests were used to test for differences between proportions.

Results: In newborn screening program for congenital hypothyroidism in Montenegro the recall rate and incidence of hypothyroidism were 1.26% and 1:2397 neonates respectively. Specificity of the program was 98% and apparent sensitivity was 100%. The mean percentage of neonatal TSH concentrations >5 mIU/L was 7.31%. Based on the proposal made by WHO, the results of our study showed a mild iodine deficiency in all but one, regions of Montenegro (prevalence range for mild thyroid deficiency, 3%–19%). Percentage of TSH values >5 mIU/L was highest (23,9%) in region of Ulcinj and indicated moderate iodine deficiency in that part of the country. Correlation coefficient between percentage of TSH values >5 mIU/L and ≥ 10 mIU/L was 0.89 ($p < 0.001$).

Conclusions: Our results suggest that population of Montenegro (e.g. pregnant women) may have mild to moderate iodine deficiency. Similar results were obtained in project organized by Ministry of health in 2007. where urinary iodine concentrations in pregnant population were not adequate. Since Montenegro is in a group of countries exposed to goitrogenic factors, the need for continued vigilance and the importance of carrying out periodic iodine deficiency monitoring studies is emphasized. Comprehensive policy should be developed for control of iodine deficiency.

SEPSIS BIOMARKERS

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Biomarkers are objectively measured and evaluated as indicators of normal biological processes, pathogenic or pharmacologic responses to a therapeutic intervention. Since sepsis is a potentially deadly medical condition characterized by a whole-body inflammatory state (called a systemic inflammatory response syndrome or SIRS) caused by severe infection, early diagnosis of sepsis is vital for adequate therapeutic treatment, prediction of sepsis progression, clinical course and outcome of sepsis patients (reduction of mortality and morbidity). Therefore, it is of great importance to define reliable sepsis biomarker. The ideal biomarker should have high sensitivity, specificity, reproducibility and good correlation with progression of specific medical state. Having in mind the complex pathogenesis of sepsis which includes a great number of proinflammatory molecules, oxidative stress, perfusion disturbances, mitochondrial dysfunction that lead to an organ dysfunction, there is a possibility to use those different molecules as a potential biomarkers of sepsis.

Today, the mostly used sepsis biomarkers are C reactive protein (CRP), procalcitonin, cytokines (IL-6, IL-8, IL-10) as well as some new biomarkers (biomarkers of endothelial dysfunction, coenzyme Q-10, proadrenomedulline, soluble triggering receptor expressed on myeloid cells-1 (sTREM-1). Thanks to its characteristics (early and rapid increase of concentration at the very beginning of infection, good correlation with sepsis progression and adequate antibiotic treatment) procalcitonin is mainly used biomarker of sepsis in biochemical laboratories.

Sepsis biomarkers can help in early sepsis diagnosis which is crucial step for better sepsis outcome, in assessment of the response to therapy, as well as in prediction of sepsis progression and identification of patients who may benefit from specific therapies. Many studies have shown that simultaneously measurement and analysis of more than one sepsis biomarkers is always better than measurement of single biomarker, giving the better sensitivity and specificity necessary for early diagnosis of sepsis.

CARDINAL PLACE OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE AND GLUTATHIONE REDUCTASE

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All life processes are dependent on biological catalyses, enzymes. The study of enzyme and enzymatic reactions begins with ancient human. The first written information and documentation on enzymatic products is the Codex of Hammurabi. Enzymes have key roles in every area of medicine as well as in our daily life.

Glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49) and glutathione reductase (GR, E.C. 1.6.4.2) enzymes have key roles in glutathione metabolism, redox state, detoxification of reactive oxygen species and xenobiotics, anabolic processes, cell survival/death, cellular homeostasis, protection of cellular molecules from environmental and cytotoxic agents, and detoxification of the cell. G6PD is the rate-limiting and first enzyme of the pentose phosphate pathway. This anabolic pathway is responsible for the reduction of NADP^+ , synthesis of ribose-5-phosphate and sugar phosphates. G6PD

deficiency is the commonest enzymopathy which is an X-linked hereditary disease resulting in nonimmune hemolytic anemia; jaundice; neonatal jaundice; drug or infection-mediated hemolytic crisis; favism; and less commonly chronic non-spherocytic hemolytic anemia, suicidal death of erythrocytes. G6PD have significant effects on erythrocyte function and survival in human. GR is a flavoprotein that catalyzes NADPH-dependent reduction of oxidized glutathione to reduced glutathione. These two enzymes act together. G6PD reduces NADP^+ to NADPH and GR enzyme oxidizes NADPH to NADP^+ .

The aim of my studies is to find out the effects of various drugs or the novel molecules on these enzymes. For this reason we have been purifying these enzymes from different tissues and doing kinetic characterization and molecular docking studies. This talk is a summary statement for these studies over the last ten years on G6PD and GR.

SEX HORMONE BINDING GLOBULIN (SHBG) AND POLYCYSTIC OVARIAN SYNDROME (PCOS)

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The polycystic ovary syndrome (PCOS) is a condition that affects 1–5% of women and is characterized by oligoovulation or anovulation (manifesting as oligomenorrhea or amenorrhea), elevated serum androgen levels or clinical manifestations of androgen excess (hirsutism, acne, or androgenic alopecia), and polycystic ovaries as defined by ultrasound examination. Patients also commonly show features of the metabolic syndrome and insulin resistance.

Nevertheless, hyperandrogenism is one of the most consistently expressed PCOS traits.

The pathogenesis of the syndrome is largely unknown, but there is increasing evidence for a strong genetic basis. Recent evidence suggests that the natural history of PCOS may originate in very early development, even in intrauterine life. Indeed, experimental animal research and clinical observations have led to the developmental origin hypothesis of PCOS. According to this hypothesis, fetal ex-

posure to androgen excess can induce changes in differentiating tissues leading to PCOS phenotype in adult.

Sex hormone binding globulin (SHBG) is a glycosylated homodimeric plasma transport protein which in humans is encoded by a single gene, *shbg*, on chromosome 17. SHBG is synthesized mainly in the liver and binds the androgens testosterone and dihydrotestosterone (DHT), with higher affinity than estradiol. The primary function of SHBG was

thought to be the regulation of free sex hormone bioavailability to target tissues. According to the "free hormone hypothesis" only steroids that are not bound by plasma proteins can passively diffuse into the target cells. Low levels of SHBG or deficient forms of SHBG may be responsible for the exposure of the female fetus to androgen excess during intrauterine life that programs the various organ systems in such a way, that they manifest the characteristics of the PCOS phenotype in adult life.

BETA-TRACE PROTEIN AS BIOMARKER OF RENAL DYSFUNCTION IN PATIENTS WITH CHRONIC KIDNEY DISEASE: COMPARISON WITH OTHER RENAL BIOMARKERS

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Beta-trace protein (BTP), also known as prostaglandin D synthase, is a low-molecular-mass protein which belongs to the lipocalin protein family. It was found to be increased in the serum and urine of patients with renal diseases. Because of its low molecular mass, constant production rate and stability, BTP has been proposed as a new endogenous biomarker of glomerular filtration rate. The aim of this study was to compare the clinical usefulness of the serum levels of beta-trace protein for detection of renal dysfunction in patients with chronic kidney disease (CKD) with levels of other renal biomarkers: creatinine, urea, cystatin C and β_2 -microglobulin (B2M) in serum and with levels of total proteins, albumin, cystatin C, α_1 -microglobulin (A1M) and B2M in urine.

The study included 134 patients with a wide range of renal function that encompassed all five CKD stages. Obtained data showed that beta-trace protein highly correlated (Spearman test) with creatinine ($r=0.890$), cystatin C ($r=0.904$) and B2M

($r=0.933$) and its levels significantly increased from CKD stage 1 to 5 in serum. ROC analyses showed that BTP had similar diagnostic accuracy for detection of reduced renal function in CKD stages as other renal biomarkers, for estimated GFRs of <30 , <60 and <90 mL/min/1.73 m². The areas under the ROC curves (AUC) for BTP, for these GFR limits, were from 0.983 to 0.917. In 24-hour urine samples from patients with CKD, BTP was also in significant correlation with all the measured specific proteins; correlation coefficients were from 0.448 to 0.871. According to the results of ROC analysis, BTP showed similar diagnostic value as cystatin C, A1M and B2M for eGFR of <30 and <60 mL/min/1.73 m².

The results of our study suggest that BTP may be a useful and reliable serum and urinary biomarker of renal dysfunction and may have place besides cystatin C and creatinine in serum and A1M and albumin in urine, as alternative biomarker for reduction of GFR, tubular damage and the progression of disease in patients with chronic kidney disease.

ANTIOXIDATIVE ENZYMES AND ALA-9VAL POLYMORPHISM OF MN-SOD GENE IN RENAL TRANSPLANT PATIENTS

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Introduction: Enhanced oxidative stress has been implicated in the development of complications after organ transplantations; especially ischemia/reperfusion injury and delayed graft function as well as acute and chronic graft rejection. Anti-oxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR), as well as glutathione (GSH) protect against the harmful effects of free radicals. The activity of SOD is genetically determined and correlates with the presence of polymorphisms in genes encoding these enzymes. The most common immunosuppressive drugs used for preventing the rejection of transplanted organs are calcineurin inhibitors (CNI) such as tacrolimus (Tac) and cyclosporine (CsA). The aim of the study was to determine activity of antioxidative enzymes and polymorphisms of MnSOD in patients with kidney transplantation (KT).

Material and methods: We measured SOD, GPx and GR activity as well as concentration of GSH in erythrocytes and whole blood. DNA samples from 55 kidney transplant patients and 36 age-matched controls were genotyped for MnSOD. Genotyping

was performed by PCR RFLP analysis using genomic DNA extracted from whole blood. We studied Ala(-9)Val polymorphisms of the Mn-SOD gene.

Results: Concentration of GSH and SOD activity were significantly increased after KT compared to the control group ($p < 0.001$ for both), GPX activity was decreased, whereas GR activity not significantly changed. Our results show that no differences in antioxidative parameters between patients treated with Tac or CsA. There was a significantly negative correlation between SOD and GPX ($r_s = -0.400$, $p < 0.001$) and SOD and creatinine clirens ($r_s = -0.336$, $p = 0.013$) and a positive correlation between GR and GPX ($r = 0.340$, $p = 0.001$). There were no difference in a distribution of SOD alleles between KT group and control group.

Conclusion: Our findings suggest that renal transplant recipients display persistent oxidative stress and changed antioxidative systems. No significant differences in antioxidative parameters were found with respect to treatment. Also, we did not find significant correlation between MnSOD genotypes and SOD activity.

MEAL-GENERATED TOTAL ANTIOXIDANT CAPACITY DECREASE IN OBES AND NON-OBES INDIVIDUALS

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TAC measurement is a global measure of the antioxidant capacity of plasma. In obese patients the signs and symptoms in many diseases are appeared

more aggressive than normal individuals. Postprandial changes acutely alter some mechanisms in body. The aim of present study was to evaluate the

effects of a standard meal that may cause acute elevations of serum glucose and lipid levels on serum total antioxidant capacity (TAC) in obese and nonobese individuals.

In this study, a standardized meal was administered to 16 (8 male, 8 female) obese [Body mass index (BMI)= $28.7 \pm 1.77 \text{ kg/m}^2$] and 14 age and sex matched non-obese [BMI= $22.2 \pm 1.57 \text{ kg/m}^2$] healthy individuals. In all subjects, blood samples were collected at baseline and after the meal (3rd and 6th hours). Standardized diet was included total energy: 700 kcal, 59% lipid, 27% protein, 14% carbohydrate. It was given after a 12–14 h fast. For plasma TAC level determinations, scavenging of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) free radicals was measured by a spectrophotometric method as previously described. SPSS (Version 11.0) for Windows XP program was used for statistical analysis.

In both of groups, there were significantly decrease versus baseline levels in TAC determinations for 3rd hour. The TAC levels in 3rd hour in obese individuals was significantly lower than baseline level ($p=0.013$) and there was a significant increase in 6th hour versus 3rd hour ($p=0.019$). 3rd hour level of TAC in obese individuals was significantly lower than non-obese individuals ($p=0.002$). TAC levels of non-obese group were $28.06 \pm 11.8\%$ (baseline level), $22.5 \pm 7.2\%$ (3rd hour), $25.6 \pm 8.6\%$ (6th hour), while these levels were $27 \pm 14.4\%$, $15.2 \pm 4.1\%$ and $23.8 \pm 9.5\%$ in obese

subjects respectively. In non-obese group, the differences between 0th and 3rd hour significant ($p=0.05$). In obese group the differences between 0th and 3rd hour and 3rd and 6th hour were also significant ($p=0.013$ and $p=0.019$) respectively). Between two groups, there were no differences for all time intervals measurements in parameters such as HDL, LDL, uric acid, urea, creatinine, total protein, albumin, total bilirubine, AST, ALT, CK, amylase, Ca, P, Fe. However cholesterol, VLDL, triglyceride, ALP, LDH, GGT levels were significantly different.

The present study shows that in obese individuals, serum TAC was lower than non-obese subjects. This study also confirms that postprandial triglyceridemia and glycemia spikes may lead to decline of TAC in 3rd hour. This finding supports the hypothesis that postprandial changes acutely induce an oxidative stress and impair the natural antioxidant defense mechanism found in the plasma in obese and non-obese individuals. Having a meal should be able to cause an oxidative stress and consumption of antioxidant capacity postprandial oxidative damage was evidently occurred in obese individuals than non-obese individuals. Moreover, these findings show that postprandial serum TAC levels can increase to baseline level in over than 6 hours. Finally, postprandial changes must be controlled to avoid various diseases and complications particularly in obese subjects.

NITRIC OXIDE AND SCHIZOPHRENIA

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Nitric oxide (NO) was initially described as a mediator of endothelial relaxation, and now its participation is recognized in numerous physiological and pathological processes. In the central nervous system NO has been identified as a widespread and multifunctional biological messenger molecule with possible roles in neurotransmission, neurosecretion, synaptic plasticity, and tissue injury in many neurological and psychiatric disorders. Some scientific evidence has pointed to a possible role for the NO in schizophrenia. NO act as the second messenger of the N-methyl-D-aspartate (NMDA) receptors and their hypofunction is involved in the major current hypothesis for schizophrenia. The importance

of NO emphasizes the fact that excitotoxicity, oxidative stress and apoptosis are potential mechanisms proposed to underlie neuroprogressive changes in schizophrenia. Studies that have examined peripheral concentration of the NO supporting apparently contradictory results: some of them suggesting an increase in NO plasma levels and another part supporting a decrease. Similarly conflicting findings have been reported for central nervous system measures: lower NO metabolite concentrations in the cerebrospinal fluid of patients with schizophrenia, but elevated concentrations in post-mortem tissue. Our results showed that schizophrenics had significantly higher plasma NO concen-

trations in comparison with healthy subjects, suggesting the existence of nitrosative stress in schizophrenia. A significant positive correlation between NO metabolite concentration and lymphocyte caspase-3 activity indicates that NO may be a potential inductor of apoptosis in patients with

schizophrenia. By testing a single nucleotide polymorphism of the human NOS1 gene, we found a significant association between this polymorphism and schizophrenia suggesting its contribution to schizophrenia pathophysiology.

DEPURINIZED MILK-EPIGENETIC EFFECT ON REGENERATIVE AND ANTI-APOPTOTIC POTENTIAL

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A number of dietary agents may contain potentially toxic compounds, which can alter human genomic and proteomic fashion, and may influence on gene expression, protein conformation and enzyme activity. They can alter tissue regenerative potential, leading to development, progression, or severity of chronic diseases. Hyperuricaemia is a biochemical basis of gout, renal urate lithiasis and inherited purine disorders, while secondary hyperuricemia, may be a result of enormous ATP breakdown or purine release, present in cardiovascular diseases, hypertension, kidney diseases, eclampsia, obesity, metabolic syndrome, psoriasis, tumor lysis syndrome or intense physical training. The possible beneficial role of dairy products in hyperuricemia dietary regimen was documented. Our experimental study was conducted to explore the effect of milk dietary regimen (commercial 1.5% fat UHT milk and patented depurinated milk-DP), in comparison to allopurinol therapy, on experimental hyperuricemia induced by oxonic acid in rats. We hypothesized that the diet regimen, as epigenetic factor, may modulate liver growth-regulatory kinases, oxidant-sensitive transcriptional factor NF- κ B and endonuc-

lease activity, what may reflect liver regenerative potential. Mean plasma UA level of rats given oxonate was more than two fold increased, while allopurinol confirmed significant hypouricemic effect. Depurinated milk (DP milk) slightly potentiated hyperuricemia, while commercial-untreated milk significantly potentiated hyperuricemia, given in proposed experimental regimen for 10 days. Among 11 parameters measured (plasma uric acid-UA and liver: NF κ B-p65, Akt kinase/phospho-Akt kinase, ERK kinase/phospho-ERK kinase, IRAK kinase/phospho IRAK kinase, p38/phospho-p38 and DNase), the Akt/phospho Akt and ERK/phospho-ERK signaling and NF κ B-p65 were the most significant variables. Of 35 toxic compounds observed in commercial milk (different sulphur compounds, aldehydes, ketones, carboxylic acid and phthalates), 13 were not detectable in depurinated milk with significant fall for all remained. In conclusion, DP milk diet would be recommended as nutritional requirement in primary or secondary hyperuricemia, accelerating liver regeneration and downregulating apoptosis.

EFFECTS OF TYPE 2 DIABETES AND GLUCOSE CONTROL ON PLASMA LEVELS OF FREE FATTY ACIDS

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Diabetes mellitus is associated with defects in proteins, carbohydrates, and lipid metabolism. Free fatty acids (FFA) represent an important link between obesity, insulin resistance, and type 2 diabetes (T₂D). Elevated plasma concentration of FFA (especially saturated FFA) are associated with impaired insulin sensitivity and glucose intolerance.

This study involved 73 patients with T₂D and 42 healthy subjects. Preparation of samples for FFA analysis was done by using a modified protocol of Lepage and Roy. Detection and quantification of FFA concentrations was done by gas chromatography. Other biochemical analyses, including glucose, glycated hemoglobin (HbA_{1c}), cholesterol, and triglycerides levels, were performed by employing standard IFCC methods.

FFA that were present in the highest concentration among a total of 19 FFA species analyzed in plasma

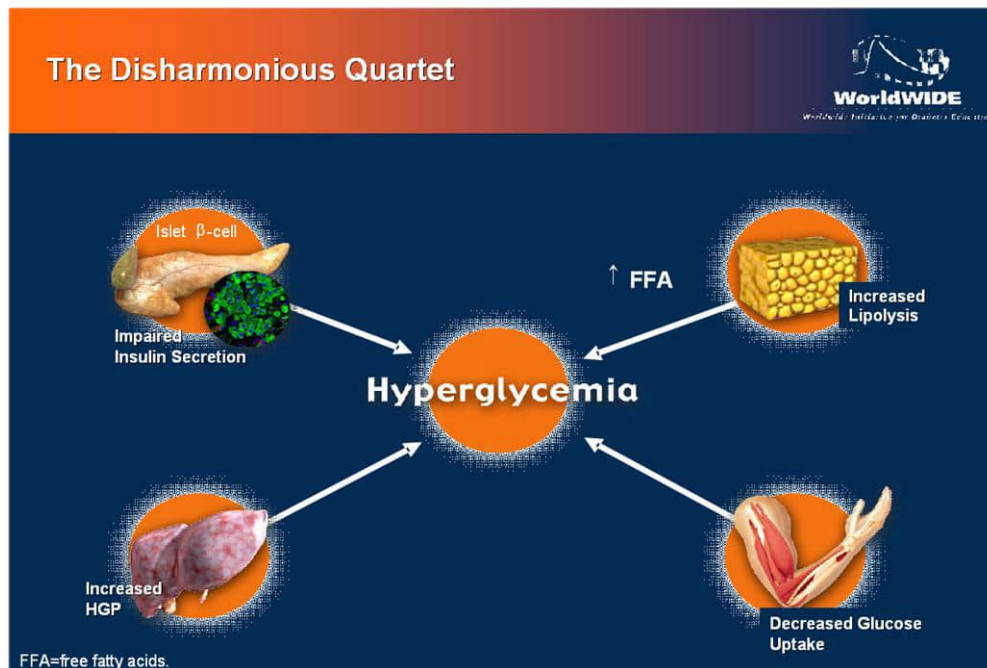
of both, diabetic and control subjects, were C16:0, C18:0, C18:1, and C18:2. Interestingly, a significant difference between T₂D and control subjects was demonstrated only for C16:0. In addition, our data also showed a significant difference in C16:0 levels between patients with poorly and well-controlled diabetes. There was a significant correlation of C16:0 with HbA_{1c} levels ($p < 0.001$) in patients with an adequate and poor T₂D control. A significant correlation of HbA_{1c} levels was also demonstrated with plasma concentration of C18:1 ($p < 0.05$), but only in patients with an inadequate diabetes control.

Thus, our data suggested that palmitic fatty acid (C16:0) could serve as a potential biomarker in development and optimal T₂D management, indicating that an individual FFA plasma profile could be crucial in predicting disease risk.

Presentation:

INTRODUCTION

- *Diabetes mellitus* is metabolic disease characterized by defective production or action of insulin and high glucose and fat levels in the blood.
- Defects in carbohydrates metabolism followed by changes in proteins and lipid metabolism.
- Free fatty acids (FFAs) play a key role in the cell membrane and insulin sensitivity.
- Elevated plasma concentration of FFAs (especially saturated fatty acids) are associated with impaired insulin sensitivity and glucose tolerance.



Pathogenesis of Type 2 diabetes mellitus

MATERIALS AND METHODS

- Participants
- This study involved 73 patients with diagnosed T2D and 42 healthy subjects.
- Fatty acids analysis
- Preparation of samples for fatty acid analysis was done by methanolysis of samples.
- Qualitative and quantitative analysis
- Performed by GC/MS, on high polar capillary column and heptadecanoic acid (C17:0) as internal standard.
- Biochemical and clinical analysis
- Biochemical and clinical parameters determined on BT PLUS 2000 analyzer using standard IFCC protocols.
- Statistical analysis

Calculations were done using SPSS 17.0 for Windows. Statistical significance was set as $p < 0.05$.

RESULTS

Biochemical and clinical parameters of study participants.

Parameters	Type 2 diabetic	Control	P-values
Participants (numbers)	73	42	-
Age (years)	61	57	<0.0001
Fasting plasma glucose (mmol/L)	9.5±0.4	5.2±0.1	<0.0001
Fasting serum TGs (mmol/L)	2.6±0.2	2.2±0.2	0.542
Total cholesterol (mmol/L)	5.5±0.1	5.7±0.2	0.742
Fasting serum HDL-cholesterol (mmol/L)	1.1±0.1	1.7±0.1	0.580
Fasting serum LDL-cholesterol (mmol/L)	3.0±0.1	3.1±0.2	0.495
HbA _{1c} (% [mmol/L])	6.7±0.1 (48±2)	4.5±0.1 (30±2)	<0.0001

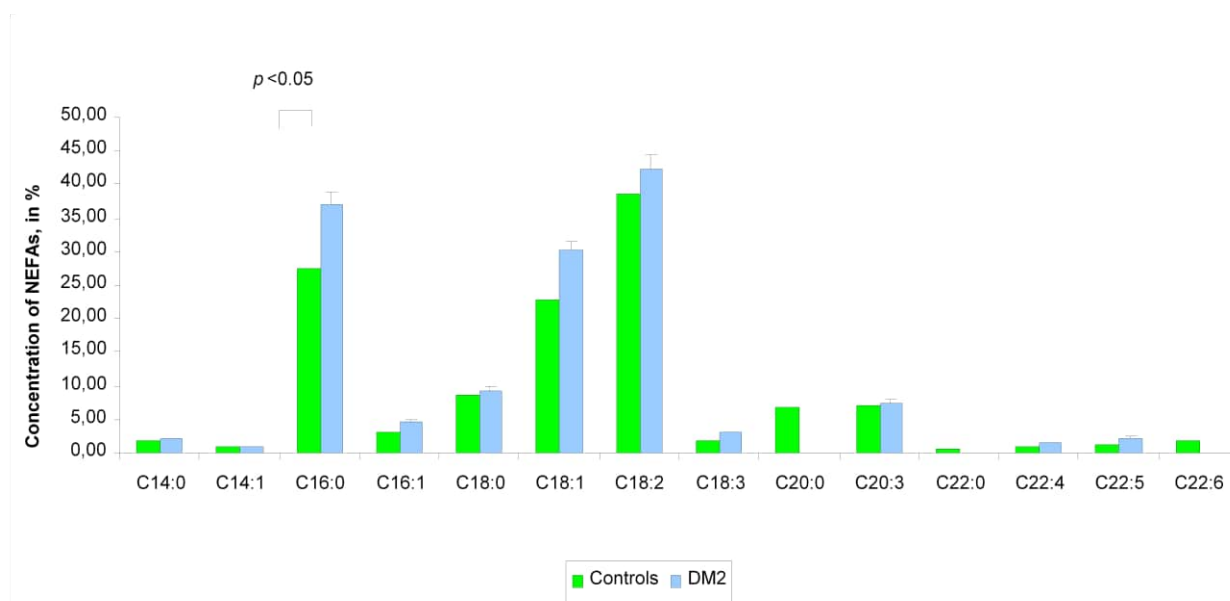
Values as mean±S.E.M.

Qualitative and quantitative analysis of FFA profile in healthy controls and T2DM patients

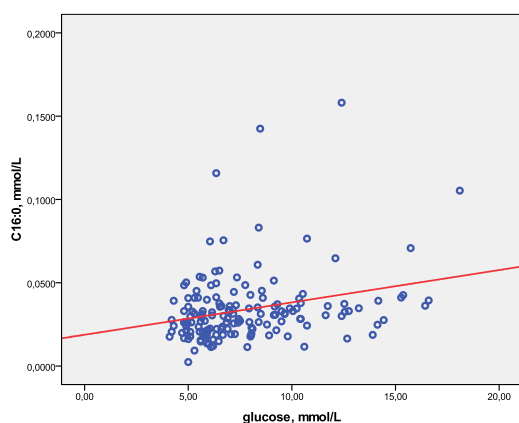
Fatty acid	Type 2 diabetic (μmol/L)	Control (μmol/L)	P-values
C14:0	9.2±1.0	7.2±0.7	<0.0001
C14:1 n-9	4.3±0.3	4.5±0.2	<0.0001
C16:0	139.1±11.0	104.0±7.4	<0.0001
C16:1 n-7	18.5±2.2	11.0±1.1	<0.0001
C18:0	31.8±2.0	29.6±2.9	<0.0001
C18:1 n-9	77.5±5.1	103.5±9.	<0.0001
C18:2 n-6	146.9±9.2	135.8±8.3	<0.0001
C18:3 n-6	7.5±0.8	6.5±0,0	0.037
C20:0	-	21.2±0,0	-
C20:3 n-3	23.9±1.3	22.6±1.4	<0.0001
C22:0	-	2.2±0,0	-
C22:4 n-6	4.4±0,0	3.0±0.1	0.001
C22:5 n-3	6.7±0.6	4.1±0.3	0.013
C22:6 n-3	-	4.2±0.3	0.005

Values as mean ±S.E.M.

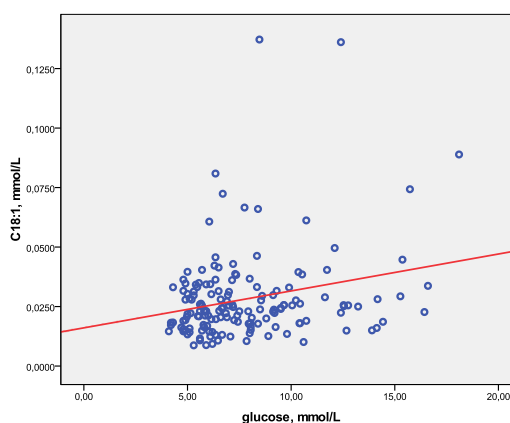
Oral Presentations



Concentrations of FFAs in study participants.

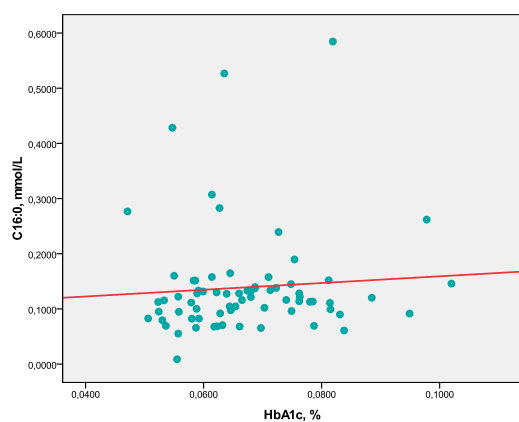


$\rho = ,274$ $p < 0,001$

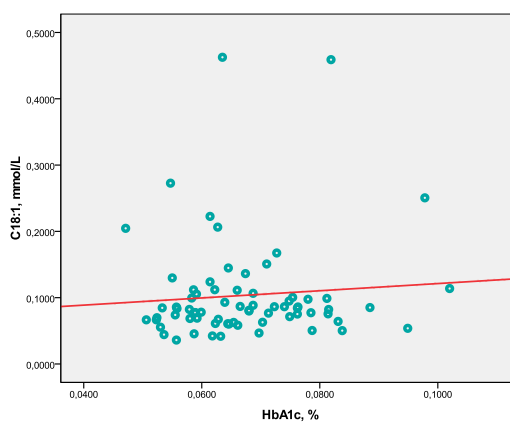


$\rho = ,184$ $p < 0,05$

Correlation between palmitic (A) and oleic acid (B), and glucose levels of study participants group.



$\rho = ,704$ $p < 0,001$



$\rho = ,230$ $p < 0,05$

Correlation between palmitic (A) in good control of disease, and oleic acid (B) with HbA1c levels in poorly control of disease.

CONCLUSIONS

- FFA that were present in the highest concentration among a total of 19 FFA were C16:0, C18:0, C18:1, and C18:2.
- A significant difference between T2DM and control subjects was demonstrated only for C16:0.
- Our data showed a significant difference in C16:0 levels between patients with poorly and well-controlled diabetes. There was a significant correlation of C16:0 with HbA1c levels ($p < 0.001$) in patients with an adequate and poor T2D control.
- A significant correlation of HbA1c levels was demonstrated with plasma concentration of C18:1 ($p < 0.05$), but only in patients with an inadequate diabetes control.
- Thus, our data suggested that palmitic fatty acid (C16:0) could serve as a potential biomarker in development and optimal T2D management, indicating that an individual FFAs plasma profile could be crucial in predicting disease risk.

ACKNOWLEDGEMENTS

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DIAGNOSTIC VALUE OF SERUM PROCALCITONIN LEVELS IN PATIENTS WITH SEPSIS. A COMPARISON WITH C-REACTIVE PROTEIN.

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Introduction: The term sepsis is used to define the systemic inflammatory response to a bacterial infection. Despite the use of new treatment and improvements in technology and increased experience, mortality rates in sepsis remain high. Early diagnosis and treatment with appropriate antimicrobial therapy may be helpful in reducing the morbidity and mortality associated with sepsis. The present study was conducted to determine the procalcitonin (PCT) level in patients with systemic inflammatory response syndrome (SIRS) and sepsis, in comparison with C-reactive protein (CRP).

Methods: 174 patients with signs of systemic inflammatory response syndrome from the emergency department of our hospital were enrolled in the study, in a period from April 2010 to April 2012. The patients were divided into 2 groups according to non suspected sepsis and suspected sepsis clinically,

in accordance with The American College of Chest Physician's Society of Critical Care Medicine consensus criteria. Blood samples were taken on the first day of hospitalization.

Results: In patients of septic group (103 patients) PCT was significantly higher with a mean of $10.22 \text{ ng/mL} \pm 18.74$ versus a mean of 0.26 ± 0.188 in SIRS group (71 patients). The mean CRP level in septic group was $148.99 \text{ mg/L} \pm 100.1$ versus 42.2 ± 45.3 in SIRS group. There is a positive correlation between PCT and CRP in 2 groups, but this relation becomes highly significant in sepsis group ($p < 0.001$).

Conclusion: PCT and CRP are helpful markers in differentiating sepsis from SIRS with a higher significance $p < 0.001$ for PCT, as a predictive marker in septic patients.

ADAPTIVE – TYPE COMPLEX SANGUINE REACTIONS OCCURING IN AERONAUTICAL PERSONNEL

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Objective: Analysis of the data obtained from the study which we initiated in INMAS in order to establish specific adaptive blood changes in the aeronautical personnel (AP).

Material and method: We microscopically examined 3378 blood smears (BS) from the AP came for the periodic medical examination in 2010 - 2012. We expanded the research in subjects with morphological blood changes to search for other associated biological reactions. We also studied the oxidative stress in some of the subjects (malondialdehyde, ceruloplasmin, albumin thiol groups, total antioxidant).

Results: Various reactive changes were observed in over 60% of the cases. We present the laboratory results focused on hematological morphology and their association with biochemical and enzymatic reactions involved in oxidative stress:

1. lymphocyte changes like: reactive lymphocytes, limfoplasmocytes, large granular lymphocytes

2. moderate basophilia+/eosinophilia+/monocytosis

3. changes in neutrophils including nuclear apoptosis cells and/or the presence of nuclear appendix

5. morphological changes of erythrocytes and of platelets

6. biochemical changes regarding the lipid profile, the urinalysis, the ESR, etc.

Regarding the oxidative stress, preliminary results show an increase in flying personnel and investigations will be pursued both by increasing the number of subjects and by dynamic determinations, in order to establish the mechanisms that cause oxidative stress.

Conclusions: Our study shows reactive-adaptive complex blood changes that occurred in the AP. The large number of subjects and the studied elements allow to appreciate that our work has a unique character in the aerospace medicine field.

*Poster presentations**Autoimmune diseases*

PT01

TWO UNUSUAL PATTERNS OF PRESENTATION OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Background: SLE is an autoimmune inflammatory disease with multi organ involvement, autoimmune reaction and unpredictable clinical course. Organ involvement in SLE occurs in most of the cases in the patient with long term disease activity. SLE remains a bizarre pathology manifested by different ways and with atypical presentation as Intracranial hypertension (ICH), thrombotic microangiopathic hemolytic anemia (TMHA), tubulointerstitial nephritis (TIN). Intracranial hypertension (ICH), looks like a pseudo-tumor cerebri is an unusual manifestation of neuro-psychiatric SLE (NPSLE). ICH characterized by increase intracranial pressure, papilledema, cephalgia (head ache) and sometimes paresis of abducent nerve in absence of space occupy lesion or cerebral ventricular enlargement and without any biochemical and haematological changes in cerebrospinal fluid.

Objectives: We will describe two patients diagnosed with SLE with ICH, and SLE with TMHA.

Results: *Case 1:* A 21 year old girl presents with an acute fever with inflammatory polyarthralgia accompanied with cephalgia, nausea, dizziness, decrease vision acuity and diplopia. Fundus oculi examination shows papilledema with hemorrhagic spots in retina and defective visual field. Neurological examination result is normal. The diagnosis of Idiopathic ICH was confirmed from the increase intracranial pressure in the absence of cerebral pathology in MRI. Cell blood count (CBC) results with anaemia and leucopenia and increase of eryt-

hrocyte sedimentation rate (ERS). Immunologic tests results positive for anti-nuclear antibody (AAN), anti ds-DNA (high titer), Anti-Sm, decreased complement titer and antibody for APS result negative. Significant proteinuria on dipstick test.

Based on the clinical, laboratory and imaging evidence the patient result with SLE and ICH.

The patient was treated with glucocorticoids with pulse therapy 1000 mg for three consecutive days and 0.5 mg/kg in the coming days with efficacy.

Case 2: A 30 years old girl presents with fever, cephalgia, fatigue, polyuria, which soon develop an altered mental status. The clinical examination reveals no inflammatory arthritis and without mucocutaneous elements. CBC resulted with anaemia, thrombocytopenia. It was also noted an increase in reticulocytes and the presence of schistocytes in peripheral blood smear. Other laboratory values show an increased LDH, increased indirect bilirubin and hyponatremia with hypoalbuminemia. Electrolytes disturbance with hypokalemia, hypophosphoremia, normal plasma calcium and increased N-acetyl-β-glucosaminidase. Immunologic tests results positive for anti-nuclear antibody (AAN), anti ds-DNA, Anti-Sm, decreased complement titer and antibody for APS result negative. Coombs test was negative. Significant proteinuria on dipstick test. Neurological examination and MRI brain result is normal.

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Based on the signs and symptoms, laboratory and imaging evidence the patient results with SLE and TMHA and TIN.

The patient was treated with glucocorticoids, cyclophosphamid, plasmapheresis with efficacy.

Conclusions: In a number of cases SLE presents for the first time in an atypical pattern with unusual signs (ICH, TMHA, TIN), especially in young patient without a history for SLE.

PT02

NITRIC OXIDE AND I-NITRIC OXIDE SYNTHASE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Nitric oxide, as the smallest ubiquitous cell signalling mediator, has an important role in immune regulation and pathogenesis of SLE. Pro-inflammatory effects involve vasodilatation, edema, cytotoxicity and mediation in cytokine-dependent processes which can possibly induce tissue destruction.

Methods: This paper investigated the status of NO system, NO concentration (Cayman Chemical test) and i-NOS activity (Bioxytech Nitric Oxidase Synthase Assay kit-Oxis International) in the blood plasma of 55 SLE patients. The patients, in acute exacerbation disease phase were divided into four groups: skin (S-SLE), neurologic (N-SLE), joint (J-SLE) and vascular (V-SLE) disease manifestation, while 20 healthy volunteers, blood-donors, comprised the control group.

Results: Highly elevated values of NO were obtained in N-SLE ($43.26 \pm 2.91 \mu\text{mol/L}$), J-SLE

($42.97 \pm 5.64 \mu\text{mol/L}$) and V-SLE ($42.48 \pm 6.36 \mu\text{mol/L}$) related to control values ($20.94 \pm 4.64 \mu\text{mol/L}$) for $p < 0.01$. The highest activity of i-NOS was registered in V-SLE ($M = 1.40 \text{ nmol/mL}$) related to controls ($M = 0.72 \text{ nmol/mL}$) ($p < 0.001$), with lower statistical significance in S-SLE ($M = 1.27 \text{ nmol/mL}$) and N-SLE ($M = 1.27 \text{ nmol/mL}$, $p < 0.01$) and, finally, in J-SLE ($M = 1.18 \text{ nmol/mL}$, $p < 0.05$).

Conclusions: Excessive NO production can be followed by the induction of i-NOS expression. The degree of induction varies in different clinical disease manifestations, depending on the degree of synergy between bacterial products and cytokines. Long-term exposure to NO may induce irreversible damage to mitochondria in the cells of SLE patients, commonly mediated by reactive oxygen radicals and peroxidant radical. Selective i-NOS inhibitors may reduce excessive NO production, and they have been recommended recently in SLE treatment.

CORRELATION OF MMP3 SERA LEVELS WITH CLINICAL AND LABORATORY DISEASE PARAMETERS IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Matrix metalloproteinases (MMPs) are key extracellular matrix-degrading endopeptidases. Disturbances in their activities underlie pathophysiology of many diseases, rheumatoid arthritis (RA) among others. MMP3, produced by many different cell types in RA joint, stimulated by inflammatory cytokines plays a pivotal role in joint destruction.

Objective: To determine MMP3 values in sera of patients with rheumatoid arthritis (RA), and to compare MMP3 with basic clinical and laboratory disease parameters.

Methods: 21 patients with RA that were diagnosed, treated with anti IL6 treatment (Actemra) and monitored at Clinic for rheumatology, Clinical Center Podgorica, Montenegro, were analyzed for following parameters: disease duration, number of swollen joints, number of sensitive joints, DAS28 score, Erythrocyte sedimentation level (ES), concentration of anti CCP antibodies (AESKU ELISA test), and MMP3 concentration (AESKU ELISA test).

Results: In all tested RA sera samples levels of MMP3 were elevated. The highest average levels were determined in samples taken immediately before start of biological therapy. Therapy induced significant decrease of MMP3 level at three month

check point ($p < 0.01$, MW). After six months we detected further slight, nonsignificant decrease of MMP3 level in more than half of treated patients, MMP3 level increment in third and unchanged MMP3 level in about 10% of patients (comparing to previous time interval). These results are not in concordance with reduction in DAS28 levels which showed significant score reduction for all treated patients. Statistical analysis confirmed that therapy induced significant changes of MMP3 levels in serial samples for every particular patient ($p < 0.01$, Wilcoxon test).

Before the start of therapy, the only estimated significant correlation of MMP3 concentration was with number of swollen joints, but MMP3 level were not significantly correlated to any of investigated laboratory parameters (ES, anti CCP levels). After therapy we estimated significant correlation of MMP3 level to number of sensitive joints, DAS28 score and ES level ($p < 0.01$, Pearson).

Conclusion: Data from our preliminary study implies that MMP3 levels in RA patient samples before therapy could be connected with inflammatory processes in affected joints, and that reduction in MMP3 levels precisely reflects reduction in disease activity, at least in early follow up.

RHEUMATOID FACTORS, ANTI-MCV ANTIBODY, ANTI-JO-1 ANTIBODY LEVELS AND MUCOPREOTEINS IN RHEUMATOID ARTHRITIS

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Introduction: In 20 patients with rheumatoid arthritis (RA) we have observed the relationship between rheumatoid factor (RF), anti-mutated citrullinated vimentin antibody levels (anti-MCV), auto antibody directed to histidyl-tRNA synthetase (Anti-Jo-1) levels and mucoproteins.

Methods: The level of rheumatoid factors was quantified using the kit Quantia-RF. Quantia-RF is a quantitative turbidimetric immunoassay for detection of rheumatoid factors of the IgM class. Serum anti-MCV antibody levels and serum anti-Jo-1 antibody levels were quantified using Elisa method, immunoblots technology produced by Orgentec, Germany. Mucoproteins were quantified with colorimetric determination of mucoproteins in serum (Winzler's fraction). Mucoproteins are separated

from other proteins by precipitation with Perchloric acid and then detected with Coomassie colouring. We have used a kit produced by Mallinckrodt Baker BV.

Patients and Methods: Serum was collected from 20 patients with suspected rheumatoid arthritis. Serum obtained at the time of clinical evaluation was tested in order to evaluate rheumatoid factors (RF), anti-MCV antibody, anti-Jo-1 antibody and mucoproteins. Anti-MCV and anti-Jo-1 were quantified in automated system Elisa Alegria. The determination of RF and mucoproteins was done with a program photometer BTS 310 Plus.

Results: The results of the measurements and simple statistical analysis are present in the table.

	Number of cases	Mean value	Deviation Standard D.S	Pathologic interval	Normal Interval	Percent of positivity
Rheumatoid Factors(RF)	20	54 IU/mL	42 IU/mL	12-96 IU/mL	0-10 IU/mL	90%
Anti-MCV	20	68 IU/mL	39 IU/mL	29-107IU/mL	0-20 IU/mL	80%
Mucoproteins	20	66 IU/mL	39 IU/mL	27-105 IU/mL	0-120 IU/mL	10%
Anti-Jo-1	20	4.5 IU/mL	1.24 IU/mL	3.3-5.7 IU/mL	0-20 IU/mL	0%

In the above table we describe the results of measurements of rheumatoid factors, anti-MCV antibody, anti-Jo-1 antibody and mucoproteins in 20 patients with Rheumatoid arthritis. Conclusion: For

the diagnosis of rheumatoid arthritis, the most specific and sensitive marker is rheumatoid factor followed by anti-MCV antibodies.

PT05

DETERMINATION OF RF, RA/CP IgG AND VCP IgG ANTIBODIES IN SUBJECTS SUSPECTED TO RHEUMATOID ARTHRITIS

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The serological diagnosis of Rheumatoid Arthritis (RA) has always been difficult because the most frequent autoantibody, rheumatoid factor (RF), is not disease-specific. It has long been known that anti-perinuclear autoantibodies, also called anti-keratine antibodies, are found in patients with RA. Recently it has been discovered that these RA-specific autoantibodies, that recognize different deiminated proteins, represent a family of antibodies collectively named "anticitrullinated peptide/protein antibodies" (ACPA). The aim of this study is to show that these antibodies (RA/CP IgG and antiVCP IgG) being highly specific markers for RA with sensitivities comparable to RF.

The study includes 30 healthy ones as control group and 36 subjects suspected to RA. We measured

levels of RF, RA/CP IgG and antiVCP IgG in the sera of all of them. RF was assayed by immunoturbidimetric method. Both RA/CP IgG and antiVCP IgG antibodies were assayed by ELISA method.

In the group of 36 subjects suspected to RA, in which the levels of RF over cut-off, in 10 of these we found increased level of both RA/CP IgG and antiVCP IgG antibodies. In the control group the levels of all antibodies are below cut-off.

Based on statistical analysis of the results, we can conclude that antiVCP IgG Ab in addition to RA/CP IgG Ab represent an important serological marker for RA, while RF is not specific for this disease.

Biochemical markers of cardiac diseases

PT57

CARDIAC MARKERS, NEW DEFINITION AND REALITY

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Background: It's almost now five years since the ACC/ESC committee announced their new definition of myocardial infarction that includes troponin as

best available cardiac marker. Still some hospitals retain their old way mainly WHO criteria for their use of laboratory cardiac markers. Despite the

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availability of that gold standard, cardiologist still retain the old way of serial measurement of cardiac markers with less dependant on that marker. Is it a matter of lack of confidence in troponin, an opinion, or the refuse to change is sill unknown?

Method: Cardiac markers for 196 (74 females, 122 males) patients were requested by physicians were involved in this case. Results were recorded, studied and compared using analyze it statistical package.

Results: Fortunately, troponin; median 1.4 ng/mL was ordered for only three patients with almost

complete dependant on old cardiac markers. AST: median 26u/L (I.Q.R 19.5–35), CK: median 124u/L (I.Q.R 86–178), CK_{activity}: median 4 U/L (I.Q.R 34–63), and LDL: median 147 U/L (I.Q.R 129.5–189). Using CK and CKMB_{activity} significant difference were found between males and females ($p<0.001$, $p=0.03$ respectively) while others didn't show any sex difference. All markers showed significant linear correlation ($p<0.001$).

Conclusion: Body mass should be taken in consideration when dealing with some old cardiac markers.

PT58

ASSOCIATION STUDY OF rs7865618 POLYMORPHISM AT 9p21.3 LOCUS IN BULGARIAN PATIENTS WITH CORONARY DISEASE

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Using a genome wide association study (GWAS), the Ottawa Heart Genomic Study and the de-CODE group simultaneously identified the first genetic risk variant, at chromosome 9p21. Later studies established that 9p21 is a risk locus for coronary artery disease (CAD). The participation of rs7865618 at 9p21 locus in the pathogenesis of CAD has been proven in many populations of European descent, but not yet in populations in the Balkan region.

We conducted a study to determine whether the rs7865618 is associated with CAD in Bulgarian patients.

We studied 760 subjects aged 22–85 years, divided into two groups: (1) patients with angiographically proven CAD ($n=276$), and (2) blood donors as a population control group ($n=484$). The rs 7865618 was genotyped by High resolution melting analysis

on Rotor-Gene Q using control DNA for each genotype.

Both the frequencies of the A allele and the AA homozygotes were higher in patients than in controls (41.3% vs. 34.1%, $p=0.05$, and 63.8% vs. 58.8%, $p=0.055$, respectively). Both the AA homozygosity (OR=1.36, 95% CI: 1.00-1.84) as well as the A allele (OR=1.23, 95% CI: 0.99-1.53) show a weak association with CAD in our population, which is expected for this polymorphism in the light of previous studies.

The polymorphic variant rs 7865618 at locus 9p21 may be considered as risk factor in the Bulgarian population. A better estimation of the risk for CAD associated with the investigated SNP in the Bulgarian population could be achieved by further analyses in increased sample of patients and controls.

PT59

THE INFLUENCE OF PHYSICAL ACTIVITY ON LIPID PROFILE OF YOUNG PEOPLE

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Introduction: Physical activity causes many changes in body, increasing blood flow to skeletal muscles improves insulin resistance, lipid profile changes (reduction in body weight and body fat redistribution, and increased enzymes involved in the metabolism of fat).

The aim of this study was to examine the levels of total cholesterol, HDL and LDL cholesterol and triglyceride levels in serum of young athletes.

Materials and Methods: In the systematic review we examined 40 male and female athletes aged 20-26 years. All are exposed to physical harm in the form of daily exercise: women (n=15) football and volleyball and men (n=25) handball and basketball. All biochemical parameters were measured by standard biochemical methods on analyzer Advia 1650. The risk factor (RF) was presented the quotient of total and HDL cholesterol and atherosclerosis index as the ratio of LDL to HDL cholesterol.

Results and discussion: Statistically processing the obtained results show a satisfactory level of triglycerides ($X=1.762\pm1.09$ mmol/L) and HDL cholesterol ($X=1.30\pm2.95$ mmol/L), while the values for total cholesterol ($X=5.856\pm1.37$ mmol/L) higher compared with the reference values adopted in our laboratory due to which both LDL ($X=3.871\pm1.05$ mmol/L), RF ($X=4.60\pm1.09$) and atherosclerosis index ($x=3.06\pm3.02$) were elevated.

Conclusion: A group of young and physically active athletes has high cholesterol and atherogenic fraction (LDL x .) Which means that the sport does not reduce cholesterol but increases the "good cholesterol" (HDL) and reduces cardiovascular risk. This suggests that, in addition to the primary point of health, laboratory measurements should help in the planning of training in order to establish optimal sports form.

PT60

CONTRIBUTION OF SOME HEMATOLOGICAL PARAMETERS IN DIAGNOSIS OR PREDICTION OF ACUTE MYOCARDIAL INFARCTION

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Objectives: Definition of acute myocardial infarction (AMI) stands on acute or sub acute ischemic state of myocardium mostly as result of atherosclerotic changes. Atherosclerosis is inflammatory process that promote immune response mostly marked as high C-reactive protein level and changes in some cardiac markers level, such as myoglobin. Thus, our study is based on dilemma if leucocytes

and hemoglobin level can also be used as signal of ischemic and inflammatory state at myocardium. Hemoglobin as oxygen supplier and depot and leucocytes as cells that are directly engage in "foam cells" degradation and elimination.

Serum samples of patients (200) with symptoms of AMI were investigated for CRP, myoglobin, hemog-

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lobin and leucocytes level with standardized methods recommended by IFCC.

For statistical analysis, 36 samples of healthy donors was included into the experiment.

Results: Measurements shows that eight-fold increasing of CRP level, as established marker for AMI diagnosis, at 44.33% of the patients, is accompanied with two-fold increased level of leucocytes at 48.45% of the patients with AMI. On the other side,

we found 6.5-fold increased myoglobin level at 61.8% patients versus almost unchanged hemoglobin level (decreasing of 0.9 fold) at small group of patients (5.15%).

Conclusion: Presented results show that it is more likely that measurement of leucocytes can be useful tool, in presence of symptoms, in AMI diagnosis. On a contrary, measurement of hemoglobin does not provide enough relevant data for precise diagnosis.

PT61

PROTEOMIC PROFILING DURING ATHEROSCLEROSIS PROGRESSION: EFFECT OF NEBIVOL TREATMENT

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Background: There is a great need for the identification of biomarker(s) for the early diagnosis of atherosclerosis and the agents to prevent its progression. The aim of this study was to explore the effect of 24 week of nebivolol treatment on serum protein profiles in Apo E^{-/-} mice during atherosclerosis progression.

Methods: Proteomic analyses were performed using surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOFMS) in the serum samples from the nebivolol treated and non-treated Apo E^{-/-} mice. The protein profiles obtained using three different chips, CM10 (weak cation-exchange), H50 (reverse phase) and IMAC30-Cu²⁺ (immobilized metal affinity capture) were statistically analyzed using the Protein Chip data manager 3.0 program.

Results: At the end of 24 week of nebivolol treatment period, the peak intensities of 662 serum protein/peptide clusters were found to be expressed differently between nebivolol treated and non-treated control mice groups (p<0.05) and the differences reached statistical significance for 95 serum protein/peptide clusters (p<0.05).

Conclusion: The proteomic profile of nebivolol treated Apo E^{-/-} mice was found different than the control group indicating a potential role of nebivolol in atherosclerosis. Our study contributes to understand the efficacy of nebivolol on serum protein/peptide profiles during atherosclerosis development.

PT62

LIPID TESTING IN ADOLESCENCE IN ORDER TO PREVENT CARDIOVASCULAR DISEASE

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Determination of lipid status in children and young people is important preventive measure in the fight against cardiovascular disease, the leading cause of death today. In 95% cases mean reason is the atherosclerosis, which starts in early childhood.

The aim of our study was to determine the value of total, HDL and LDL cholesterol and triglycerides in a group of 54 adolescents of both sexes aged 14–19 years. They are sent from Child health center with a diagnosis ZOO-Notice by medical examination, asymptomatic, but with some risk of developing cardiovascular disease.

We examined levels of lipid parameters in serum of 27 boys and 27 girls on the Advia 1800 analyzer, commercial assays company Siemens. Averages ($\pm$ sd) the whole group and by sex expressed in

mmol/L were: total cholesterol 4.27 ± 0.67 (girls 4.43 ± 0.55 , boys 4.1 ± 0.75), HDL cholesterol 1.22 ± 0.31 (girls 1.34 ± 0.36 , boys 1.11 ± 0.19), LDL cholesterol 2.53 ± 0.58 (girls 2.55 ± 0.52 , boys 2.51 ± 0.64), triglycerides 1.11 ± 0.41 (girls 1.15 ± 0.38 , boys 1.08 ± 0.44). Whole group value of total cholesterol shows a low correlation with values of HDL, middle with HDL and triglycerides. Statistical significantly difference HDL in boys and girls, $p < 0.05$.

According to the recommendations of the National guidelines for diagnosis and treatment of lipid disorders, a value of 4.43 total cholesterol for girls is on the edger of risk. HDL cholesterol values were not at high risk, but significantly lower than desirable. This points to the necessity for preventive measures and different lifestyle.

PT63

MEAN PLATELET VOLUMEN (MPV) – COULD IT BE A PROGNOSTIC MARKER FOR CORONARY ARTERY DISEASE?

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Introduction: Platelet aggregation is a major cause of coronary artery disease, finally manifested by acute myocardial infarction (AMI), which is a leading cause of morbidity and mortality in developed countries. The aim of this study was to determine if MPV as widely used parameter may predict increased platelet aggregability and consequently AMI.

Methods: We measured MPV (Cell Dyn 1800, Abbott Diagnostics, USA) and platelet aggregation (Multiplate, Dynabyte, Munich, Germany) in 105

patients with coronary artery bypass grafting (CABG) and 30 healthy volunteers. All patients received acetylsalicylic acid (100 mg/day) as an anti-aggregation therapy, while healthy volunteers did not receive any therapy.

Results: There were not significant differences between MPV in patients and healthy volunteers ($p = 0.4144$). In patients there was not correlation between MPV and platelet aggregation as we expected because of acetylsalicylic acid therapy ($r = 0.217$; $p = 0.0261$; $R^2 = 0.047$) and in healthy volun-

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teers there was a weakly correlation but not statistically significant ($r=0.256$; $p=0.1719$; $R^2=0.066$).

Conclusion: In this study we concluded that MPV is not in relation to platelet aggregation and therefore

it is not a good prognostic marker for platelet reactivity and coronary artery disease.

PT64

THE CONCENTRATION OF Mg IN SERUM AND ERYTHROCYTES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background: Cardiovascular effects of magnesium include coronary and systemic vasodilatation, inhibition of platelet and anti arrhythmic action and protection from myocardial ischemia. Because of the importance of magnesium in acute myocardial infarction, our goal was to review the imbalance Mg^{2+} ions in serum and erythrocytes, and the determination of significant differences in specific disease groups.

Method: The study included 35 patients with acute myocardial infarction. The control group consists of 20 healthy volunteers, age 18–55. Determination of Mg^{2+} concentration in serum was provided by spectrophotometric method and concentration of erythrocytes by indirect method.

Results: Statistical analysis demonstrated a significant difference of magnesium in serum for patients

with acute myocardial infarction ($p=0.01$). Mg^{2+} concentration of erythrocytes in patients with acute myocardial infarction was significantly low ($p<0.001$).

Conclusion: There is a significant decrease of Mg^{2+} in serum and erythrocytes in patients, compared to the control group. The reduction of Mg^{2+} in serum is accompanied by decrease of Mg^{2+} in erythrocytes in patients with acute myocardial infarction. The most striking statistical difference compared to the control group, is in patients with acute myocardial infarction. Having in mind that importance of biochemical Mg^{2+} , is not negligible giving of magnesium per os in the treatment of acute myocardial infarction in the case of the verified reduced concentration of Mg^{2+} .

PT65

HOMOCYSTEIN IN MALES WITH CORONARY HEART DISEASE AND OVERWEIGHT

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Background: Elevated homocystein (HCY) is associated with onset of early atherosclerosis independent of other risk factors. The study is aimed to investigate HCY in males with overweight after myocardial infarction or with stable angina or aorto-coronary bypass.

Material and methods: Fifty men at mean age 53.9 years with ischaemic heart disease or aorto-coronary bypass, BMI over 25 kg/m² were tested for HCY, serum lipids, blood glucose, uric acid, blood cell count, RBC sedimentation rate and hsCRP. Biochemical and hematological parameters were measured by common commercial methods; hsCRP by hemiluminiscent immunometry; HCY by two different methods: mass selective detection gas chromatography and competitive immuneassay.

Results: Lipid parameters evidenced moderate dyslipidemia, while all other parameters studied were within the reference values. HCY measured by the immuneassay method was 13.26±0.95 µmol/L, and measured by gas chromatography 14.67±1.09 µmol/L. Significant correlation (r=0.870, p<0.001) between the two methods was established.

Conclusion: 40.81% of the patients studied had elevated HCY unassociated with other lipid and nonlipid risk factors. Vitamin B₆, B₁₂ and folic acid supplementation added to the main cardio- and antilipid therapy could be considered in patients with ischemic heart disease and overweight for risk reduction for primary or secondary myocardial infarction.

PT66

FIBRINOLYTIC AND INFLAMMATION MARKERS IN YOUNG PATIENTS WITH MYOCARDIAL INFARCTION

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Background: Inflammation and disturbances in various components of fibrinolytical system are important in pathogenesis of myocardial infarction (MI). Concentration of fibrinogen and CRP and activity of plasminogen and plasminogen activator inhibitor (PAI) are compared between young patients with MI and healthy persons.

Methods: The study enrolled 60 patients with MI, younger than 46 years, while the control group comprised 60 healthy persons, matched by age. Turbidimetric, coagulometric and photometric tests were employed. Results were compared using Mann-Whitney U-test.

Results: Patients had significantly increased fibrinogen (P=0.000) and CRP (P=0.013) when compared

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with controls. Differences in activities of plasminogen and PAI did not reach statistical significance. A significant difference in fibrinogen concentration ($P=0.000$) was observed between male patients and healthy men, while such observation was absent in female population of our study. Gender had no impact on CRP, plasminogen and PAI levels in both groups.

Conclusion: According to our results we can conclude that fibrinogen and CRP are higher in young patients with MI, while the differences in plasminogen and PAI activities are not of statistical importance. Such conclusion may mark inflammation markers as valuable prognostic markers in young patients with MI. Nevertheless, larger studies are needed to evaluate the utility of determination of fibrinolytic parameters in young patients with MI.

PT67

ARE ADIPONECTIN LEVELS RELATED TO NT-proBNP LEVELS IN CHRONIC RENAL FAILURE PATIENTS ON PERITONAL DIALYSIS

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Aim: Cardiovascular events are the leading cause of mortality in chronic renal failure (CRF) patients. It was proposed that normal/high levels of adipokine, adiponectin might prevent cardiovascular diseases and complications in healthy subjects. However, recently it was reported that serum adiponectin levels increased paradoxically in chronic heart failure in accordance with an independent predictive role for mortality. B type natriuretic peptide (BNP) is accepted as the most sensitive and specific marker of cardiac dysfunction. In the present study we investigated relations of adiponectin with NT-proBNP and hsTnT, a marker of cardiomyocyte necrosis, in CRF patients on peritoneal dialysis (PD).

Methods: Serum adiponectin, NT-proBNP and hsTnT levels were measured in 51 CRF patients on PD (21 women, 30 men, 50.9 ± 16.5 years) and 41 healthy controls (22 women, 19 men, 45.7 ± 11.6 years) using ELISA for the former and ECLIA for the latter.

Results: Adiponectin (44218 ± 6702 vs. 9703 ± 813 ng/mL, $p < 0.0001$), NT-proBNP (5382.00 ± 1115.00 ng/mL vs. 77.00 ± 9.99 , $p < 0.00001$) and hsTnT (0.070 ± 0.010 ng/mL vs. 0.004 ± 0.001 , $p < 0.00001$) levels were significantly higher in CRF patients on PD compared to controls. Adiponectin levels in CRF patients on PD correlated significantly with NT-ProBNP ($r = -0.465$, $p = 0.001$), body weight ($r = -0.465$, $p = 0.001$) and BMI ($r = -0.464$, $p < 0.001$).

Conclusions: Early diagnosis and treatment of heart failure are important in reducing mortality and morbidity in these patients. Significant relations of adiponectin with NT-proBNP and hsTnT suggested that adiponectin levels might be helpful in diagnosis and treatment of cardiac failure in CRF patients on PD.

PT68

SOLUBLE ST2 LEVELS IN HEART FAILURE PATIENTS

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Introduction: ST2 (for growth STimulation expressed gene 2) is a member of the interleukin-1 (IL1) receptor family of proteins, which play a central role in the regulation of immune and inflammatory response. In the heart, ST2 has a biological role in the innate immunological process and is also directly involved in a cardiac signaling pathway, which, under healthy conditions, serves to protect the heart during pressure overload or stretch.

Methodology: We use The Presage ST2 Assay produced by Critical diagnosis to measure ST2 serum levels in 75 patients with heart failure in order to predict adverse outcome. We compare their results to NT-ProBNP levels and evaluate the predictable ability of ST2 in mortality.

Results: Patients can show elevated ST2 levels, and increased risk of death, as early as 2–3 hours after

an ischemic event or cardiac injury, with elevated risk persisting throughout the follow-up period. Patients with heart failure illustrates the significance of a high ST2 value for predicting clinically relevant outcomes in patients with heart failure. Patients with ST2>35 ng/mL have a 2.8x higher risk of adverse outcomes within 30 days than patients with low ST2 concentrations.

Conclusion: Soluble ST2 is an emerging marker that has been shown to predict adverse outcomes in individuals with established heart failure and is also a powerful prognostic marker for future cardiovascular disease in the general population. Higher levels of circulating ST2 can be detected in apparently healthy individuals and precede adverse outcomes. Circulating ST2 is a sensitive marker of cardiac stress or ischemic injury.

PT69

CORRELATION OF SERUM HIGH SENSITIVE C-REACTIVE PROTEIN, FERRITIN, SOLUBLE TRANSFERRIN RECEPTORS (sTfR), VALUE OF sTfR INDEX WITH ACUTE CORONARY SYNDROME

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Background: Association between serum high sensitive C-reactive protein (hsCRP), ferritin, soluble transferrin receptor (sTfR), value of sTfR index and acute coronary syndrome (ACS) assessed with coronary angiography were examined by authors.

Methods: This study included 25 patients (9 women and 16 men) with angiographically proven ACS and 25 healthy controls (14 women and 11 men). Blood

samples were obtained on the day before angiographic procedure and hsCRP, ferritin, sTfR were measured by nephelometry (BNII, Dade Behring). sTfR index was calculated (sTfR/log ferritin).

Results: The values (median) of serum hsCRP were statistically significantly higher for patients compared to controls (3.65±3.16 mg/L versus 1.24±0.65mg/L, t=3.64, p<0.01). The same conclu-

sion was obtained for ferritin ($98.42 \pm 36.37 \mu\text{g/L}$ for cases versus $67.59 \pm 30.01 \mu\text{g/L}$ for controls, $t=3.20$, $p<0.01$). Concentrations of sTfR ($1.35 \pm 0.46 \text{ mg/L}$ for cases versus $1.30 \pm 0.35 \text{ mg/L}$ for controls, $t=0.39$, $p>0.05$) and sTfR index (0.73 ± 0.29 for cases versus 0.71 ± 0.16 for controls, $t=0.28$, $p>0.05$) did not differ significantly.

Conclusions: Our study supports the hypothesis that higher iron stores, measured by serum ferritin are associated with risk for ACS. But, in view of higher CRP levels in cases, we concluded that inflammation was present that might be responsible for elevated ferritin levels. sTfR concentrations were not related with developed of ACS.

PT70

ASSOCIATION OF LACTATE CLEARANCE WITH INFLAMMATORY BIOMARKERS PCT AND CRP IN PATIENTS UNDERGOING CARDIAC SURGERY

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Background: Lactate clearance, a surrogate for magnitude and duration of global tissue hypoxia is used for diagnostic, therapeutic and prognostic purposes. Recent studies documented that serial lactate measurements over time or lactate clearance may be clinically more reliable than single lactate measurement for risk stratification in different pathological conditions. This study examined the association between lactate clearance with inflammatory biomarkers (PCT and CRP) in patients undergoing cardiac surgery.

Methods: We measured PCT, CRP and Lactate levels in 50 adult patients undergoing cardiac surgery. The measurements were performed after the surgery (T1) and then on second (T2), third (T3), fourth (T4) and fifth postoperative day (T5). Determination of Lactate concentration was provided by spectrophotometry on ARCHITECT c 8000 (Abbott, USA), PCT by ECLIA on Cobas e 411 (Roche, Germany) and CRP by immunonephelometry on the Dade Behring BN II (Siemens, Germany). All data are expressed as median and standard deviation. The Kruskal-Wallis test was used to assess changes over time of variables. $P<0.05$ was considered statistically significant.

Results: PCT values were highest at T1 ($0.47 \text{ ng/mL} \pm 1.27$) then at T2, T3, T4, T5 ($0.35 \text{ ng/mL} \pm 0.72$; $0.28 \pm 0.98 \text{ ng/mL}$; $0.21 \pm 0.65 \text{ ng/mL}$ and $0.16 \text{ ng/mL} \pm 0.37$) PCT value slowly decreased; $p<0.001$. CRP increases over time till third day after the surgery (T1- $46.0 \text{ mg/L} \pm 27.60$; T2- $156.0 \text{ mg/L} \pm 52.62$; T3- $179.0 \text{ mg/L} \pm 61.80$) and then started to decrease (T4- $152.0 \text{ mg/L} \pm 53.45$ and T5 - $111.0 \text{ mg/L} \pm 45.28$), $p<0.001$. Lactate values have shown unusual dynamic over time: at T1 values were highest, $3.0 \text{ mmol/L} \pm 1.79$, then started to decrease: T2- $2.7 \text{ mmol/L} \pm 0.76$, T3 - $2.34 \text{ mmol/L} \pm 0.40$ and then raised again fourth day after the surgery: T4 - $2.43 \text{ mmol/L} \pm 0.53$; T5 - $2.58 \text{ mmol/L} \pm 0.68$; $p<0.001$. Lactate clearance from first and second day after surgery was $11.21\% \pm 26.68$, second and third: $12.65\% \pm 19.08$, third and fourth: $-5.69\% \pm 22.39$ and fourth and fifth day: $-6.37\% \pm 28.59$; $p<0.001$.

Conclusion: PCT values statistically decreased from first to fifth day after surgery. CRP values, as expected, showed raise just after the surgery and then slowly decreased. In our study, Lactate values and lactate clearance showed the same dynamic in five postoperative days.

PT06

RENAL OSTEODYSTROPHY IN CHRONIC HEMODIALYSIS PATIENTS

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Renal osteodystrophy is one of the most common complications of chronic hemodialysis and one of the risk factors for increased mortality among haemodialysis patients. The aim of this study was to estimate the appearance of renal osteodystrophy in chronic hemodialysis patients in the Institute of Nephrology, Struga and to estimate the influence of certain demographic, clinical and biochemical parameters (intact Parathyroid hormone (iPTH), total calcium, phosphorus and alkaline phosphatase). In the study 74 patients were included: 40 males and 34 females with average age of 57.9 years and average hemodialysis duration of 11.2 years. Fasting patients' serums were used. The serum concentration of iPTH was measured using two-step sandwich immunoassay on automated immunoanalyser. Based on the results, the patients were divided into two groups with iPTH below 300 pg/mL and iPTH above 300 pg/mL. The values of total calcium,

phosphorus and alkaline phosphatase were measured by colorimetric method of automatic clinical analyzer. For statistical analysis Student t-test was used. 63.5% of the patients had secondary hyperparathyroidism, 16% hypercalcemia, 47% hyperphosphatemia and 26% had increased alkaline phosphatase. By comparing the values of average age of total calcium and phosphorus, no statistically important difference was discovered. Statistical significance was found comparing the two groups in relation to the hemodialysis duration and the alkaline phosphatase values. The patients are characterized by high percentage of hyperphosphatemia which contributes to the development of secondary hyperparathyroidism and renal osteodystrophy. The results indicate the need of better medicinal treatment and diets in relation to phosphorus intake.

PT07

BONE METABOLISM IN POSTMENOPAUSAL WOMEN WITH RHEUMATOID ARTHRITIS

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Introduction: Patients with rheumatoid arthritis (RA) are suffering from an imbalance between bone formation and bone resorption, leading to generalized bone loss. As a result of disturbed bone metabolism, postmenopausal women with RA are high-risk population for osteoporosis and incidence of fractures including hip fracture.

The purpose of this study was to determine through the concentration of bone markers osteocalcin (OC) and CTX to display bone metabolism in postmenopausal women with RA.

Methods: The study included a control group of 66 postmenopausal women with mean age (63.9±9.1

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years) and a group of 65 postmenopausal women with RA mean age (61.5 ± 9.5 years). The determination of OC and CTX was done with the electrochemiluminescence method on the automatic immunoanalyzer Elecsys 2010.

Results: Mean values of OC between the control group and patients with RA were statistically significantly different ($p=0.0001$). Patients with RA have significantly lower values of OC (20.73 ± 5.8 ng/mL) compared with the control group (33.3 ± 9.7 ng/mL). Also, highly significant difference occurs at mean values of CTX ($p=0.00014$) among the tested groups. Patients with RA have higher values of CTX

(0.765 ± 0.195 ng/mL) compared with the control group (0.497 ± 0.132 ng/mL).

Conclusion: The results obtained in this study indicate impaired bone turnover in postmenopausal women with RA. Bone formation is suppressed and bone resorption was increased compared with the control group. This disorder of bone metabolism leads to rapid development of bone loss, the risk of osteoporosis and bone fracture. The results may indicate the need to introduce antiresorptive therapy with bisphosphonates in postmenopausal women with RA.

PT08

DEVIATION OF VALUES FOR PARATHYROIDHORMONE IN RECRUITES YEAR 1985-1986 IN MONTENEGRO COMPARED TO THE REFERENCE VALUES

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Introduction: Parathyroid hormone (PTH) is synthesized in parathyroid glands and secreted into the bloodstream. It is a single chain polypeptide is constructed of 84 amino acids. Only the N-terminal end (first 34 amino acids) shows biological activity, while other parts of molecules biological inert.

Parathyroid hormone participates, along with vitamin D and calcitonin in the regulation of the metabolism of calcium, phosphate and magnesium. Changes in the secretion of PTH may lead to changes in concentrations of these important minerals and it is important to determine the Reference Values of the hormone in the blood.

Materials and methods: We conducted our inquiries among the Military Medical Center in Podgorica for six months. Processed 110 recruits aged 18–19 years without symptoms such as hypo- or hyperfunction of the parathyroid glands.

The values obtained by measuring PTH on the device "ELESCYS 2010" chemiluminescence method ranged from 13.0–52.0 pg/mL.

The test for determining intact PTH uses the principle of sandwich test where biotin monoclonal antibodies react with the N-terminal fragment (1-37 amino acids), and monoclonal antibodies tagged with ruthenium complex react with the C-terminal fragment.

Results and discussion: Since study was conducted among healthy young males, baseline PTH to the manufacturer's test (Roche) does not correspond to the values obtained in our homogeneous sample.

Since Serada on healthy young males, baseline PTH to the manufacturer's test (Roche) does not correspond to the values obtained in our homogeneous sample of this age.

PT09

CEA AND CA 19-9 AS PROGNOSTIC INDEX IN COLORECTAL CANCER

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Introduction: The use of a combination of tumor markers may be an important tool in the early diagnosis of colorectal cancer, which is the key to improving prognosis. The study aim was to investigate the diagnostic value of carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) levels in patients suspected and with colorectal cancer.

Methodology: Blood samples were collected from 280 patients (61, 22% confirmed high levels of tumor markers) and 30 healthy in control group. An ELISA assay was used to measure the serum CEA and CA19-9. The normal reference values were CA19-9 (0–39 U/mL) and CEA (0–3.4 ng/mL).

Results: Mean serum CA19-9 and CEA levels in patients with colorectal cancer (89.55 ± 21.18 U/mL; 18.43 ± 2.25 ng/mL) were significantly higher ($p < 0.001$) than those in healthy examinees

(14.34 ± 1.95 U/mL, 2.17 ± 0.37 ng/mL). At the time of diagnosis, 22% of the patients (N=61) had elevated serum levels of CEA and 15% of CA 19-9 (N=42). Relapse was observed in 51 patients, 18.2% of whom had elevated CEA and/or CA 19-9 levels. Among patients without relapse, 68% and 73% had normal values of CEA and CA 19-9, respectively. The sensitivity of the combined detection of CA19-9 and CEA (92.31%) was significantly higher ($p < 0.05$) than that of either marker alone (79.49%, 71.79%, respectively).

Conclusion: The combined detection of CA19-9 and CEA could overcome the deficiency of using single marker detection by improving the sensitivity to diagnose of colorectal cancer. At the same time, CA19-9 and CEA detection could be used to assess mesenteric artery invasion and the metastasis of lymphatic's and distant organs in pancreatic cancer.

PT10

CLINICAL USEFULNESS OF FREE PSA/TOTAL PSA RATIO IN EARLY DIAGNOSIS OF PROSTATE CANCER

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Introduction: The aim of this study was to analyze the role of total prostate-specific antigen (PSA) and free-PSA levels in the diagnosis and clinical following of prostate cancer.

Methodology: We determined total PSA serum concentration and free-PSA in 253 patients (age of 50–60, 60–70 and 70–79 years), 203 with benign

prostate hyperplasia (BPH) and 50 with prostate cancer. Total and free PSA concentration was measured with the ELISA methods. Prostate volume was determined by transrectal ultrasound. The diagnostic validity of tPSA, %fPSA, and PSAD was evaluated by receiver operation characteristic (ROC) curve analysis.

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Results: Free-PSA affords little additional diagnostic advantage compared with total PSA in the screening population. The receiver operating characteristic curves for diagnostic accuracy were ranked: (1) PSA density; (2) total PSA and (3) f-PSA. Median total PSA serum concentration was 3.7 ng/mL in patients with BPH and 31.4 ng/mL in patients with prostate cancer ($p < 0.001$). When total PSA is in the range of 4.0–10.0 ng/mL, a free/total PSA ratio < 0.10 indicates 50–68% risk of prostate cancer depending on age; a free/total PSA ratio > 0.25 indicates a 9% to 16% risk of prostate cancer, depending on age. The determination of f/t was useful in the cases with a serum total PSA of 5.1–10 ng/mL; the specificity

was 60% with a sensitivity of 90% at an f/t of 0.148. The positive predictive value for diagnosis of prostate cancer also increased to 54% from 34% of that in total PSA alone. In the range of 4.1–10 ng/mL, the cut-off value of f/t was 0.155 for obtaining relatively high specificity; sensitivity was 85% and specificity was 56.5%.

Conclusions: Thus, the determination of f/t was considered to be an effective tool for discriminating the non-prostate cancer cases from those of prostate cancer. For good prognosis metastatic prostate cancer patients under anti-androgen treatment, PSA response at 6 months with cut-off levels of ≤ 1 ng/mL and ≤ 10 ng/mL is prognostic for survival.

PT11

DNA TURN-OVER ENZYME ACTIVITIES IN ORAL CAVITY CANCER TISSUES

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Oral cavity cancers are the most common in the head and neck cancer and has a high mortality. Carcinogenesis studies emphasizes that carcinogenic substances are needed to be metabolized by the body's enzyme systems. As this accepted hypothesis, the chemical strum to the electrophilic metabolites (radicals) in the body and, the lead to the activation of ontogenesis caused by DNA damage. Nitric oxide (NO) and nitricoxidesynthase (NOS) play important roles in carcinogenesis, with the effects to inflammation process and oxidant and antioxidant effects. ADA (adenosinedeaminase) and XO (xanthine xidase) are the purine metabolic pathway enzymes. ADA is known to be essential for cell-mediated immunity, in carcinogenesis this enzyme is also important in terms of increased cell prolifera-

tion. XO (xanthinc oxidase) plays a role as a basis of free radicals in the mechanism of carcinogenesis. In this study, oral cavity carcinomas were compared with tumour-free control tissues; ADA, XO, NO, NOS activities were evaluated in terms of difference. Seventeen patients with cancerous tissue and adjacent normal mucosa were obtained during resection. In tumour tissues, NO and NOS activities were observed as decreased but not statistically significant, ADA activities were observed as statistically significant increased. Increased ADA activity which increased with increase in cellular proliferation in tumour tissue, is a result of purine metabolism. In this context, an inhibition of ADA, is thought to be a glimmer of hope in patients with oral cavity cancer.

FLUORENYLSPIROHYDANTOIN DERIVATIVES: SYNTHESIS AND BIOACTIVITIES

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Introduction: Hydantoin derivatives have important biochemical and pharmacological properties. Several compounds are known as anticonvulsant and antiarrhythmic drugs. There not many studies researching their anticancer effects.

In the present study, we have examined cytotoxic activity of four hydantoin compounds:

- (1,3-dihydroxymethylene-(9'-fluorene)-spiro-5-hydantoin;
- 4'-bromo-1,3-dihydroxymethylene-(9'-fluorene)-spiro-5-hydantoin;
- (9'-fluorene)-spiro-5-(2,4-dithiohydantoin) and
- 4', 5'-diaz-(9'-fluorene)-spiro-5-hydantoin) on retinoblastoma cell line (WERI-Rb-1).

Material and Methods: A series of hydantoin derivatives has been designed and synthesized. The compounds were studied by IR, ATR FT, ¹H-, ¹³C NMR and Raman spectroscopy. The cytotoxic effect of four derivatives on retinoblastoma cell line was assessed using WST-1 assay. Cells were seeded in duplicates in 96-well plates at a density of 5x10⁴

cells/well. After cultivation period of 24h, the compounds were added at a concentration 500 µM and incubated for 24 and 48h respectively. WST-1 was added to the cells at these time points and incubated for 4h. Absorbance was measured on ELISA SUNRISE Reader.

Results: Our preliminary results showed that all the compounds exhibit a time-dependent cytotoxic effect on human retinoblastoma cell line. We found that prolonged incubation periods affect cell viability remarkably. These data suggest that four hydantoin derivatives induce cytotoxicity on the tested cells, however, the effect was more prominent when 4'-bromo-1,3-dihydroxymethylene-(9'-fluorene)-spiro-5-hydantoin and 1,3-dihydroxymethylene-(9'-fluorene)-spiro-5-hydantoin were used.

Conclusion: Our investigation is the first to reveal that the synthesized hydantoin derivatives have anticancer properties. Further studies are required to understand the exact mechanisms of their action.

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PT13

GENE EXPRESSION AND SERUM YKL-40 LEVELS IN HIGH-GRADE GLIOMAS

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Brief introduction: Malignant gliomas are the most common type of primary malignant brain tumours. They are characterised by extreme proliferation and aggressive invasion. There is evidence for overexpression of the YKL-40 gene in high-grade gliomas. The high serum levels of the glycoprotein are associated with poor prognosis of various inflammatory and tumor process.

In this study we investigate YKL-40 mRNA expression and serum levels in glioma patients and search correlation between them.

Material and Methods: Thirty seven patients with glial tumors were included in the study: 14 patients with glioblastomas, 23 patients with high-grade gliomas. Brain tissue was collected from the patients during surgical removal of the tumor and post mortem from suddenly deceased individuals with no clinical data of disease. Real-time RT-PCR and ELISA were used to measure YKL-40 mRNA in brain tissue and protein expression in serum. The control

group of 33 age-matched healthy people was examined for YKL-40 serum levels.

Results: The mean serum value of YKL-40 in healthy subjects was 84.52 ± 13.65 ng/mL, while the mean serum concentration in high-grade gliomas was 118.49 ± 15.49 vs 139.68 ± 22.39 ng/mL in glioblastoma patients. Serum YKL-40 level in glioma patients was significantly higher compared to that of healthy controls ($P \leq 0.05$).

The gene analysis revealed that YKL-40 mRNA in glioma patients was over-expressed versus normal brain. In three of the particular cases the gene expression was increased significantly. These patients had shorter survival time compared to other glioblastoma patients.

Conclusion: YKL-40 expression and secretion is associated with malignant clinical-pathological features in brain tumours and might serve as a valuable prognostic biomarker.

PT14

INFLUENCE OF INFLAMMATION ON SERUM LEVEL OF PROSTATE-SPECIFIC ANTIGEN IN BENIGN PROSTATIC HYPERPLASIA

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Objective: Investigating the effects of inflammatory response over synthesis and secretion of prostate-specific antigen (PSA) in patients with benign prostatic hyperplasia (BPH).

Methods: 60 males were monitored, with an average age of 56.3 years (30-BPH, 30-control). PSA (ng/mL), ferritin (ng/mL), CRP (mg/dL), C₃ (mg/dL), C₄ (mg/dL), transferrin (mg/dL), albumin (g/dL) were

measured by standard methods of analysis (photometric, immunoturbidimetric, ELISA).

Results: We have obtained statistically significant differences ($p < 0.05$, CI=95%) between BPH and control: PSA (3.85 ± 0.07 and 0.9 ± 0.7), CRP (0.36 ± 0.33 and 0.07 ± 0.07), C_3 (116 ± 37 and 92 ± 9), C_4 (23 ± 6 and 16 ± 7), albumin (3.72 ± 0.26 and 3.96 ± 0.57), transferrin (193 ± 42 and 218 ± 56). A positive relationship was observed between PSA and CRP ($r = 0.787$, $p < 0.01$), PSA and C_3 ($r = 0.502$, $p < 0.01$), PSA and C_4

($r = 0.311$, $p < 0.05$), PSA and ferritin ($r = 0.179$, $p < 0.05$) and a weak negative association between PSA and albumin ($r = 0.114$, $p < 0.05$), PSA and transferrin ($r = 0.237$, $p > 0.05$).

Conclusion: These results provide the evidence that the inflammatory response induces changes to serum PSA. The authors estimated that eradication of inflammation could be an accurate way of monitoring and staging of BPH by quantifying PSA.

PT15

ROLE OF ESTRADIOL AS A DIAGNOSTIC MARKER IN PATIENTS WITH COLORECTAL CARCINOMA IN NORTH INDIA

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Introduction: Globally more than 1 million people suffer from colorectal cancer (CRC) per annum, resulting in about 0.5 million deaths. The role of estrogen in CRC is being researched with great interest; expression of estrogen receptors (alfa and beta) is being explored.

Objective: Our objective was to compare the serum estradiol levels in diagnosed male patients of colorectal cancer, with age-matched controls; and to study the estradiol levels across the different stages of CRC.

Methodology: A cross-sectional study was conducted from January, 2012 to March, 2013 at a tertiary care hospital in north India. 51 male pre-operative CRC patients were enrolled along with 50 age-matched male controls. Ethical approval and informed written consent from each participant were duly obtained. CRC patients were staged as per

TNM (I, II, III and IV) criteria. Serum estradiol level was measured by Chemiimmunofluorescence method (normal=11.6–41.2 pg/mL).

Result: The mean serum estradiol level among CRC patients (43.4, sd=27.1) was significantly higher than that among controls (mean=24.7, sd=17.5), ($p < 0.0001$). Across the four TNM stages of CRC patients, mean estradiol level was highest in Stage II (55.9, sd=15.5); followed by Stages III (44.1, sd=24.9), IV (36.3, sd=30.0) and I (26.4, sd=38.8). However, significant difference was obtained only between Stages I and II.

Conclusion: Our study reveals that serum estradiol level may be considered as an important diagnostic marker in Indian male CRC patients. However, larger prospective research is warranted to corroborate this finding, and to understand the role of estradiol across different TNM stages of CRC.

TUMOR M2 PYRUVATE KINASE (TUMOR M2-PK) – DIAGNOSTIC CAPABILITIES

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Introduction: Colorectal cancer is one of the most frequent tumor diseases in Europe.

The quantitative determination of tumor M2-PK in feces provides a new, sensitive screening method for the diagnosis of colorectal carcinoma by direct detection of tumor derivative of an enzyme that is released into the intestinal lumen. The aim of our study is to present the diagnostic possibilities of tumor marker M2-PK in feces.

Material and methods: 40 samples from 11 clinically healthy and 29 patients with pathology (bowel polyps, colorectal cancers, inflammatory bowel disease: Crohn's disease and Ulcerative colitis) were examined for Tumor M2-PK and occult bleeding. The patients were separated into groups after colonoscopies were performed. The analysis of M2-PK was performed with manual ELISA-method of ScheBo®Biotech. The analysis of occult blood was performed by qualitative immunoassay test (DIMA-Diagnostika, Germany).

Results and conclusions: The clinical sensitivity of M2PK in the total group of patients is 68.97%. This

is due to the high % negative results for patients with bowel polyps and inflammatory bowel diseases in remission. The clinical sensitivity of M2PK in the group of polyps is 37.5%, whereas the same in the group of colorectal carcinoma is 100%. The clinical sensitivity of the test for occult blood in the common group of patients is 62.07%. In the group with polyps is 12.5%, while in the group with colorectal carcinoma is 90%.

In our study, higher values for clinical specificity were obtained in the assay for occult blood.

- Tumor M2-PK in stool is a candidate marker for early diagnosis of colorectal carcinoma.
- Tumor M2-PK in stool is a useful marker in screening of patients, before a further diagnostics using more costly and invasive procedures - colonoscopy.
- Unlike the test for occult blood, tumor M2PK was detected in patients without bleeding.

Diabetes mellitus and metabolic syndrome

PT17

EFFECTS OF EXPERIMENTAL DIABETES MELLITUS ON SOME ACUTE PHASE PROTEINS AND TRACE ELEMENTS IN RATS

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In the present study, the levels of CRP, ceruloplasmin, haptoglobin, iron, copper and zinc were investigated in rats with experimentally induced Diabetes mellitus. For this purpose, 27 female wistar rats were enrolled. Diabetes mellitus was induced in 16 rats using streptozosin 50 mg/kg intraperitoneally and control animals (n=11) received equal amount of buffer citrate. The blood glucose levels were measured on 21 days after injection. Blood samples were collected in rats and serum CRP, ceruloplasmin, haptoglobin, iron, copper and zinc levels were determined. The results were conducted with ap-

propriate statistical methods. The result of this study revealed that CRP and haptoglobin levels were significantly affected in rats with experimentally induced diabetes. It was observed that Fe and Cu levels were elevated in diabetic rats in comparison to control group rats. In conclusion CRP and haptoglobin may be used for monitoring of inflammatory process and for early diagnostic purpose in cases of determining complications of diabetes. Fe, Zn and Cu levels were altered in diabetes mellitus and those parameters may be important for prognosis of the disease.

PT18

EVALUATION OF RATE OF RENAL FUNCTION DECLINE IN PATIENTS WITH DIABETES MELLITUS TYPE 2 ACCORDING TO THE RATE OF ALBUMINURIA AND GLOMERULAR FILTRATION RATE

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Introduction: Decline of glomerular filtration rate (GFR) in diabetic patients depends of many factors. We evaluated rate of this decline according to the rate of albuminuria and GFR.

Methods: Patients with diabetes mellitus type 2 (n=113) divided into three groups according to the albuminuria and GFR: I 41 with reduction of GFR above 20% and with pathologic albuminuria; II 34 with reduction of GFR below 20% and with pathologic albuminuria; III 38 with normal GFR and normal albuminuria. At the beginning and every 12

months during two years we measured serum levels of creatinine, urea, uric acid (standard biochemical methods), cystatin C (immunoturbidimetry), creatinine clearance, and proteinuria (pyrogallol red) and albuminuria (sandwich-immunometric method).

Results: At the beginning, level of GFR was 53.6 ± 3.38 in I, 100.5 ± 3.71 in II, and 93.1 ± 3.51 mL/min/1.73m² in III group. Average reduction of GFR/year in I ($6.9 \pm 1.6\%$) and II group ($6.1 \pm 1.5\%$) were significantly higher compared to III ($2.0 \pm 1.2\%$) ($p=0.016$)

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and $p=0.03$, respectively), but it was similar in I and II group. After two years follow-up average GFR reduction of the all patients was 10.1% (53.1% of first, 36.7% of second, and 24.1% of third group, 22% in macroalbuminuric, 12% in microalbuminuric, and 4% in normoalbuminuric patients). Risk for decline of GFR is 4 times higher in patients with proteinuria >1 g/dU.

Conclusion: Decline of GFR is faster in those with macroalbuminuria and/or proteinuria >1 g/dU. Baseline albuminuria and proteinuria are important risk factor for kidney failure in diabetic patients, and provides a means to identify patients at greatest risk.

PT19

SERUM VASPIN CONCENTRATIONS IN HUMAN OBESITY AND TYPE 2 DIABETES

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Introduction: Vaspin was identified as an adipokine with insulin-sensitizing effects, which is predominantly secreted from visceral adipose tissue. It has recently shown that vaspin mRNA expression in adipose tissue is related to parameters of obesity and glucose metabolism. The regulation of vaspin serum concentrations in human obesity and type 2 diabetes remains to be established.

Methodology: For the measurement of vaspin serum concentrations, we use an enzyme-linked immunosorbent assay (ELISA). Using this ELISA, we assessed circulating vaspin in a study of 82 subjects with a wide range of obesity, body fat distribution, insulin sensitivity, and glucose tolerance and in 40 individuals with normal glucose tolerance (NGT) and diabetes type 2.

Results: Vaspin serum concentrations were significantly higher in female compared with male subjects. There was no difference in circulating vaspin between individuals with NGT and type 2 diabetes. In the NGT group, circulating vaspin significantly correlated with BMI and insulin sensitivity.

Conclusion: In this study we found a sexual dimorphism in circulating vaspin levels. Elevated vaspin serum concentrations are associated with obesity and impaired insulin sensitivity. Type 2 diabetes shows no correlation between increased circulating vaspin, higher body weight, and decreased insulin sensitivity.

PT20

DECREASED PARAOXONASE ENZYME ACTIVITY AND INCREASED SMALL DENSE LDL REVEALS FORMATION OF AN ATHEROGENIC RISK IN DIABETIC GUINEA PIGS

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Introduction: Cardiovascular disease (CVD) is the leading cause of death in diabetes mellitus (DM). Low density lipoprotein (LDL) phenotype, enzyme alterations in reverse cholesterol transport and remodeling of plasma lipoproteins can contribute to CVD. Therefore, this study aimed to investigate LDL subfraction distribution as well as serum cholesteryl ester transfer protein (CETP), lecithin-cholesterol acyltransferase (LCAT) and paraoxonase (PON1) activity in diabetic guinea pigs.

Methods: Male guinea pigs were given a single intraperitoneal (ip) injection of streptozotocin (STZ) and animals having fasting blood glucose levels greater than 200 mg/dL, measured 3 days after STZ injection, were considered diabetic. Protein levels of LCAT and CETP were determined via ELISA. Paraoxonase activity was measured kinetically by the formation of phenol while LDL subfraction analysis was done by disc polyacrylamide gel electrophore-

sis. Results: Plasma glucose and high density lipoprotein (HDL) cholesterol were significantly increased while total cholesterol and LDL cholesterol were significantly decreased in diabetic guinea pigs compared to controls. LDL subfraction analysis revealed a significant decrease in non-atherogenic LDL-2 subfraction and a significant increase in atherogenic LDL-4 subfraction in diabetic guinea pigs compared to controls. Plasma CETP and PON1 levels were significantly decreased while LCAT showed no significant difference in diabetic guinea pigs compared to controls.

Conclusion: Decreased non-atherogenic LDL-1, LDL-2 subfractions, increased small dense LDL-4 subfraction and decreased PON1 activity, which protects against LDL oxidation, reveals formation of an atherogenic risk in diabetic guinea pigs. The decrease in CETP levels supports the observed increase in HDL cholesterol levels in diabetic guinea pigs.

PT21

HYPERBARIC OXYGEN THERAPY EFFECTS ON GLUCOSE, TRIGLYCERIDES AND CHOLESTEROL SERUM LEVELS IN DIABETES MELLITUS TYPE II PATIENTS

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Introduction: Previous studies indicate a favorable effect of the hyperbaric oxygen therapy (HBOT) application on glucoregulation and inflammatory

markers. The aforementioned effects of HBOT in patients with diabetes mellitus (DM) may mean decrease in atherogenesis progression. The results

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of this pilot study showed the effect of HBOT on fasting serum glucose, cholesterol and triglyceride levels in DM.

Methods: The study included 41 patients with diabetes mellitus type II. All patients underwent HBOT with 100% oxygen under high pressure for one hour during two weeks, five days a week. Fasting serum glucose, cholesterol and triglycerides levels were measured before the first HBOT treatment and after the tenth treatment.

Results: After two weeks of HBOT decrease of glucose (9.7 vs. 8.0 mmol/L; $p=0.022$), total cholesterol

(6.62 vs. 6.06 mmol/L; $p=0.015$) and LDL cholesterol (4.99 vs. 4.4 mmol/L; $p=0.013$) serum levels were found in patients with DM type 2. At the same time the changes in HDL cholesterol (1.22 vs. 1.37 mmol/L; $p=0.66$) and triglycerides (1.76 vs. 1.58 mmol/L, $p=0.089$) serum levels were insignificant.

Conclusions: HBOT decreases fasting serum glucose levels as well as proatherogenic lipids levels, which might have multiple beneficial effects in patients with diabetes mellitus type 2.

PT22

ELEVATED ADENOSINE DEAMINASE ACTIVITY IN DIABETIC DISTAL SYMMETRICAL POLYNEUROPATHY

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Diabetes mellitus is a major cause of peripheral neuropathy, commonly manifested as distal symmetrical polyneuropathy. Adenosine, a degradation product of ATP, protects the organ systems against ischemia-induced damage and also limits the degree of vascular injury after ischemia by reducing the release of reactive oxygen species.

Adenosine deaminase (ADA) is the enzyme which catalyses the deamination of adenosine and plays an important role in modulation of insulin action on glucose metabolism in various tissues, but its clinical significance in diabetic polyneuropathy is still studied.

This study involved 42 patients with type 2 diabetes mellitus and signs of distal symmetrical polyneuropathy, as well as the control group of 40 healthy subjects. The evaluation of diabetic distal symme-

trical polyneuropathy (DDSP) was based on physical examination and nerve conduction studies.

Laboratory analyses involved blood glucose and HbA_{1c} levels, as well as plasma ADA activity. Serum glucose and HbA_{1c} levels were significantly higher in DDSP patients versus the control group ($p<0.001$). The activity of ADA revealed a significant increase in the plasma of patients with DDSP opposite to healthy donors ($p<0.001$). There was statistically significant positive correlation between ADA activity and glucose value ($p<0.001$). A significant positive correlation was noticed between the activity of ADA and HbA_{1c} levels ($p<0.01$).

The obtained results suggest a probable pathogenic role of elevated ADA activity in the sera of patients with diabetic distal symmetrical polyneuropathy.

PT23

INSULINE RESISTANCE IS ASSOCIATED WITH NONALCOHOLIC FATTY LIVER DISEASE AND METABOLIC SYNDROME IN OBESE SUBJECTS

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is considered to be an integral part of the Metabolic Syndrome (MS) with insulin resistance (IR) as a central pathogenic factor. The aim of our study was to investigate whether IR is associated with NAFLD and MS in obese subjects.

Materials and Methods: A cross-sectional study performed on 118 consecutive subjects (37 males, 81 females, median age 44, and range 26 – 68 years) in Tirana, Albania, during 2010; mean body mass index (BMI) 38.8 ± 3.7 kg/m². Serology was negative for viral and autoimmune hepatitis; alcohol consumption was less than 20 g/day in all subjects. No evidence of metabolic liver disease was found on any subject. Insulin resistance (IR) was calculated according to homeostatic model assessment (HOMA), when HOMA was >3. The diagnosis of NAFLD was based on ultrasonography and serum ALT (>2xN). MS was defined by ATP III, as a combi-

nation of 5 risk factors [waist circumference >102 cm (M) or >88 cm (F), fasting glucose ≥ 110 mg/dL, triglycerides ≥ 150 mg/dL, HDL-cholesterol <40 mg/dL (M) or <50 mg/dL (F) and blood pressure $\geq 140/90$ mmHg]. Three or more of these criteria identified MS.

Results: NAFLD was found on 57 (48%) of our subjects and MS on 71 (60%) of them. IR was significantly associated with NAFLD (HOMA 12.11 ± 3.4) and MS (HOMA 2.3 ± 3.3). Presence of NAFLD and MS increased with BMI. Predictive value for NAFLD were calculated by BMI >35 kg/m², ALT level >2.5 N and triglycerides >171 mg/dL; while for MS, BMI >40 kg/m², triglycerides >179 mg/dL and fasting glucose >121 mg/dL.

Conclusions: These results suggest that IR is significantly associated with NAFLD and MS in obese subjects.

PT24

IMPORTUNES OF REGISTERING HbA_{1c} AND ELECTRONEUROGRAPHY IN PATIENTS WITH DIABETIC NEUROPATHY

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Background: Diabetes mellitus (DM) is one of the most widespread chronic non-infective diseases of the modern age. To assess long-term metabolic controls, beside glycemia findings, the analysis of glycosylated hemoglobin (HbA_{1c}) is most significant. The aim of our study was to assess the correlation

between the parameters of good metabolic control, primarily HbA_{1c} and neurophysiologic parameters in patients on intensified insulin therapy.

Methods: We assessed 81 eligible patients (27 women, 54 men), with diabetes mellitus type 1 with

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poor metabolic control. Metabolic control parameters (glycemia, HbA_{1c}, cholesterol, HDL, LDL and triglycerides) and electrophysiological investigation (motor conduction velocity (MCV) on the peroneal, tibial and median nerves, sensory conduction velocity (SCV) on the sural nerves, and late response latencies) were assessed twice during a three months period.

Results: Metabolic control parameters at the beginning of study: glycemia 9.10 ± 3.69 mmol/L, HbA_{1c} 65 mmol/mol, cholesterol 5.66 ± 1.79 mmol/L, HDL-Cholesterol 1.64 ± 0.46 mmol/L, LDL 3.14 ± 0.91 mmol/L and triglycerides 1.67 ± 1.04 mmol/L. After 3 months: glycemia 7.88 ± 2.79 mmol/L, HbA_{1c} (49 mmol/mol), cholesterol 5.21 ± 1.47 mmol/L, HDL-Cholesterol 1.49 ± 0.42 mmol/L, LDL 2.89 ± 0.93 mmol/L and triglycerides 1.62 ± 0.99 mmol/L. There was no statistically signifi-

cant difference in glycemia findings after 3 months of follow-up, but from clinical view it is near by good regulation value (from 9.10 up to 7.88 mmol/L. Mean insulin dosages, HDL and LDL showed a statistically significant difference. Mean HbA_{1c} rate showed a statistically significant difference ($p < 0.05$). Lowered HbA_{1c} (from the initial 8.12-6.63% (65–49 mmol/mol) as highly significant from the clinical aspect. Taking into consideration the rates of HbA_{1c} and glycemia we could be satisfied with their lowered values and regulation within the follow-up period of 3 months. Statistically significant improvement was found for MCV for tibial and median nerve, for SCV and for H-reflex latencies.

Conclusion: We can conclude that three months period of the metabolic parameters regulation could be related to the registered improvement of electrophysiological parameters.

PT25

THE RELATION BETWEEN SERUM FRUCTOSAMINE AND ISCHEMIA-MODIFIED ALBUMIN LEVELS

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Aim: Mechanisms of diabetic complications are not clearly elucidated although factors including toxic effects of high glucose levels, oxidative stress and hypoxia-ischemia have been proposed to be responsible. Control of hyperglycemia in diabetes (DM) is important in prevention of morbidity and mortality. Serum fructosamine level, a biochemical parameter of level of glycosylated plasma proteins, reflects the mean glucose level in previous 2–3 weeks in DM patients. Ischemia-modified albumin (IMA) levels have recently been used frequently as a marker of ischemia in several diseases. In the present study we investigated relation between serum IMA and fructosamine levels in DM patients.

Material and Methods: We measured serum IMA and fructosamine levels with spectrophotometric methods in 73 type 2 DM patients and investigated the correlation between their levels.

Results: Fructosamine levels were increased (>285 mmol/L) in 24 patients and within normal limits (<285 mmol/L) in 49 patients. Mean ages of patients with increased and normal fructosamine levels were similar (57.2 ± 8 vs. 51.3 ± 14 years, $p > 0.05$). Mean fructosamine (350.5 ± 62.2 vs. 239.2 ± 30.9 mmol/L, $p < 0.001$) and IMA (106.6 ± 39.9 vs. 90.5 ± 22.5 U/mL, $p < 0.05$) levels were significantly higher in patients with increased as compared with normal fructosamine levels. Serum fructosamine and IMA correlated significantly ($r = 0.53$, $p < 0.01$) only in patients with increased fructosamine levels.

Discussion: In the present study we found an increment in ischemic status in parallel with deterioration of glycemic control in type 2 DM suggesting that serum IMA levels might be a useful parameter in follow up and evaluation of glycemic control and ischemia-induced complications in DM.

PT26

MICROALBUMINURIA AT PATIENTS WITH DIABETES MELLITUS

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Introduction and objectives: Microalbuminuria (MA) is defined as excretion of between 30 mg and 300 mg of albumin per day in the urine in at least two subsequent samples. Presence of permanent microalbuminuria represents the earliest phase of kidney damage in diabetes, which precedes the clinically manifested diabetes nephropathy. The objective of this paper is to determine the influence of the patients' age and the duration of diabetes to the appearance of the microalbuminuria at patients with T₁DM and T₂DM.

Methods: 150 patients have been examined, 80 with T₁DM while 70 with T₂DM. Microalbuminuria was determined in 24 h collected urine using the method of nephelometry immunoassay (DCA 2000).

Outcomes: Microalbuminuria has been diagnosed at 36% of the patients with T₁DM and 45% of patients with T₂DM. Patients with T₂DM and MA are statistically significantly older than other ones

($\bar{x}_{T_2DM}=59.20\pm11.60$; $\bar{x}_{T_1DM}=31.3\pm11.32$ yrs), while DM has had the longer duration ($\bar{x}_{T_2DM}=11.60\pm8.20$; $\bar{x}_{T_1DM}=9.52\pm6.42$ yrs). The occurrence of microalbuminuria among T₁DM patients gets higher with the duration of the disease. It also has prognostic values: at 80% of patients with T₁DM and MA, albumin excretion in urine increases 10-20% per year followed by clinic development of proteinuria, which with time lead to the final phase of kidney disease. 20-40% patients with T₂DM and MA progresses into early nephropathy but in 20 years time only about 20% of the patients develop the final form of kidney diseases.

Conclusion: Patients with DM and MA are at a higher risk of developing CVD. Annual MA determination for the patients with no signs of clinical proteinuria should be started during the period of puberty or after the period of 5 years after the diagnosis of T₁DM has been set, and at the moment of T₂DM diagnostics.

PT27

VITAMIN D (25 HYDROXY) LEVEL AND OTHER MARKERS IN PATIENTS WITH NEWLY DIAGNOSED DIABETES MELLITUS (DM)

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Introduction: A low 25-OH vitamin D level has been associated with increased risk of diabetes mellitus, with an epidemic character in obese population.

Objective: To estimate the significance of low 25-OH vitamin D level in patients with newly diagnosed DM.

Methods: Our study included 32 patients who were diagnosed with DM during last six months in Clinical Center of Montenegro, Podgorica, 5 with type 1 (T₁DM) and 27 with type 2 (T₂DM), 23 women and 9 men, from 16 to 84 years old and a control group of healthy people with similar characteristics. We used body mass index (BMI) to evaluate a degree of obesity (≤ 18.5 kg/m² malnutrition, 18.5–24.9 kg/m²

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normal range, 24.9–30 kg/m² overweight, ≥30 kg/m² obesity). The diagnose of DM was established on fasting blood glucose ≥7.0 mmol/L, or postprandial blood glucose ≥11.1 mmol/L. Serum 25-OH vitamin D level is measured by using electrochemiluminescence immunoassay (ECLIA). Depending on 25-OH vitamin D serum levels patients were divided into three groups, deficiency ≤50 nmol/L, insufficiency 50–75 nmol/L and referent values ≥75 nmol/L. We have determined for all their general biochemical status with standard methods, from fresh serum, which was in limits of expected values. Cystatin C (mg/L) and hs CRP (mg/L) have been determined by immunonephelometry.

Results: Low 25-OH vitamin D level was found in all patients with newly diagnosed T₁DM: 60% (3) had deficiency and 40% (2) insufficiency. All of them had BMI in normal range. Concerning patients with

T₂DM low 25-OH vitamin D level was established in 83.9% (26), 69.2% (18) had deficiency and 30.8% (8) had insufficiency. Low 25-OH vitamin D level was found in even 53.1% (17) obesity patients with T₂DM, 58.8% (10) had deficiency and 41.2% (7) had insufficiency. Mean value for HbA_{1c} (mmol/mol) is 59.34, KV 18.4% vs 32.24, KV 8.63% (p<0.01); hs CRP (mg/L) is 1.03±0.77 vs 0.89±0.57 ±0.57 (p<0.05); Cystatin C (mg/L) is 0.99±0.32 vs 0.76±0.15 (p<0.01).

Conclusion: Our study showed that patients with both T₁DM and T₂DM had low 25-OH vitamin D level. Vitamin D supplementation may play an important role in prevention of DM. By measuring of the sensitive biochemical markers it is possible an early recognition of the disease and so a better chance for improvement and prognosis of the condition.

PT28

CORRELATION BETWEEN hsCRP AND LIPIDOGRAM IN DIABETIC AND NON-DIABETIC HEMODIALYSIS PATIENTS

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Introduction: The cardiovascular system in hemodialysis patients is under the influence of a number of well-known risk factors dependent uremia and dialysis. All of these risk factors predispose microinflammation, endothelial dysfunction and atherosclerosis, underlying cardiovascular disease.

Aim: The aim of this study was to examine the correlation between hsCRP, as a marker of microinflammation, and lipidogram in diabetic and non-diabetic hemodialysis patients.

Methods: The research was conducted on 53 patients (M:F 25:28) who are regularly treated by hemodialysis, the average age 56.47±11.79 years, average length of dialysis 5.33±4.48 years and average KT/Vsp index 1.12±0.15. Patients were separated in two groups: Diabetes mellitus (DM) group (10 patients-treated by statins) and non-diabetes (N) group (43 patients). In DM group: the average value

of serum hsCRP 5.50±9.70 (mg/L), HbA_{1c}-DCCT 6.93±1.71 (%), Chol 4.02±0.73 (mmol/L), Trig 1.10±0.43 (mmol/L), LDL 2.40±0.64 (mmol/L), HDL 1.11±0.40 (mmol/L) and apoB 0.71±0.22 (g/L). In N group: the average value of serum hsCRP 6.07±8.85(mg/L), HbA_{1c}-DCCT 5.22±0.31 (%), Chol 4.25±1.04 (mmol/L), Trig 1.50±0.61(mmol/L), LDL 2.66±0.88 (mmol/L), HDL 0.92±0.22 (mmol/L) and apoB 0.76±0.25 (g/L). Statistical analysis of the results was performed by the Spearman test for rang correlation and Students t-test.

Results: There is a high statistically significant negative correlation between the total concentration of hsCRP and HDL levels (p<0.01), but there is no correlation between levels of hsCRP and Chol, Trig, LDL and apoB in N group (p>0.05). There is no statistically significant correlation between the total concentration of hsCRP and levels of Chol, Trig, LDL, HDL and apoB in DM group (p>0.05). There is a sta-

tistically significant difference between levels of HDL, between patients in DM and N group (HDL 1.11 ± 0.40 vs 0.92 ± 0.22 mmol/L) ($p < 0.05$), but there is no difference between levels of hsCRP, Chol, Trig, LDL and apoB in these two groups (Chol 4.02 ± 0.73 vs 4.25 ± 1.04 mmol/L, Trig 1.10 ± 0.43 vs 1.50 ± 0.61 mmol/L, LDL 2.40 ± 0.64 vs 2.66 ± 0.88 mmol/L, HDL 1.11 ± 0.40 vs.

0.92 ± 0.22 mmol/L, apoB 0.71 ± 0.22 vs 0.76 ± 0.25 g/L) ($p > 0.05$).

Conclusion: hsCRP as a marker of microinflammation, endothelial dysfunction and atherosclerosis is a risk factor for development of cardiovascular complications in patients treated by hemodialysis. Non-diabetic group has higher levels of hsCRP, Chol, Trig, LDL and apoB, but lower levels of HDL.

PT29

ANALYSIS OF COMMON GENETIC POLYMORPHISMS ASSOCIATED WITH DYSLIPIDEMIA IN TYPE 2 DIABETIC PATIENTS FROM BOSNIA AND HERZEGOVINA

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The primary defect underlying lipoprotein abnormalities in Type 2 diabetes (T_2D) is insulin resistance, which leads to increased levels of triglycerides, dominance of small, dense low-density lipoprotein (LDL) and low high-density lipoprotein (HDL) levels. Recent genome-wide association studies identified approximately 60 loci influencing T_2D risk, with a particular interest in those affecting plasma lipid levels.

In this study, we examined the associations of variants in three candidate loci, including rs3890182 in ATP-binding cassette transporter A1 gene (ABCA1), rs1800775 in cholesteryl ester transfer protein (CETP), and rs17321515 in TRIB1 gene, with dyslipidemia and T_2D in population from Bosnia and Herzegovina. A total number of 136 subjects (61 T_2DM patients and 75 nondiabetic participants) were recruited in this study. Subjects were genotyped for

ABCA1 rs3890182, CETP rs1800775, and TRIB1 rs17321515 SNPs by using the MassArray Sequenom iPLEX platform.

Interestingly, our data demonstrated an association of TRIB1 rs17321515 polymorphism with lower LDL ($p = 0.012$) and total cholesterol levels ($p = 0.007$) in T_2D patients. Furthermore, ABCA1 rs3890182 and CETP rs1800775 polymorphisms showed a significant association with lower HDL-cholesterol levels in nondiabetic subjects ($p = 0.013$ and $p = 0.010$, respectively).

In conclusion, our data suggested a protective effect of TRIB1 variation in regards to the plasma lipid profile in diabetic patients. However, ABCA1 and CETP variants appeared to be associated with decreased HDL-cholesterol levels and thus, could be considered as risk alleles for lipoprotein abnormalities in T_2D .

PT30

COMPARATIVE DETERMINATION OF ANTI-GAD65/IA2 ANTIBODIES AND ANTI-ZnT8 ANTIBODIES IN PATIENTS WITH DIABETES MELLITUS

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Autoantibodies to pancreatic beta-cell antigens are important serological markers of DM1. The antigens recognized by these antibodies include insulin, glutamate-decarboxylase (GAD65), tyrosine-phosphatase-related protein islet antigen 2 (IA2) and Zinc Transporter 8. On contrary to GAD65 and IA2, ZnT8 is highly specific for beta-cells, so the goal of this study is to show if DM1 patients possess detectable level of ZnT8Ab comparing to antiGAD65/IA2Ab levels. In that way it would be possible to determine the importance of ZnT8Ab and its measurement as DM1 serological marker.

The study includes 58 subjects. 28 of these were control group healthy ones, 21 with DM1 and 9 with

DM2. AntiGAD65/IA2Ab and ZnT8Ab are specified by ELISA method.

Regarding the subjects in control group and those with DM2, in which the levels of both antibodies were below the cut-off, patients with DM1 almost all had increased levels of antiGAD65/IA2Ab (18 of 21) and among 15 of these had increased anti-ZnT8Ab. Statistical analysis of the results showed that there is a significant difference of antibodies levels between DM1 patients and control group, as well as between DM1 and DM2 patients.

According to statistical analysis of the results we can conclude that determination of ZnT8Ab is very important serological marker for DM1.

Hematology and haemostasis

PF01

UREMIC HEMOLITIC SYNDROME IN FOUR BROTHERS

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Introduction: Four male patients, brothers, coming from an unfavorable social economic group (indigents) and residents in the neighboring area were studied.

Their ages were 19 years old, twins 18 years old and 16 years old. At the start of this study they all showed general neglect as regards personal hygiene and health, presenting hematological values totally

altered: severe anemia, microcytosis with leucosis, accompanied with severe thrombocytosis. Also the values concerning urea, creatinine, LDH, CPK, phosphorus in blood and plasmatic ionogram were rather high accompanied with high fever.

The first measure was to move the children to a relatives home where they could have better hygienic, nourishing and building conditions.

Also their diet was radically changed.

Materials and method: During the first days they were helped with folic acid to raise the hemoglobin level, whose value was between 7 and 8.20 g/dL, to perform peritoneal dialysis to every child so as to improve the renal function and to prescribe antibiotic treatment.

At the next blood control it was clear-sited that slowly and gradually the values of uremia, creatinine and plasmatic phosphorus which dropped almost to normal values. That's why it was decided not to suspend the folic acid but to keep it until was confirmed by hematological values.

Results: After 30 days, when the corresponding hematological blood control was done, the children

had got the expected analytical values: hemoglobin values between 12 and 13 g/dL accompanied with normocytic elements and values of count of thrombocytes between $300-400 \times 10^9$.

Also the values of urea, creatinine and phosphorus were as expected.

Conclusion: By means of the diet a period in which these patients got folic acid they recovered their health, vitality and basically their right to fully enjoy a better quality of life thus achieving psycho-social stability.

It was successfully reached by the author's free attention concerning treatment, supply of medicines and the following of the corresponding medicines prescribed.

PF02

THROMBOCYTOPENIA IN LONGEVOUS PATIENTS UNDER TREATMENT WITH SODIUM ALENDRONATE

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Introduction: 20 old-aged patients diagnosed with osteoporosis and treated with sodium alendronate were studied. They are all above 65 years old. They belonged to middle class strata of society and were residents in an old people's home in the urban area, having poor nourishing habits.

At the start of this study 85% of the patients presented moderate to severe anemia, severe thrombocytopenia accompanied with microcytic elements and values of plasmatic homocysteine, CPK, LDH very superior to normal ones.

In the first place, it was decided to suspend the sodium alendronate, modify the nourishing diet inducing the ingestion of proteins, vegetables and dairy products nutrients and move the old people to another residence.

Materials and methods: During the first 40 days they were helped with succinic acid + ferrous succinate to raise the hemoglobin level which was between 9 and 9.80 g/dL and a minimum dose of

acenocoumarol to prevent thrombotic events, without using corticoids.

At the next blood control it was clear-sighted that the hematological homocysteine dropped to almost normal values. For this reason it was decided to suspend the succinic acid + ferrous succinate, but not to suspend the acenocoumarol which was kept in the time (adjusted to INR) and to allow the new diet to perform the corresponding supply.

Results: After 90 days, when the corresponding hematological blood control was done it was shown that the patients had got normal analytical values, the plasmatic homocysteine values had dropped to normal ones, the values of hemoglobin were between 12 and 13 g/dL, accompanied with normocytic elements and values of count of thrombocytes between $250-400 \times 10^9$.

Conclusion: The 80% of the patients in the case study who were under instructed treatment improved their prognosis as regards osteoporosis.

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Most important to get the hematological and expected serum values back to normal which resulted in significant improvement in the longevous pa-

tient's quality of life, their rights and psycho-social stability.

PF03

CYTOCHROME P450 (CYP2C9*2,*3) & VITAMIN K EPOXIDE REDUCTASE COMPLEX (VKORC1 1173C>T) GENE POLYMORPHISMS & THEIR EFFECT ON ACENOCUMAROL DOSE REQUIREMENT IN BULGARIAN PATIENTS

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Oral anticoagulant therapy based on vitamin K antagonists is the current mainstay for the prevention and long-term treatment of a variety of thromboembolic disorders. Many genetics determinants involved in the metabolism of acenocoumarol have been shown to influence the anticoagulant dosage.

The aim of this study was to determine the occurrence of CYP2C9*2,*3 and VKORC1 1173C>T gene polymorphisms and to study their effects on the dose of acenocoumarol required to maintain a target International Normalized Ratio (INR) in Bulgarian patients on anticoagulant therapy.

The genotypes of CYP2C9 variants including CYP2C9*2 and CYP2C9*3, and VKORC1 1173C>T were assessed for the association with acenocoumarol dosing in 99 patients whose data were collected for acenocoumarol dose; INR and other clinical characteristics. Determination of the studied

polymorphisms was done by High Resolution Melting Analysis.

The mean acenocoumarol daily dose requirement was higher in CYP2C9 homozygous wild-type patients, compared to those with the variant *2 allele ($P<0.001$), similar to those with the variant *3 allele ($P=0.044$) and highest in patients with the VKORC1 1173 CC genotype compared to those with the CT genotype and the TT genotype ($P<0.001$). Age was the only demographic factor significantly affecting acenocoumarol dose ($p=0.01$). In a multivariable analysis of variance CYP2C9 and VKORC1 genotypes and age explained 38.8% of acenocoumarol dosing variability.

The study findings point towards the role of CYP2C9 and VKORC1 gene polymorphisms in determining the inter-individual dose variability of acenocoumarol in the Bulgarian patients on anticoagulant therapy.

PF04

HYPERHOMOCYSTEINEMIA IN PATIENTS WITH DEEP VEIN THROMBOSIS

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Introduction: In patients with homocysteinuria, 50% of vascular complications account for vein thromboembolism. A large number of studies that have analyzed the association between hyperhomocysteinemia and venous thrombosis have shown an increased total homocysteine (tHcy) concentration in patients with deep vein thrombosis (DVT). The aim of this study was to determine the tHcy concentration by spectrophotometric cyclic enzymatic method, which nowadays is still not regularly used in routine biochemical laboratories in R. Macedonia, in healthy subjects and patients with deep vein thrombosis.

Material and methods: The investigation comprised a total of 160 subjects divided in two main groups:

80 healthy subjects (control group) and 80 patients with deep vein thrombosis. Concentration of tHcy was determined by spectrophotometric cyclic enzymatic method. Statistical analysis of the results was made by means of the Statistica version 7.1 program.

Results: The results obtained for plasma tHcy in the control group were $11.62 \pm 3.43 \mu\text{mol/L}$, while tHcy level was significantly higher in patients with deep vein thrombosis as compared to the control group, $15.19 \pm 3.63 \mu\text{mol/L}$ ($p < 0.001$).

Conclusion: Concentration of total homocysteine in patients with deep vein thrombosis was statistically significantly increased in comparison with healthy subjects.

PF05

MARKED MEGALOBLASTOSIS AS THE FIRST PRESENTATION OF ACUTE ERYTHROID LEUKEMIA (M6). Case report

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A 74 year old man was hospitalized with a two weeks history of severe weakness, anorexia, weight loss. He suffered of diabetes mellitus type II insulin dependent, and arterial hypertension. The physical examination showed cutaneous and conjunctival pallor, large ecchymosis in the low extremities and hepatosplenomegaly. CBC showed pancytopenia with: WBC $1900/\text{mm}^3$, RBC $1.040.000/\text{mm}^3$, Hb 4.1 g/dL , PLT $8000/\text{mm}^3$, MCV $126 \mu\text{m}^3$, MCH 36 pg ,

MCHC 37 g/dL , bands 8%, neutrophils 60%, lymphocytes 16%, monocytes 16%, normoblasts 4:100 and reticulocyte count 3%. Serum B12 and folates were elevated. DTC negative, ITC negative, LDH 1660 U/L , total bilirubin 1.1 mg/dL , total proteins 5.6 g/dL , glucose 388 mg/dL , and other parameters within the normal range. The bone marrow aspiration revealed: mieloblasts 6%, promyelocytes 1%, myelocytes 6%, metamyelocytes 6%, bands 5%, neutro-

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phils 4%, proerythroblasts 4%, basophilic normoblasts 4%, polychromatophilic normoblasts 4%, promegaloblasts 7%, basophilic megaloblasts 10%, polychromatophilic megaloblasts 36%, orthochromatic megaloblasts 7%

The rate myeloid/erythroid=1/3, showing the predomination of the erythroid line which constitute 65% of all cells.

The proportion of proerythroblasts and basophilic erythroblasts was 38% of all erythroblastic cells. The

proportion of megaloblastic forms was 80% of all erythroblasts.

PAS staining of bone marrow smears showed typical cytoplasmatic positivity of pathologic erythroblasts: granular in more immature cells and diffuse in more mature ones, confirming the diagnosis of erythroleukemia. Retrospectively even the course of the disease, support this diagnosis, as the patient had an extremely aggressive clinical deterioration and died ten days after admission.

PF06

EFFECTS OF OCT4, SOX2 AND NANOG EXPRESSION ON IMATINIB RESISTANCE IN CML

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Overview: OCT4 and NANOG are homebox transcription factors essential to the self-renewal of stem cells and are expressed in several cancers, the role of OCT4/NANOG signaling in tumorigenesis is still elusive. Infinitely self-renewing cells could conceivably account for the immortal nature of tumor cells at the population level, and accumulating evidence supports the notion that ESC self-renewal and pluripotency genes, including the transcription factor triad OCT4, SOX2 and NANOG serve as neoplastic engines driving oncogenesis. Chronic myeloid leukemia (CML) is a clonal disorder of the pluripotent hemopoietic stem cell, in which a reciprocal translocation t(9;22)(q34;q11) forms a Philadelphia (Ph) chromosome and creates a novel fusion gene, BCR-ABL. Imatinib mesylate is one of the most widely used drugs for CML. Imatinib resistance, occurring in nearly 33% of patients, has been associated with a variety of mechanisms, subclassified as BCR-ABL gene expression or mutations in

the BCR-ABL gene. This study is planned to determine the role of genes which engaged in self-renewal and pluripotency in imatinib resistance of K562r cells.

Methods: K562s (sensitive) and K562r (resistance) were grown in RPMI-1640. K562r cells were maintained in RPMI-1640 medium supplemented with 5μM imatinib. RNA isolation, cDNA synthesis, RT-PCR was performed respectively.

Results: The results showed that NANOG (3.7), OCT4 (2.7) and SOX2 (4.7) mRNA expression levels in K562r cells was higher than levels in K562s cells.

Discussions: According to our findings, enhancement of imatinib doses in K562r treatment increase expression level of NANOG, OCT4 and SOX2 (CSC biomarkers), so it can be suggested that up-regulation of these genes and CSC are responsible of imatinib resistance.

PF07

IMPORTANCE OF LABORATORY PARAMETERS ON PERY-PROCEDURAL BLEEDING AMONG PATIENTS WITH ACUTE MYOCARDIAL INFARCTION UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

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Introduction: Major bleeding is one of the most frequent and most severe complications of the primary Percutaneous Coronary Intervention (PCI). The aim of this study was to investigate laboratory parameters which are important for predicting the bleeding in patients treated for acute myocardial infarction with ST elevation (STEMI), who were subjected to primary PCI.

Methods: All consecutive STEMI patients (643) who were subjected to primary PCI between 11/2006 and 7/2009 were analyzed. Major bleeding was defined according to the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) study criteria. In this study, basic laboratory parameters were analyzed.

Results: Major bleeding occurred in 30 patients, of total 643 STEMI patients who were subjected to

primary PCI (4.7%). Patients with major bleeding were older, more frequently female had lower body weight, hemoglobin level and creatinine clearance, as well as elevated white blood cell count at admission. Multivariate logistic regression was used to distinguish laboratory parameters as predictors of major bleeding: low hemoglobin (Hb) levels on admission (<120 g/L for women and <130 g/L for men) (OR=4.01; 95% CI for OR 1.57-10.22; p=0.004), low creatinin clearance (<60 mL/min) (OR=3.36; 95% CI for OR 1.47-7.71; p=0.004) and elevated white blood cell count at admission (OR=1.11; 95% CI for OR 1.01-1.22; p=0.029).

Conclusion: We demonstrated that low hemoglobin level, low creatinine clearance as well as higher white blood cell count at admission are at particularly high risk of bleeding.

PF08

RETICULATED PLATELETS AS A MEGAKARYOCYTIC ACTIVITY

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Platelets play fundamental role in haemostasis. They are formed by fragmentation of megakaryocytes, during maturation of this progenitor cells. Group of cells, called reticulated platelets, represent the youngest form of circulated platelets.

This study gave us important information about megakaryocytic activity in acute and chronic immune thrombocytopenia.

Results were obtained from 26 patients, divided in two groups according to the duration of low plate-

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let count: 14 patients with chronic ITP and 12 in acute state of disease and 20 healthy volunteers, as control group. Total platelet count and percentage of reticulated platelets were measured on Cell Dyn Sapphire, Abbott Diagnostics, by flow cytometry.

In control group mean values for total platelet count (PLT), mean platelet volume (MPV) and percentage of reticulated platelets (rPLT) were $232.0 \times 10^9/L$, 8.06 fL and 1.776%. PLT was significantly lower in patients with acute ITP ($14.37 \times 10^9/L$) and with chronic ITP, also ($64.73 \times 10^9/L$). Differences in MPV between participants with acute (12.32 fL) and chronic ITP (10.55 fL) were not statistically sig-

nificant ($p=0.173$). Percentage of rPLT was significantly higher in patients with acute (18.780%) compared to those with chronic ITP (5.571%).

Percentage of rPLT might be of high significance in understanding the pathophysiologic pathway of thrombocytopenia because, as reported in recently published expert recommendations, ITP is now recognized as a disease that involves both platelet destruction and suboptimal platelet production. Standard diagnostic algorithm for patients with low platelet count should include determination of reticulated platelets as a measure of platelet synthesis.

PF09

ANEMIA IN I.V. DRUG USERS FROM 14-26 YEARS OLD AT “QUO VADIS VLORA CENTER”

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Background: The blood parameters control in i.v. drug users from 14–26 years old at “Quo vadis Vlora Center” is important for discovering and treating the anemia on time. The aim of this study: is to evaluate the values of Hb, HCT and MCH, MCHC, RDW, Ferritin and iron as parameters for identification of anemia.

Material and methods: In our study we have 40 i.v. drug users for exams of blood parameters, separated in 2 groups. The first group contains 35 boys from 14–26 years and the second group is 5 girls from 14–26 years. The parameters are measured using an ABX micro 60 cell-counter analyze and EKO autoanalyzer. Anaemia is defined by the World Health Organization as haemoglobin (Hb) <120 g/L in woman and Hb <130 g/L in men.

Results: For 30 i.v. drug users from the first group we took this values: for Hb=130 g/L, for HCT=39% and for MCV=80 μ^3 , and for the second group we took for Hb=104 g/L, for HCT=31.2% and for MCV=72 μ^3 . In 5 i.v. drug users from the first group we have discovered these values: Hb<110 g/L, HCT<33% and MCV=70 μ^3 , In the second group 2 of them have these values: HB<100 g/L, HCT<30% and MCV<55 μ^3 .

Conclusion: We can conclude that like hepatitis B, C, AIDS, even anaemia is found in the i.v. drug users. It's necessary to make regular controls of blood parameters in i.v. drug users, where the anemia is not so rare, in order to realize adequate treatment of it and to insure healthy condition.

PF10

ANTERIOR PITUITARY HORMONES (SOMATOSTATIN, PROLACTIN) REGULATE ANTICOAGULATION MARKERS IN THE RAT

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Introduction: Growth hormone and prolactin are postulated to exert multiple effects on various organs and systems. However, little is known about their interaction with haemocoagulation and anticoagulation.

This study was aimed to investigate growth hormone (GH), somatostatin (SST) and prolactin (PRL) effects on some factors of the anticoagulation system in the rat.

Methods: GH (0.2 mg/kg body mass), SST (0.1 mg/kg body mass), GH combined with SST-pre-treatment in the same doses, and prolactin (0.2 mg/kg body mass) were injected subcutaneously in 6 groups male Wistar rats for three consecutive days. Integral indexes of hemocoagulation: activated partial thromboplastin time (APTT), thrombin time (TT), reptilase time (RT), thrombin-coagulase time (TCT) and anticoagulation factors:

antithrombin III (ATIII), residual thrombin activity (RTA) were estimated by Dade Behring, and Stago Diagnostica tests and CoagMateXM coagulometer.

Results: SST, GH+SST, and PRL shifted all general coagulation indices investigated towards hypercoagulability ($p < 0.001$), while GH applied alone illustrated opposite effect on the same parameters ($p < 0.001$). SST, GH+SST and PRL decreased significantly ATIII and increased RTA ($P < 0.001$). GH applied alone elevated ATIII ($P < 0.001$) and suppressed RTA ($p < 0.001$).

Conclusion: The data received, indicate the GH, SST, PRL are substantially involved in anticoagulation, as GH applied alone favors hypocoagulability at least through activation of anticoagulation factors ATIII and heparin, while SST, PRL and GH with SST-pre-treatment, exert the opposite effects.

PF11

THYROID FUNCTION IN THALASSEMIA MAJOR

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Background: Thalassemia major is one of the most common pathologies in the Western Lowlands of Albania. The combination of therapy and transfusion has significantly prolonged the life of thalassemic patients. On the other hand, frequent blood transfusions can lead to increased levels of ferritin which is associated with endocrine complications. The aim of this study was to identify the prevalence

of endocrine disorders in thalasemic patient's correlation between levels of ferritin and hemoglobin, ferritin and thyroid hormone.

Methods: 101 patients that had received frequent transfusion in Hemoglobinopathies Center in Lushnje participated in this study. All patients were examined; Hemogram was determined by Mythic

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18, ferritin, TSH, FT4 were measured by ECLIA method using Elexys 2010 by Roche Diagnostic. The level of ferritin and hemoglobin were followed for 1 year period May 2012 to May 2013. The blood was collected in K3EDTA and heparin tubes. Patients were divided into four groups based on their age respectively 1–13 years old, 14–24 years old, 25–44 years old and >44 years old.

Results: Group I has presented with lower Hb levels, 6.5 ± 1.62 gr/dL. There is a strong correlation be-

tween FE–FT4 on group III, ($r=0.6$), there is no correlation between FE–FT4 ($r=0$) on group IV, FE–Hb ($r=-0.67$) on group III. The prevalence of hypothyreosis, is more strongly expressed on group IV (16%) whereas that of hyperthyreosis is expressed more on the patients of group I (16%).

Conclusion: In the Western Lowlands of Albania, endocrine disorders don't correlate with the frequency of transfusions.

PF12

THE TRANSFERRIN ROLE AS TRADITIONAL INDICATOR OF IRON STATUS IN THE DIAGNOSTIC OF IDA IN TERMS OF INFLAMMATION

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Introduction: Transferrin is iron-binding blood plasmaglycoprotein that control the level of free iron in biological fluids. Transferrin, as a protein that transports iron, is elevated in iron-deficiency anemia, indicating that the body needs more iron. Transferrin is also associated with the innate immune system and the level of transferrin decreases in inflammation as a protein of acute phase reacted. This fact made difficult to diagnose IDA in terms of inflammation.

Materials and methods: In our study are included 187 subjects, 156 patients and 31 control group. Anemia was defined as hemoglobin below 12 g/dL in females and 13 g/dL in males. Anemic patients were further evaluated for the type of anemia with iron profile, peripheral blood smear, reticulocyte count. The laboratory measurements were performed in ABX, Olympus AU 640, and Immulite 1000. Independent Sample Test, Anova test, Chi-Square Tests was used for statistical analysis.

Results: From the comparison of the concentration of transferrin in the serum between ACD and IDA, ACD and ACD/IDA, and of these groups with the

control group, we have noted that these values were higher in IDA (344.2 ± 54.6) vs ACD (241.0 ± 51.8) vs ACD/IDA (225.6 ± 48.9). 68.4% of the cases with IDA had increased value of transferrin and 31.6% normal values, whereas in ACD 25% had decreased values and 72.7% normal values. In ACD/IDA 46.2% had decreased values and 53.8% normal values. So we see that we have cases where the difference of ACD and IDA is light based on the values of transferrin, but there are other cases when these values don't differentiate these two kinds on anemia because they are normal in both cases. This diagnosis becomes impossible when we compare ACD with the cases when IDA is an associate of ACD. In this case, based on the ANOVA test, these changes aren't significant ($p>0.05$). This is also confirmed by the fact that for the values of transferrin ≥ 361 mg/dL we have a low sensitivity to discover IDA (52.6% and a 100% specificity based on ROC curve, AUC:0.904)

Conclusion: the concentration of the transferrin in the serum doesn't contain a disputable value in the discovery of IDA in the conditions of inflammation.

PF13

THE EVALUATION OF THE PLATELET NUMBER IN THE HEMOGRAM OF PATIENTS DIAGNOSED WITH ACUTE MALARIA IN ALBANIA (OUR EXPERIENCE)

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Anemia, thrombocytopenia and leucopenia isolated or combined occur frequently in the hemogram of patients with acute Malaria.

Purpose of the study: The study of platelet number in the hemogram of patients diagnosed with acute Malaria.

Material and method: We studied hemograms of 15 patients diagnosed with acute Malaria in the Clinical Laboratory of UHC "Mother Theresa" during March 2010-May 2013. 3 of the patients studied were diagnosed incidentally with Malaria (Vivax) during routine examination of peripheral blood smears. These cases were imported from Greece. 12 other cases of which 11 are diagnosed with Falciparum Malaria and 1 with Oval Malaria were clinically suspected because patients were returning

from Guinea. These patients were diagnosed after examination of the thin film and thick film of peripheral blood for Malaria. All blood cell parameters of all patients were measured in cell counter SYSMEX.

Results: Thrombocytopenia was present in 100% of cases studied. The number of platelets ranged from 48×10^3 — $110 \times 10^3/\text{mm}^3$. In patients with Malaria caused by Plasmodium Falciparum the platelet number ranged from 48×10^3 — $79 \times 10^3/\text{mm}^3$.

Conclusion: Thrombocytopenia is a common finding in the hemogram of patients diagnosed with acute Malaria. Thrombocytopenia is more pronounced in patients with Malaria caused by Plasmodium Falciparum.

PF14

MEAN RETICULOCYTE VOLUME IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA

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Background: Over half of oncology patients develop anemia and it is always present in hematological neoplasia. In these patients the anemia is multifactorial and several mechanisms may occur simultaneously in the same patient. Thus, in some cases the exact etiology of the anemia is not well estab-

lished and new laboratory parameters are continuously reported as potential tools for anemia diagnosis. Parameters related to reticulocytes provide valuable information about erythropoietic activity and seem to be a much more sensitive indicator of sudden erythropoietic changes than conventional

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red blood cell indices. In this work we evaluated the mean reticulocyte volume (MRV) in patients with B-cell non-Hodgkin lymphoma (B-NHL).

Material and Methods: 17 male patients with diagnosed B-NHL and with haemoglobin <10 g/dL were processed for reticulocyte counts in a Beckman Counter. Reticulocytes were also evaluated for 25 patients with diagnosed iron deficiency anemia (IDA) and 15 healthy men (control group).

Results: Obtained results have show that B-NHL patients presented an MRV of 108.5 ± 7.2 fL and Iron

Deficiency Anemia group an MRV of 78.8 ± 6.3 fL. The statistical analysis demonstrated that MRV was significantly higher in the B-NHL patients than in the IDA patients ($p < 0.001$).

Conclusions: The results suggest that MRV may be used as an additional tool in establishing the anemia etiology in B-cell non-Hodgkin lymphoma patients and distinguishing from patients with Iron Deficiency Anemia.

Laboratory endocrinology

PT32

PREVALENCE OF THYROID ANTIBODY IN PATIENT WITH THYROID DYSFUNCTION

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Introduction: Hypothyreosis is one of majority of thyroid disease. Most of this problem is autoimmune occur approximately ten times more frequently in women than in man. We determined values of Anti-TPO (antiperoxidase thyroid antibody) in suspect in patient clinically for disorders in thyroid function

Methods: In period from January 2011 till January 2013 we have examined 325 patients in total, clinically suspected for damage of thyroid function. We

have been determined values of TSH, anti-TPO. The technique used is chemiluminescence offering from instrument IMMULITE and the range normal was for anti-TPO <35 IU/mL, TSH 0.4–4.2 IU/mL.

Results: 107 from 325 cases clinically suspected for damage of thyroid function had high titer of anti-TPO, from those 13 were man and 94 were women

Conclusion: Our study goes in favor of using of anti-TPO in screening of thyroid disorders.

PT33

INDIRECT DETERMINATION OF REFERENCE INTERVALS CONFIRM AGE- AND POPULATION-SPECIFIC CHANGES IN THYROTROPIN VALUES

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Introduction: Traditional determination of thyrotropin (TSH) reference intervals does not reflect age- and population-specific changes. The aim of this study is to estimate TSH reference intervals with indirect method.

Methods: We analyzed 66842 results for TSH that was stored in our laboratory information system between 2008 and 2011. We calculated 2.5th and 97.5th centiles separately for male, female, inpatients, outpatients and decades of life.

Results: We found no statistical significant difference in 2.5th and 97.5th centiles for male and fe-

male. Analyzed inpatients had significantly lower 97.5th centile than outpatients (2.92 mIU/L vs. 3.92 mIU/L, $p < 0.05$). Overall results indicate significant increase in 97.5th centile with ageing, taking into account relationship between calculated z and critical z values for patients older than 70 (25.16 vs. 10.95, $p < 0.05$).

Conclusions: With indirect determination of TSH reference intervals laboratory can provide clinicians more accurate age-and-population-specific TSH values, which should improve patients care.

PT34

FOLLICLE STIMULATING HORMONE (FSH) AS A PREDICTOR OF LOW OVARIAN RESPONSE IN ASSISTED REPRODUCTION

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Background: Women with an elevated FSH level have poor response to ovarian stimulation leading to a lower pregnancy rate in treatments of assisted reproduction. The aim of this study was to evaluate and compare the role of serum FSH concentration among patients who had a normal basal FSH level with outcomes of patients with an increased FSH level on third day of cycle.

Methods: For this research, 20 patients undergoing In vitro fertilization (IVF) cycle were analyzed on third day of the cycle. Of these, 10 had normal basal FSH levels (< 10 IU/L), and 10 had increased basal FSH levels (≥ 10.00 IU/L). We used Immulite 1000 (Siemens), for quantitative measurement of FSH in

serum. Oocyte retrieval was performed 36 hours after hCG injection. Embryo transfer was performed 2–3 days later.

Results: This study showed that women with higher FSH levels were associated with less number of oocytes retrieved, less number of oocytes fertilized and less number of embryos obtained ($p < 0.05$). In group with increased FSH the average number of retrieved oocytes was low, (± 2.6), fertilized oocytes (± 1.6) and embryos (± 1.2) comparing the group with normal FSH which had higher average number of retrieved oocytes, (± 8.1), fertilized oocytes (± 6.2) and embryos (± 4.5). Implantation rate was higher in

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group with normal FSH (30%), comparing with group with increased FSH (10%).

Conclusion: FSH can be used to identify women who are very likely to perform poorly in assisted

reproduction process, probably because of diminished ovarian reserve.

PT35

NEWBORN SCREENING – WIDE PANEL, TESTING IN R. OF MACEDONIA

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PHI Adrialab Synlab, R. of Macedonia

Newborn screening for congenital hypothyroidism is obligatory in R. of Macedonia and it is covered by the Insurance Found. Beside the governmental screening program it is introduced, 4 years ago, by Synlab, wide panel of a newborn screening - detection of 24 metabolic disorders with one drop of blood on filter paper.

Robert Guthrie is pioneer for the earliest screening for phenylketonuria in 1960s using blood samples on filter paper obtained by pricking a newborn baby's heel on the second day of life to get a few drops of blood. Congenital hypothyroidism was the second disease widely accepted for newborn screening protocols in the 1970s. Guthrie and colleagues also developed bacterial inhibition assays for the detection of maple syrup urine disease and classic galactosemia. But the development of tandem mass spectrometry screening, as very sensitive method, in the early 1990s led to a large expansion of potentially detectable congenital metabolic diseases that can be identified by characteristic patterns of amino acids and acylcarnitines.

Wide panel of newborn screening is performed on the second pediatric examination of the newborn 36–72 h after delivery. Peripheral blood drop is used as sample material and it is collected on filter paper. Dry filter card is then used in the laboratory for testing. Reference laboratory for Adrialab is

Synlab Weiden, Germany. Few methods are used for analysis: tandem mass spectrometry, fluorescent immunoassay, radio immune assay and spectrophotometry. Results are performed within 7 days and with specialized comment are send back to the pediatricians. Positive results are not diagnosis. Additional confirmatory tests are provided as next step to final diagnosis.

Using this approach, 24 metabolic disorders are tested: amino acid disturbances (phenylketonuria, maple syrup disease, glutaraciduria etc.) defects in the metabolism of carnitine (fatty acids metabolism-MCAD, LCHAD, VLCAD etc.), adrenogenital syndrome, biotinidase deficiency, cystic fibrosis, galactosemia and hypothyroidism.

During period of 4 years 338 newborns were tested in Adrialab-Synlab. Positive were 4 newborns, two for hypothyroidism, one for biotinidase deficiency and one for phenylketonuria. Confirmation of the results and therapy with hormones/biotin/special diet, accordingly, followed the positive results.

Benefit from the wide programs for newborn screening are evident. Patients are detected in time and with adequate therapy serious consequences are avoided. We encourage the idea of obligatory wide panel for newborn screening for all newborns in R. of Macedonia. Our first results, we hope will be positive impulse in that direction.

PT36

DETERMINATION OF TWENTY-FOUR HOUR URINARY C-PEPTIDE

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Introduction: C-peptide is a polypeptide molecule built of 31 amino acids that is excreted in approximately equimolar amounts with insulin. It is eliminated unchanged and, due to its characteristics could be determined in twenty-four hour urine representing approximately 10% of the secretory capacity of β -cells of the pancreas.

Our study aims to assess the applicability of the method for determining this specific analyte as a biochemical marker of the secretory capacity of the endocrine pancreas.

Methods: This study included 44 patients suffering from type 2 diabetes (mean age 63.0 ± 8.0 years). Blood samples were taken from all the participants due to determination of glucose, insulin, HbA_{1c} and calculation of HOMA-S (secretory) and HOMA-R (resistance). Twenty-four hour urine was collected in order to measure c-peptide (automated system

ADVIA CENTAUR XP). All the results were statistically processed by Data Analysis package.

Results: Mean concentration of assayed urinary c-peptide was $37.58 \pm 37.00 \mu\text{g/dU}$. In diabetics, it was determined a statistically significant degree of linear correlation between the calculated values of HOMA-S and measured urinary C-peptide ($p < 0.01$). Also, there was a statistically significant linear correlation between the measured values of insulin and urinary C-peptide ($p < 0.01$).

Conclusions: Twenty -four hour urinary c-peptide could represent a reliable parameter and the accurate measure of endogenous secretion of β -cells in diabetics. It is especially suitable for patients who require longer monitoring of secretory capacity of the endocrine pancreas, patients with difficult venous access or children.

PT37

HYPERTHYROIDISM IN FEMALE POPULATION COMPARED TO THE OTHER THYROID DISEASES IN OHRID-STRUGA IN R. MACEDONIA

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Background: Hyperthyroidism is a condition of high concentration of thyroid hormones T3 and T4 in serum. In Macedonia we don't have official data for incidence of hyperthyroidism in female population. We have done this study to see the frequency and presence of hyperthyroidism in females in Ohrid – Struga region. Here we display our results for percentage participation of patients with hyperthyroidism compared to the other thyroid diseases,

derived from diagnoses which were carried out in our private medical institution and laboratory.

Methods: The diagnostic assessment of the thyroid gland done by thyreodologist was consisted of physical exam, thorough anamnesis of the patients. Thyroid ultrasound exam was done on every each of the patients and thyroid hormonal status was done be measuring the TSH and FT4 in blood serum (Abbott, Axsym, reference range for TSH

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0.47 mIU/L–4.64 mIU/L; FT4 9.14 pmol/L–23.81 pmol/L). For processing of obtained values for concentration of TSH and FT4, age of the patient and gender, and diagnosis, was used SPSS program for statistical data processing (IBM SPSS version 19).

Results: In a period of one year (01.2011–02.2012) in our laboratory were examined 826 patients, from which 473 were excluded due to insufficient data.

353 patients were analyzed from which 87.5% were female at age 13 to 85 year old. As the final result, 16.8% were diagnosed with hyperthyroidism.

Conclusion: As predicted at the start of the study, we can conclude that hyperthyroidism in our region is present in high percentage with emphasize on female population.

PT38

DEHYDROEPIANDROSTERONE SULPHATE AS A MARKER FOR SCHIZOPHRENIA

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Background: Studies suggest higher serum concentrations of dehydroepiandrosterone sulfate in patients with schizophrenia and it is assumed that this steroid has a role in the pathophysiology of schizophrenia.

Aim: To investigate the role of serum dehydroepiandrosterone sulfate in the pathophysiology of schizophrenia.

Material and method: In this clinical study were assessed for prospective morning serum concentrations of dehydroepiandrosterone sulfate in 50 subjects suffering from schizophrenic disorder, and 40 healthy subjects identical by sex and age. Dehydroepiandrosterone sulfate was determined by immunometric chemiluminescent assay (CLIA). Intensity of psychopathology was evolved with psy-

chiatric rating scales: PANSS (The positive and negative syndrome scale) and HAMD (Hamilton scale).

Results: Patients with schizophrenia showed significantly higher concentrations of dehydroepiandrosterone sulfate compared with healthy individuals. ($p < 0.001$). Values of dehydroepiandrosterone sulfate in the control group (3.7 ± 0.46) were lower than values of dehydroepiandrosterone sulfate in patients diagnosed with schizophrenia (4.99 ± 0.95). Study shows a positive correlation of the intensity of positive and negative schizophrenic symptoms. As the intensity of the depression.

Conclusion: Serum concentrations of dehydroepiandrosterone sulfate can be used as a biological marker for the diagnosis of schizophrenia.

PT39

THE VISFATIN LEVELS IN THYROID DYSFUNCTION

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Abnormal thyroid function is accepted to be associated with disordered production of hormones released by adipose tissue. Recent studies have shown multiple roles of hormones on visfatin expression and down regulation of visfatin expression by T3. In this study we aimed to investigate the relationship between thyroid functions and visfatin, an adipose tissue-derived protein. Twenty-seven patients with hyperthyroidism, 27 patients with hypothyroidism and 31 euthyroid subjects as control group were selected. Serum TSH, fT3, fT4, fasting glucose, lipid profile and visfatin levels were determined.

Serum visfatin levels were markedly higher in the hypothyroidism group (8.96 ± 4.27 ng/mL) as compared with the hyperthyroidism (5.8 ± 3.78 ng/mL) and control (3.57 ± 2.24 ng/mL) groups ($p < 0.0001$).

Groups of hyper- and hypothyroidism demonstrated a statistically significant difference

($p = 0.005$), hyperthyroidism and control groups showed no significant difference ($p > 0.0167$), hypothyroidism and control groups exhibited a significant difference ($p < 0.001$). According to this result, hypothyroidism group was found to show statistically significant difference from other groups in visfatin levels.

Visfatin, positively correlated with TSH, total cholesterol, LDL-C and negatively correlated with fT3 and fT4. We obtained parallel results with the studies of Ozkaya et al. and Caixas et al. visfatin is thought as a partly mediator to the effect of hyper/hypothyroidism on several metabolic parameters. Thyroid dysfunction may affect the visfatin clearance and may trigger visfatin secretion from visceral adipose tissue. The decrease in visfatin level due to thyroid hormones can be an additional result of the influence of thyroid hormones on the whole body metabolism.

Markers of liver and gastrointestinal diseases

PT40

HAEMOSTASIS INVESTIGATIONS AT PATIENTS WITH HEPATIC CIRRHOSIS

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Background: Cirrhosis hepatis is a chronic progressive liver disease. The morphological changes of the injured liver cause damages with different intensity in its various functions. These damages directly af-

fect the appearance of haemostatic disorders due to the liver role in the synthesis of clotting factors.

Aims: To show the haemostatic disorders in patients with advanced disease stadium (ascites) who

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were treated in our Daily transfusion hospital and in the Internal and Infection Diseases ward Units, with plasma components (fresh frozen plasma and human albumin - 20%).

Methods: From the Eastern part of Macedonia in the last nine years, 147 patients were hospitalized and treated with plasma components and at the same time hematological and biochemical analyzes were made of them. Also, haemostatic basic tests were made, such as: number of thrombocytes, the bleeding time (Duke), the prothrombin time (Quick) shown as prothrombin activity in percent and INR, the thrombin time activated partial thromboplastin time (aPTT), capillary resistance and level of fibrinogen in serum. Reagents from Siemens Company were used.

Results: In all these patients there was a decreased number of thrombocytes under $150 \times 10^9/L$ (in range of $90-115 \times 10^9/L$); the bleeding time (Duke) mildly extended up to normal (3–6 min.); protrombin time (Quick) strongly extended from 25.5 to 27.5 sec. or (45 to 40%) and INR from 2.16 to 3.5; the thrombin time and aPTT were in normal range; capillary resistance positive with 2++ and fibrinogen level in serum under normal (1-2 g/L).

Conclusions: With the disease advancement of hepatic cirrhosis, the haemostatic disorders become more and more manifested. Bruisings and bleedings are present due to the decreased productions of factors of prothrombin complex and also decreased thrombopoietin from progressive hepatosplenomegaly.

PT41

LIVER CIRRHOSIS AND HAEMOSTATIC ABNORMALITIES

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Aim: The liver has a central role in the clotting process and an altered haemostasis is common in advanced liver disease. The aim of this study was to evaluate the incidence and risk factors for thrombotic events (deep vein thrombosis and portal vein thrombosis) in patients with liver cirrhosis.

Material and methods: We studied 79 patients (aged 37–78) diagnosed with liver cirrhosis in Institute of Infectious and Tropical diseases between January 2012.–December 2012. The following variables were measured: platelets, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen level, antithrombin, protein C, protein S, plasminogen, D dimer, using standard coagulation tests.

Results: Platelet, antithrombin, protein C, protein S, plasminogen in group with liver cirrhosis were found lower but PT, aPTT, D-dimer were higher than the control group.

Conclusions: Our findings suggested that the abnormalities of coagulation and fibrinolysis system have some role in provoking thrombotic events in patients with liver cirrhosis.

PT42

HAEMOSTATIC DISORDERS AT SICK PEOPLE WITH LIVER CIRRHOSIS

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Introduction: Liver cirrhosis is a chronicle progressive disease of liver. Morphological changes make lower function of the cirrhosis at sick people (with liver cirrhosis) which directly reflex with present haemostatic disorders because of the basic function of the liver in making factors of coagulation.

Purpose: To identify haemostatic disorders at sick people with liver cirrhosis, which were threatened at daily transfusion hospital with plasma components - fresh frozen plasma and 20% human albumin.

Material and methods: At 73 people sick with liver cirrhosis were made the basic laboratory biochemical analysis and haemostatic tests: number of thrombocytes, bleeding time (according Duke), protrombin (according Quick) as protrombin activity given in percents and INR, trombin time and activated partial thromboplastin time, capillary resistance and retraction of the coagulum.

Results: At all sick people there was lower number of thrombocytes thrombocytopenia under $150 \times 10^9/L$, as result of the present congestive splenomegalia, as if the lower thromboprotein from the damaged hepar. Bleeding time, according Duke, gently extended to normal (3 min–6 min), capillary resistance with two plas, protrombin time, according Quick, hardly extended from 25.5–27.5 min in percents 45%–40% and INR from 2.16–3.5, trombin time and activated partial tromboplastin time in limits of normal. Basic laboratory biochemical analysis as: bilirubin, aminotransferases AST and ALT, GTT and serum Fe, were with higher values. The same were made in biochemical laboratory.

Conclusions: As if disease develops, complication at sick people can show. Haematomas and bleeding are shown as a result of the lower production of factors of coagulation and congestive hepatosplenomegalia.

PT43

SUCCESSFUL ANTIVIRAL TREATMENT DIMINISHES HEPATOCYTE NECROSIS IN PATIENTS WITH CHRONIC HEPATITIS C

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The goal of antiviral treatment in patients with chronic hepatitis C is a virologic cure preventing liver-related complications. The beneficial impact of therapy is in the decrease of aminotransferases levels to normal values. The aim of this study was to evaluate the occurrence of normalization of alanine

aminotransferase (ALT) activity as indicator of diminishing of hepatocyte necrosis and prediction of sustained viral treatment response. The data of 249 patients treated in the Clinic for Infectious and Tropical Diseases were analyzed. The results showed significant correlation between the normal

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ALT value and viral negativity (71.5% and 73.1%, respectively, $P < .0005$). The binary logistic regression revealed several significant variables for normalization of ALT: age older than 40, viral genotype 1, liver cirrhosis and high viral RNA as negative variables, and genotype 3 as the positive variable. Age over 40 was the most significant negative variable ($P < .0005$; OR=0.331; .122-.454) in multivariate regression

analyses. The difference between pre-therapy ALT values and normalization of ALT after therapy was not significant. This study confirmed that successful antiviral treatment causes lowering of ALT activity reflecting the diminishing of liver necrosis in the majority of patients. Also, normalization of ALT can be cautiously taken into account as the positive predictor for viral elimination.

PT44

BIOCHEMICAL AND CLINICAL MANIFESTATIONS OF THR PATIENTS GROUP SUSPECTED AS CARRIER OF HEMOCHROMATOSIS

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Aims: Serum iron load and ferritin parameters of the patients affected by liver disease and suspected for hemochromatosis disease.

Materials: 20 patient hospitalized in the Clinic of Gastrohepatology in Tirana Hospital, affected by liver disease (cirrhosis) (18 males and 2 females) were analyzed for serum iron load and ferritin using 5 mL blood from each patients. Moreover were collected 5 mL blood with K3 EDTA for each patient to be used for DNA test of HFE.

Methods: DNA extraction for EDTA blood was performed from 20 patients using QIAGEN extraction kit. Blood serum was collected and ferritin was analyzed using VidasFerritin kit (an automated quantitative test using the ELFA technique Enzyme Linked Fluorescent Assay) and miniVidas equipment. Serum iron load was analyzed using Colorimetric Chromazurol B, End point method and Minitecno analyzer.

Results: 4/20 20% of the patients represented increased ferritin and iron load (ferritin >1200 ng/mL and iron load from 171 mg/dL up to 214 mg/dL). 12/20 60% of the patients represented increased ferritin levels (from 151–1200 ng/mL). 6/20 30% of the patient represented increased iron load (171–214 mg/dL). Clinical manifestations were identified as cirrhosis and steatosis.

Conclusions: This group of patients is a part of about 100 patients that should be analyzed for hemochromatosis by DNA tests. We were interested to identify the presence of HFE mutations and correlation with ferritin and iron load in the patients affected by cirrhosis and other liver diseases. We have identified about 12 patients with increased ferritin levels (from 151–1200 ng/mL), that will be the first group analyzed by DNA HFE test.

PT45

LIPID PROFILE STATUS IN EROSIVE AND NON-EROSIVE REFLUX DISEASE

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Introduction: Lipid disorders are known risk factors for gastroesophageal reflux disease (GERD) -especially for erosive reflux disease (ERD) - has recently come to be recognized as a serious clinical problem. We sought to investigate the lipid profile status in ERD and non-erosive reflux disease (NERD) patients.

Material and Methods: Eighty five ERD patients (44 male/41 female) and 69 NERD patients (23 male/46 female) were enrolled. ERD and NERD patients were those who had heartburn and/or regurgitation with and without erosion(s), respectively. Blood samples were drawn for measurement of total Cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL). Data was compared among ERD and NERD patients.

Results: There were no significant differences between ERD and NERD patients in age (42.2 ± 14.3 vs.

43.4 ± 16.9 year; $p=0.653$), BMI (25.7 ± 4.7 vs. 25.3 ± 4.8 kg/m²; $p=0.573$), systolic blood pressure (87.0 ± 17.9 vs. 83.4 ± 11.9 mmHg; $p=0.174$) and diastolic blood pressure (131.7 ± 20.7 vs. 127.3 ± 16.5 mmHg; $p=0.176$). There was a significant increase in the levels of TC in NERD patients (212.0 ± 49.1 mg/dL) when compared to ERD patients (188.4 ± 52.3 mg/dL; $p=0.011$). No statistically significant difference was observed between ERD and NERD for TG (72.7 ± 39.3 vs. 86.3 ± 57.1 mg/dL; $p=0.119$), LDL (81.6 ± 28.7 vs. 82.3 ± 28.1 mg/dL, $p=0.894$) and HDL (33.6 ± 12.1 vs. 35.8 ± 12.2 mg/dL, $p=0.336$).

Conclusion: GERD could be taken as an indicator for the presence of metabolic abnormalities. We revealed that cholesterol disorders are associated with NERD symptoms and it seems to be a disease triggered by lipid imbalance.

Molecular diagnostic

PT46

MOLECULAR BIOMARKERS OF GLIOMA MALIGNANCY

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Glioblastoma constitute the most frequent and deadliest brain tumors of astrocytic origin. The average survival time is only 12–15 months.

The intra- and intertumor heterogeneity is a major obstacle in the treatment of malignant glioma. Knowing the exact molecular pathways involved in

the formation of these brain tumors could lead to the development of an individualized therapeutic approach.

The aim of this pilot study is to identify potential biomarkers indicative of the grade of malignancy in different forms of glioma. RT2 qPCR was used to

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analyze the expression of four genes (NF κ B, PIK3CG, PTEN, MAPK1) whose protein products play an important role in tumor proliferation and invasion. Tissue samples derived from the tumor lesion were studied in 20 patients – 4 with astrocytoma grade II, 7 with astrocytoma grade III and 9 with glioblastoma.

Only slight overexpression of the NF κ B gene was detected in high-grade glioma. Expression levels of PIK3CG were high but noticeably reduced in advanced-stage glioma in comparison to astrocytoma grade II. Interestingly, this was not governed by PTEN, a tumor suppressor and negative regulator of the mTOR pathway, since it showed normal levels in low-grade tumors. MAPK1 was underexpressed in

high-grade glioma suggesting an inhibitory crosstalk between the mTOR and the MEK/ERK pathways. Nevertheless, PIK3CG overexpression in astrocytoma grade II could not be explained by downregulation of the MEK/ERK pathway, since MAPK1 levels in these patients were normal.

Our results show that the relative quantitative transcriptional analysis of genes regulating tumor proliferation and invasion could provide important clues for determining an adequate therapeutic strategy in different patient groups.

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PT47

MEASUREMENT OF THE EXPRESSION VALUES OF BCR- ABL MBCR FUSION GENE TRANSCRIPT IN CML PATIENTS AT DIAGNOSIS

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Introduction: The development of new molecular markers and their application to everyday clinical practice improves the medical strategy of individualized therapy and translational medicine. The accurate measurement of BCR- ABL fusion gene expression in patients with chronic myeloid leukemia (CML) lead to individualized approach to each patient and tailored therapy using the first and second generation tyrosine kinase inhibitors, introducing the concept of pharmacogenetics into the disease prognosis.

Patients and methods: We analyzed the RNA isolated from peripheral blood of 19 CML patients, newly-diagnosed at the Clinics for Hematology, Clinical center of Vojvodina (KCV), applying the standardized molecular methods and quantitative real- time reverse transcriptase polymerase chain reaction (qRT-PCR), as recommended by European LeukemiaNet (EU organization of physicians and

biomedical scientists) in order to detect presence and provide normalized expression of the BCR- ABL MbcR gene in each sample.

Results: The BCR-ABL standard curve and the ABL standard curve equations were used to transform raw data into BCR-ABL MbcR and ABL copy numbers (CN). The ratio of these CN values gives the normalized copy number in diagnostic samples (NCN_{DX}), which is important pretreatment characteristic and a basis for monitoring response to initial therapy.

Conclusions: The main result of application of molecular methods in diagnostics of haematological malignant diseases is the improvement of the secondary prevention, adequate individualized therapy, including chemo-, radio- and stem-cell therapy, the minimal residual disease detection, before the clinical symptoms of relapse appear, as well as the improvement of the quality of life of patients.

PT48

THE INCIDENCE OF ELEVATED SWEAT TEST IN CLINICAL CENTER OF NIS

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Background: Cystic fibrosis (CF) is the most common autosomal recessive disorder among Caucasians, with an incidence of 1 in 2500. Patients with CF have an increased concentration of chloride and sodium in their sweat as a consequence of reduced sweat reabsorption in the distal sweat glands.

Methods: In the period between January 2011 and May 2013, in Central Laboratory of Clinical Center Nis, performed 180 sweat tests in children (with age rang of 5 months to 7 years) referred for suspect CF. Sweat test were performed by stimulation using pylocarpine iontophoresis, sweat collection and measurement of sweat Cl^- mmol/L by titration (colorimetric end-point). The minimum acceptable sweat weight was 65 mg. Some children had more of one testing.

Results: 130 sweat tests were negative (<40 mmol/L), thirty-six subjects had chloride concentrations in the border-line range (40–60 mmol/L) and positive sweat test had fourteen samples (>60 mmol/L). Median sweat chloride values was 22.83 ± 8.60 in group with negative, 49.72 ± 9.32 in border-line group and 84.12 ± 12.0 mmol/L in group with positive sweat test. Five children had multiple examinations because they had consecutive positive tests. Three of them were finally diagnosed as cystic fibrosis in further genetics testing (CFTR mutations).

Conclusions: A sweat test performed in a reliable laboratory is still a good measure of CFTR function in most cases.

PT49

NEPHROCALCINOSIS IN MACEDONIAN CHILDREN: ETIOLOGY, GENETICS AND OUTCOME

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Introduction: Renal tubular disorders and some extrarenal diseases (primary hyperoxaluria, parathyroid adenoma) may present with nephrolithiasis or nephrocalcinosis. Many of these diseases have genetic background and therefore pediatric patients with nephrocalcinosis should undergo extensive biochemical, metabolic and genetic evaluation in order to establish correct diagnosis, tailor appropriate management and perform prenatal diagnosis.

Materials and Methods: In order to establish etiological diagnosis, genetics and to analyze the outcome of Macedonian children with nephrocalcinosis all children who had radiological or ultrasound evidence for bilateral nephrocalcinosis were included in this study.

Results: During the period 1993–2013 there were 38 children who presented with nephrocalcinosis. In 22 children the primary diseases was renal tubulopathy: distal renal tubular acidosis (dRTA) (12) -

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complete form 8, incomplete form 4; familial hypomagnesemia with hypercalciuria and nephrocalcinosis (4); Bartter syndrome (4); Hypophosphatemic rickets as a complication of therapy (3). Extrarenal disorders: primary hyperoxaluria (4), Leprechaunism (1), idiopathic infantile hypercalcemia (4), parathyroid adenoma (1), sarcoidosis (1). The etiology was not clarified in four children (one with familial low renin hypertensive syndrome and in three with normocalcemic hypercalciuria). In 18 children the diagnosis was confirmed with mutational analysis of the respective gene(s). There were

six deaths: dRTA (3), primary hyperoxaluria (1), neonatal Bartter syndrome (1), Leprechaunism (1). One child has mildly impaired glomerular filtration rate, two children progressed to terminal uremia and one was transplanted.

Conclusion: Our study revealed that dRTA is still the most common tubulopathy leading to nephrocalcinosis in Macedonian children. The factors associated with unfavourable outcome were non-compliance with the treatment, complication of primary metabolic disorder and progressive renal insufficiency.

PT50

LIPID STATUS AND PARTURIENS INJURY OF NEWBORN CHILDREN FROM MOTHERS WITH GESTATIONAL DIABETES

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Gestational diabetes mellitus (GDM) is the most common complication during pregnancy and it is complication for both, mother and the child. This study was aimed to determine lipid status of neonates from mothers affected with GDM as well as to compare the obtained results with the lipid status and frequency of respiratory disorders with control group. The tests were performed on 30 infants of mothers affected with GDM and 45 infants of healthy mothers. Gestational age of all the infants ranged between 37.6 GW to 39.2 GW. The following parameters were determined in maternal blood samples obtained immediately before delivery as well as in neonatal umbilical blood samples at the moment of delivery: glycemia, cholesterol, HDL, LDL, triglycerides, Apo A, Apo B and Lp(a). The re-

sults indicated that infants of mothers affected with GDM had lower values of glycemia ($x=3.733$ mmol/L), in comparison to the control group of healthy mothers ($x=4.434$ mmol/L). Statistically significant difference ($p<0.05$) was obtained upon comparison of the mean LDL cholesterol values. Mean LDL cholesterol value recorded at infants of healthy mothers was 1.086 mmol/L in comparison to 0.875 mmol/L evidenced at neonates of mothers affected with GDM. Respiratory disorders were observed in 6% of infants from mothers affected with GDM, while it was not recorded in control group of infants from healthy mothers. Macrosomia was evidenced in 71.5% of infants of mothers affected with GDM.

PT51

DO INHALED CORTICOSTEROIDS INFLUENCE ASTHMA CHILDREN'S GROWTH?

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Background: Asthma is considered the most widespread chronic disease, usually being initiated in early childhood, and its control and treatment imply application of inhaled corticosteroids (ICS). Their potential adverse effects on children's growth and development have been subject to numerous epidemiological and anthropological studies.

The objective of this paper is to determine whether the population of school children suffering from asthma in Montenegro is different from the group of their healthy peers in terms of the height, having in mind that treatment implies recommended conventional doses of ICS.

Materials and methods: The sample accounts for 708 examinees, out of which 354 suffer from asthma, and the rest is a control group. Both groups

have been divided according to the gender and age, and afterwards according to the level of disease. Anthropological analysis comprised measurement of the height in all examined categories.

Results: Statistical data processing and comparison have shown the results indicating that the average value of the height, in all categories of the experimental group, is higher compared to the control group, but the observed differences have no statistical importance ($p > .1$). Significant differences between the examined groups have neither been perceived in relation to the level of the disease.

Conclusion: Inhaled corticosteroids, if applied in accordance with recommended doses, do not inhibit the growth of children who are under asthma treatment.

PT52

S100B IN ASPHYXIATED FULL-TERM NEWBORNS AS A USEFUL TOOL FOR EARLY DETECTION OF POSTASPHYXIA BRAIN

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Introduction: The purpose of this study was to investigate whether measurements of serum levels of S100B in asphyxiated full-term newborns may be a useful tool for early detection of postasphyxia brain damage as already we know that S100B protein is in the brain within the prenatal development and the early neurodevelopment follow up at 3–6 months.

Methods: All risk neonates with severe asphyxia, within the first hours after the admission of the

delivery were eligible for inclusion in the study. One serum blood sample was obtained from each patient at the 24h post-injury time-point. S100B levels were measured using Electro-Chemi-Luminescence Immuno Assay-Elecsys 2010-RocheDg. The results were correlated with the presence or absence of neurologic abnormalities at age 3-6 months.

Results: One 119 neonates were recruited in NICU. The average serum S100B levels for the control

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group (N=48) was 0.12 µg/L(-1) (cut-off point). S100B levels were significantly higher in asphyxiated term neonates N=29; M=0.64, premature neonates N=30; M=0.18, IUGR (intrauterine growth retardation) N=9; M=0.03 and neonates with neurological defect N=3; M=1.73.

Conclusion: The first 24h after birth S100B protein in term neonates was significantly higher compared

to all of the other groups, except the neonates with neurological defects, which showed higher levels of S100B protein that showed no statistical significance. S100B protein is good indicator of brain damage in term neonates especially in the first 24h after birth. Longitudinal S100B protein measurements in serum soon after birth are a useful tool to identify which asphyxiated infants are at risk of long-term neurologic sequelae.

PT53

DISTINGUISHING SEGMENTAL OVERGROWTH SYNDROME AN CLOVE SYNDROME

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Patient is a 6 year old girl, the first and only child of young parents, born with significant enlargement of the 1–3 rays of the left foot. She was born at full term after an uncomplicated pregnancy, with birth weight 2550 g, and length was 47 cm, and her OFC is not available. She was born with larger foot, bigger toe, syndactily (2–3th finger), the 4th and 5th finger were smaller. In the first year the foot, the leg, the left buttock and the left labia started to enlarge. She underwent surgery two times: amputation of the 2nd and 3rd fingers of the left food which stood vertically (in the same time there was a syndactily between them) and a debulking procedure of the fat in the same region, while the second time

liposuction of the left foot and the left pubic region excised 110 mL of fat. She has normal height and weight. A renal and heart ultrasound were uneventful. Exome sequencing revealed the cancer-associated mutation encoding p.His1047Leu in PIK3CA, the gene that encodes the p110a catalytic subunit of PI3K. In contrast to the CLOVE syndrome no cutaneous vascular malformation was present although the syndromes share the alteration of the same gene. It is obvious that additional study is necessary to determine if the segmental overgrowth syndrome is a clinical entity distinct from CLOVES syndrome.

PT54

AQT90 ANALYZER AS A POINT OF CARE

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Introduction: A lot of interest toward point-of-care testing for biochemical markers of myocardial cell necrosis in addition to traditional testing is found in clinical field. Since this technology introduced a wide range of tests to be performed simply and quickly.

However, rapid patient triage, decreased length of stay and improvement in turnaround time, lack of precision, sensitivity, increased cost and no demonstration in improvement in patient outcomes gave a mixed opinion regarding the issue of POC that precludes their routine use and recommendation.

We tested AQT90 analyzer/Radiometer as a random access immunoassay system “whole blood” for patients with suspected cardiac symptoms “Troponin I, CKMB, Myoglobin, and NT- pro BNP and D-Dimer”.

Method: Blood samples from suspected cardiac patients or coagulation abnormalities were collected using EDTA as standard sampling tube. Ci-

trated tube was used for D-Dimer to be compared with latex agglutination method” DIMERTEST, American Diagnostica” and EDTA samples. Comparison with Beckman Access for Troponin I was also done.

Results: 147 patients, 75 males & 72 females were involved randomly. Citrated and EDTA D-Dimer tests correlated significantly $P < 0.0001$. D-Dimer tests > 1000 ng/mL showed positive results with latex agglutination. Interestingly, D-Dimer correlated with NT-pro BNP, $p = 0.001$. Troponin correlated with CKMB $p = 0.035$, NT-pro BNP $p = 0.03$ but not Myoglobin $p = 0.4$ or D-Dimer $p = 0.9$. Troponin I (median_{AQT90} 0 ng/mL), however, failed to show correlation with Beckman access (median_{Access} 0.01 ng/mL).

Conclusion: AQT90 could be a valuable POC testing in addition to traditional testing for cardiac patients using EDTA samples only. More samples are needed for Troponin comparison.

Pregnancy and prenatal testing

PF15

SECOND TRIMESTER SCREENING FOR FETAL ANEUPLOIDIES - A FOUR YEAR EXPERIENCE

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Introduction: Triple screening test is a combination of maternal age, weight, ethnicity, gestation of pregnancy, biparietal diameter (BPD) and serum total HCG, AFP and unconjugated estriol for second trimester. In this paper we wanted to demonstrate the importance of using correct procedures that allow detecting high number of positive cases of trisomy 21, 18 and 13, and lowering the unnecessary amniocentesis.

Method: Screening analysis was performed between 2009–2013. We analysed 312 serum samples from pregnant women by Immulite 1000 (Siemens, risk assessment software PRISCA 5). The distribution of estimated risks for trisomy 21 was determined and sensitivity and false-positive rate for a risk cut-off 1 in 250 were calculated.

Result: The average maternal age was 30 (range 16–44) years and in 53 (17%) the age was 35 years or more, the average gestation at screening was 15.9 (14–22) weeks and average of BPD was 35 (range 29–56) mm. The estimated risk for trisomy 21 based on mentioned parameters was 1 in 250 or greater in 3.2% (10 of 312) of pregnancies. For each of pregnant women with risk, cytogenetic tests were determined and showed no evidence of fetal aneuploidies.

Conclusion: Our result of screening for chromosome abnormality by measuring of fetal BPD and maternal biochemical parameters are similar to those reported in literature.

PF16

PRISCA-PRENATAL RISK CALCULATION SYSTEM

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Some children are diagnosed with chromosomal abnormality after birth, but the most of them are diagnosed prenatal using screening and diagnostic test. PRISCA provides statistical risk assessment for Down syndrome (TR21) and Edwards and Patau syndrome (TR18 and TR13) in the first and second trimesters of pregnancy.

We examined 148 pregnant women in first trimester with PAPP-A and free beta HCG as biochemical markers (immunoassay tests) and NT and CRL as echographic markers, also 6 pregnant women in second trimester with alpha FP, HCG and unconjugated E3 immunoassay tests, and BPD as echographic measurement. The age of pregnant women are 25.5 to 46.9 years, CRL among 47–81.4 mm, and NT 0.9–3.5 mm. These parameters we put in PRISCA

– user-friendly program operating under Windows98/2000/XP on a network.

We found 4 pregnant women with high risk of T21 in all 154 of them or 2.6% and low risk for T18 and T13 for all of them. The high risk for T21 present risk >1:50 and suggested other diagnostic test which requires invasive procedures such as amniocentesis or chorion villus sampling. The other 97.4%

pregnant women have low risk of T21, T18 and T13 in ratio among 1:460 to <1:10 000.

PRISCA calculates chromosomal and neural tube defect risk based on age-related, biochemical and sonographic information for first and second trimester screening. PRISCA offers security via multiple user levels and back up and archive functions.

PF17

THE ASSOCIATION OF SERUM VISFATIN AND ADIPONECTIN LEVELS WITH THE SEVIRITY OF GLUCOSE INTOLERANCE IN WOMEN WITH PREVIOUS GESTATIONAL DIABETES

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Objectives: Gestational diabetes is a state of carbohydrate intolerance during pregnancy. In this study, clinical and laboratory data (CRP, IL-6, TNF- α , visfatin, PAI-1, adiponectin and lipids) were evaluated to investigate the risk of atherosclerosis, carbohydrate intolerance (CI), and Insulin Resistance Syndrome (IRS).

Material and Methods: 80 women with GDM diagnosed by the criteria of the National Diabetes Data Group at 24–28 weeks of gestation were comprised in the study. The postpartum assessment, 41 women were stratified with OGTT as a reactive hypoglycemia (n=27), impaired glucose tolerance (n=10) and diabetes mellitus (n=4) according to the ADA criteria.

Serum triglyceride, total and HDL cholesterol and insulin levels measured with Roche autoanalyzer by colorimetric and electrochemiluminescence me-

thods. Visfatin, adiponectin, IL-6, TNF- α and plasma PAI levels were determined by ELISA method.

Results: In postpartum assesment, visfatin were significantly lower, HbA_{1c} and fasting insulin were higher in prediabetic women than those of normal women (p=0.004, p=0.023, and 0.017). CRP, fasting glucose, 2nd hour glucose and insulin were increased (p=0.005, p=0.001, p=0.002), visfatin was decreased (p=0.05) in women with IGT compared to normal women. In reactive hypoglycemics, visfatin was lower (p=0.006), 1st and 2nd hour insulin, glucose were different (p=0.001). CRP and adiponectin were higher in reactive hypoglycemic patients, fasting glucose, 2nd and 3rd hour glucose and insulin were lower than IGT. Conclusion: Visfatin is associated with IGT and reactive hypoglycemia, adiponectin is found noticeably significant in reactive hipoglycemic subjects. GDM is attenuated with serum visfatin or adiponectin measurements during pregnancy.

PF18

FIRST-TRIMESTER BIOCHEMICAL MARKERS OF ANEUPLOIDY AND THE PREDICTION OF FETAL GROWTH RESTRICTION

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Introduction: To examine the clinical utility of the first-trimester markers of aneuploidy in their ability to predict fetal growth restriction (FGR). Preliminary studies have shown that reduced levels of these biochemical markers – particularly PAPP-A – may be of potential value in identifying those pregnancies that may result in adverse outcome such as fetal growth restriction.

Methods: We examined 50 singleton pregnancies with no chromosomal abnormality and with complete outcome data that had undergone screening for trisomy 21 by a combination of fetal nuchal translucency (NT) thickness, maternal serum free β -human chorionic gonadotropin (free β -hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 10 and 13 weeks' gestation. The biochemical markers were converted to multiples of the expected normal median for a pregnancy of the same gestation (MoM). Pearson's correlation coefficient was used for association between variables.

Results: During the study period, 50 women met the inclusion criteria. Analysis of crossing categories of PAPP-A MoM findings of pregnant women in relation to the distribution by FGR showed that there was no statistically significant difference ($\chi^2 = 0.712$, $df=1$, $p>0.05$), as in all categories PAPP-A MoM (i.e. in values up to and over 2.5) percentage of the FGR incidence was nearly equal. Analysis of crossing categories of free β -hCG MoM findings in pregnant women in relation to the distribution by FGR showed that there was no statistically significant difference ($\chi^2=0.254$, $df=1$, $p>0.05$), as in all free β -hCG MoM categories (i.e., in values up to and over 2.5) the percentage of FGR incidence was nearly equal.

Conclusions The clinical value of using these hormonal levels to predict and manage fetal growth restriction requires further investigation.

PF19

REDEFINITION OF GESTATIONAL DIABETES MELLITUS: EXPERIENCES IN CENTER OF LABORATORY DIAGNOSTICS IN PRIMARY HEALTH CARE CENTER IN PODGORICA

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Background: An increasing prevalence of obesity and type 2 diabetes mellitus worldwide, as well as a more advanced maternal age has increased the number of women with undiagnosed gestational

diabetes mellitus (GDM). So, early diagnosis and appropriate management of both GDM and pre-existing diabetes in pregnancy are of utmost importance in avoiding adverse pregnancy outcomes.

International Association of Diabetes and Pregnancy Study Groups (IADPSG) have recently issued recommendations on the diagnosis and classification of hyperglycaemia in pregnancy, and American Diabetes Association (ADA) adopted them and revised the previous criteria.

To evaluate the prevalence of GDM according to IADPSG/ADA recommendations and to compare them with the World Health Organization (WHO) and the old ADA criteria.

Methods: The study included 208 women age between 16 and 42 years (mean age 30.2 ± 5.40 years) who underwent a two-hour oral glucose tolerance test (OGTT) with 75 g glucose between 24 and 28 weeks of gestation, without previously diagnosed overt diabetes, in a period from November 2012 to July 2013.

According to IADPSG/ADA criteria, the diagnosis of GDM is made if at least one value of glucose concentration is equal or exceeds the thresholds of ≥ 5.1 mmol/L, ≥ 10.0 mmol/L, and ≥ 8.5 mmol/L (for fasting, one-hour and two-hour post load glucose values respectively), after performing a 75 g OGTT. The test was scheduled in the morning, after an

overnight fast of at least 8 hours. Exclusion criteria were pregnant women who met overt diabetes criteria (fasting glucose ≥ 7.0 mmol/L or 2h glucose ≥ 11.1 mmol/L) ($n=2$), and those who were not completed the test ($n=8$). Fasting glucose was measured by standard enzymatic procedure.

Results: Based on IADPSG/ADA criteria, GDM was diagnosed in 44 women (21.1%) who were significantly older than their healthy counterparts (33.2 ± 5.42 vs. 29.4 ± 5.12 ; $p=0.0001$). Only 12 women with GDM were younger than 30 years old, whereas a total of 32 women with GDM were older than 30 years. A total of 17.3% women had one value, 3.4% had two, and only 0.5% had all of the three values equal or exceeding the thresholds. On the other hand, using WHO criteria, GDM was diagnosed in 10.1% women, and only 2.4% women were diagnosed with GDM based on the old ADA criteria.

Conclusions: As expectedly, the use of new diagnostic glucose values increases the prevalence of GDM, as compared with WHO and former ADA criteria, primarily because only one abnormal value, but not two (former ADA criteria), is sufficient to make the diagnosis. Preventive measures should be aimed by Public Health Initiatives for reducing this trend.

Proteins and proteomics

PT71

TYPE DISTRIBUTION OF PARAPROTEIN IN SERBIAN PATIENTS WITH MONOCLONAL GAMMOPATHIES

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Monoclonal gammopathies represent a group of disorders characterized by the proliferation of one or more clones of plasma cells. They are characterized by the presence of a monoclonal protein (paraprotein) in the serum or urine. Protein electrophoresis is used for the detection and identification of paraproteins. Immunofixation (or immunoelectrophoresis) is necessary for determining the type of the paraprotein (heavy and light chain subclass).

The aim of this study was to investigate type distribution of paraprotein in Serbian patients with monoclonal gammopathies.

The investigation was carried out on laboratory data base from Center of Medical Biochemistry, Clinical Center of Serbia in Belgrade for period of one year. There were 1243 patients with detected paraprotein in serum and/or urine. The type of paraprotein was determined by agarose gel immuno-

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fixation (Sebia, France). The distribution of paraprotein type was as follows: IgG lambda (23.1%), IgG kappa (35.2%), IgM lambda (5.9%), IgM kappa (9.4%), IgA lambda (5.9%), IgA kappa (6.0%), lambda light chains (11.3%) and kappa lambda light chains (2.4%). IgD lambda was identified in one patient, 6 patients had biclonal gammopathies and heavy chains gammopathy was identified in 3 patients for period of one year. The presence of paraprotein in

urine was detected in 303 patients: Bence Jones proteins were found in 46.9% patients and intact immunoglobulin in 51.8% patients.

The most frequent paraprotein type was IgG kappa in Serbian patients with monoclonal gammopathies. The correct classification of type of paraprotein is important for diagnosis and treatment of monoclonal gammopathies.

PT72

THE VALUE OF ALBUMIN DETERMINATION VERSUS TOTAL PROTEIN IN THE CSF OF PATIENTS WITH ACUTE BACTERIAL MENINGITIS

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Bacterial meningitis continues to be life threatening for the patients. The choice of sensitive and with high specificity tests helps for quick and precise diagnosis.

Purpose of the study: Determination and comparison of the values of total protein and albumin in the CSF of patients with acute bacterial meningitis.

Material and method: We studied 50 patients over 18 years of age presented at the emergency service of Infectious Diseases Hospital in the period May 2006–May 2010 in the clinical context of an acute encephalopathy and with final diagnosis acute bacterial meningitis. To all patients was performed lumbar puncture and in lumbar CSF obtained along with the number of leukocytes was determined the total protein and albumin. Determination of total protein and albumin was conducted in Olympus AU 640 analyzer with colorimetric method. For data analyses was used SPSS 17.0 statistical package

Results: Total protein was found increased in 98% of cases. The average value of total protein was 465.98 mg/dL (SD 376.14), the minimum value 99 mg/dL while the maximum value was 1900 mg/dL. Increased albumin was found in 84.0% of cases. The average value of albumin in the CSF was 247.84 mg/dL (SD 248.34), the minimum value 99 mg/dL while the maximum value was 1200 mg/dL. We found that the sensitivity of total protein was 98% and specificity 42%, while albumin had 92% sensitivity and 84% specificity for bacterial meningitis.

Conclusion: Determination of albumin in the CSF of patients with bacterial meningitis is a sensitive test and with higher specificity than total protein. Determination of albumin in the CSF should be included in the LCS standard protocol for suspected infection of the CNS structures.

Quality assessment, standardization, traceability and accreditation

PF22

MEASUREMENT UNCERTAINTIES FOR SERUM ELECTROLYTES USING TOP-DOWN METHOD

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Introduction: The uncertainty of measurement (UM) is a parameter obtained from measurements, which serves, to characterize a range of values for the true value of the measurand. Estimating and reporting the UM is required of laboratories accredited to ISO/IEC 17025 and ISO/IEC 15189 and is important for medical laboratories, mostly to make patient results comparable, irrespective of where the testing is done.

Aim of the study: To demonstrate top-down approach as a method estimation of UM for serum sodium, potassium and chlorides measured by direct ISE. Data from internal quality control and EQAS were used in: estimation of $\%u_{rw}$ from intralab imprecision (reproducibility and repeatability) as well as calculations of $\%u_{bias}$, combined uncertainty $\%u = (\%u_{rw}^2 + \%u_{bias}^2)^{1/2}$ and expanded UM (with a 95% confidence).

Results: Results were similar for both control levels used and higher values are presented. These are:

- for sodium: $\%u_{rw}=3.1$, $\%u=3.9$ and UM (mmol/L)=9;
- for potassium: $\%u_{rw}=4.4$, $\%u=5.5$ and UM (mmol/L)=0.4 and
- for chlorides: $\%u_{rw}=5.0$, $\%u=7.8$ and UM (mmol/L)=14.

Conclusions: In cases where no demands have been published, a guiding principle that was accepted in this study could be that expanded MU should be approximately equal to, or less than 2 times the reproducibility. Our results showed just a little bit higher UM. So, the main goal that arose from the study was to reduce the uncertainty. A few recommendations could be accepted and implemented: use of calibration facilities with the smallest uncertainties, corrections to compensate for known errors, checking the measurements by repeating them and check calculations.

PF23

REFERENCE INTERVALS FOR CHOLESTEROL AND GLUCOSE (THREE YEAR OLD CHILDREN IN KOTOR)

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Reference values for some biochemical parameters in the pediatric population, especially the lower age are not available. This is supported by the data

analyzed for cholesterol (Chol) and glucose (Glu) obtained by reviewing 202 healthy children (95 girls and 112 boys) aged three years in a pediatric outpa-

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tient medical center in Kotor. Children were examined in the period 2009–2012 year. Our current reference range in children is an important marker for early-onset hypercholesterolemia and hyperglycemia, which will help to assess early cardiovascular disease (CVD) and diabetes prevention.

Chol and Glu concentrations were determined by BioSystems reagents, the apparatus A-25 of the same company.

Analyses of variance were compared to data obtained for Chol and Glu from year to year, for girls and boys. There was no statistically significant difference between the groups (Chol-f $V = 0.57$ Glu-f $V = 0.52$, Chol-m $V = 0.41$, Glu-m $V = 0.49$). Student's t-test values were compared between boys and girls for each year separately. Not yield statistically significant differences between gender, age. Comparing all boys and girls get a significant difference in Chol (f-4.56, 4.35 m-t=1.91, $p < 0.05$) and Glu (4.52 f-,

m-4.72 t=2.13, $p < 0.05$) at the poles. Reference interval, 95.47% of the value in the $X \pm 2SD$ Chol-w 3.04 to 6.08, m-2.91-5.79 and Glu-w 3:24-5.80, m-3.32 to 6.12 clustering data, 95% of the concentration of Chol for girls is in the range 3.15-5.94 mmol/L, and 92% for boys within 3.15 to 5.54 mmol/L. The concentrations of glucose for girls 95% are within 3.40–5.79 mmol/L, a concentration of 92% for boys is between 3.60–5.99 mmol/L.

The results show that girls have a higher concentration of Chol and boys Glu, which is consistent with studies obtained for other age groups and other geographic populations of children. Three girls and four boys had higher values for Chol than the average, while glucose only one girl and three boys more than the average. We conclude that research needs to continue in order of prevalence of CVD and diabetes is on the rise, according to research in the pediatric population.

PF24

INTERNATIONAL STANDARDS IN MEDICAL LABS - NEED AND NECESSARY

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Medical laboratories have high moral and ethical responsibility in the health system. They generally receive a copy of the patient from which to develop analysis, the results will help in making the correct diagnosis and treatment. Or even more, In Vitro diagnosis is very important factor is the duty of each laboratory to facilitate it. Therefore, it is necessary the certification and accreditation of medical laboratories. The certification process is to ensure that the third party process, the service and the result is in accordance with certain requirements such as ISO 9001:2008. ISO 17025 specifies general requirements for the competence to carry

out tests and calibration using standard laboratory-developed methods. Implementation of 15189 resulted in significant improvement in laboratory competence as personnel, facilities, equipment, test methods and traceability of measurements. Accreditation is a procedure by which the accreditation body provides official confirmation that the laboratory meets the criteria and procedures specifically developed to determine her competency. Medical laboratories should develop a system of quality management that will provide a high degree of accuracy, reliability, timeliness and efficiency, so everything is done well the first time.

Renal diseases and transplantation

PF62

EVALUATION OF TWO BIOMARKERS (NGAL AND CYSTATIN C) IN PATIENTS WITH CHRONIC HEART FAILURE USING DIGOXINIsiksacan N.¹, Erturk M.², Akturk IF.², Koser M.¹, Yazan S.², Yildirim MR.², Bakir I.³¹*Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Biochemistry Department, İstanbul, Turkey*²*Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Cardiology Department, İstanbul, Turkey*³*Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Cardiovascular Surgery Department, İstanbul, Turkey*

Introduction: Digoxin is frequently used in treatment of heart failure and is mainly excreted in urine. This drug has a narrow therapeutic range and a toxic potential. Dosage monitoring of digoxin is more important for elderly patients with impaired renal functions. Glomerular filtration rate is a highly specific and valuable test in determining renal functions, however difficulties in collecting 24 hours urine samples leading investigators to find another biomarkers especially for elderly patients. NGAL, and Cystatin C are biomarkers, which can be used for evaluation of renal functions.

The aim of this study is to evaluate the renal functions of elderly patients with chronic heart failure (CHF) for monitoring digoxin levels to avoiding toxic effects.

Methodology: 100 patients (mean age: 67±12 years) from cardiology outpatient clinic diagnosed as CHF enrolled to this study, who has a digoxin treatment duration of minimum one month 0.25 mg daily. Digoxin, creatinine, cystatin C and NGAL levels were measured from venous blood samples in 2 hours after collection. The methods and manufacturers are chemiluminescence (Architect i2000-Abbott), kinetic colorimetric jaffe method- (cobas c501-Roche), immunoturbidimetric method- (cobas

c501-Roche) and immunofluorescence method- (Triage-Biosite) respectively. All the cases received written inform on the study including Ethics Committee Approval before the study was initiated. SPSS (Statistical package for Social Science) for Windows 11.5 was used for statistical analysis. Spearman Correlation Analysis is performed.

Results: Serum digoxin levels ranged from 0.3 to 3.2 ng/mL, creatinine level ranged from 0.4 to 3.2 mg/dL, cystatin C level ranged from 0.927 to 3.109 mg/L and NGAL levels ranged from 60 to 605 ng/mL. A correlation was found between NGAL and creatinine ($r=0.520$, $p<0.001$), between cystatin C and creatinine ($r=0.599$, $p<0.001$) and between NGAL and cystatin C ($r=0.561$, $p<0.001$). Digoxin levels were significantly associated with renal function tests, $r=0.482$, $p<0.001$, $r=0.341$, $p<0.001$, $r=0.468$, $p<0.001$ between digoxin and NGAL, creatinine and cystatin C respectively.

Conclusion: Results of this study indicates that NGAL and cystatin C can be used to evaluate renal functions in patients with CHF. Venous blood collection may be easier for elderly patients than 24 hours urine sample collection. Dosage adjustment for digoxin related to renal function is necessary to improve patient's life quality.

PF63

RELATIONSHIP BETWEEN SERUM NON-ESTERIFIED FATTY ACIDS (NEFA) AND GRAFT FUNCTION IN RENAL TRANSPLANTATION

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Introduction: Nonesterified fatty acids (NEFA) are associated with the development of insulin resistance and atherosclerosis. Fatty acids have also been shown to be important mediators of the immune response in transplantation. This study aimed to assess the relevance of serial postoperative serum NEFA measurements for predicting graft function after transplantation.

Patient and Methods: We studied 50 kidney transplant recipients. Blood samples were collected immediately before and after surgery at day 1, day 7, month 1 and month 3. Serum NEFA levels were measured by enzymatic colorimetric method using a commercial kit (Wako HR, NEFA-HR 2, Wako Chemicals, USA). Serum creatinine levels were analysed by modified Jaffe method in Cobas 8000 analyser. Patients were assigned to 2 groups: defined slow graft function (SGF) as a reduction in serum

creatinine by <70% on day 7 and immediate graft function (IGF) as ≥70%. Data were expressed as mean ± Standard deviations. Mann-Whitney U and Student's t tests were done.

Results: Among 50 recipients, 6 had SGF, and 44 had IGF, no patient had delayed graft function (DGF; required dialysis within 1 week of transplant). Serum NEFA levels were statistically different between groups before transplantation ($p < 0.05$). Serum NEFA levels on days 1 and 7 were significantly higher in SGF compared to IGF ($p < 0.05$).

Conclusions: Our findings suggest that increased NEFA levels early and accurately predicted allograft function after renal transplantation. The sequential monitoring of serum NEFA in kidney transplant recipients might be recommended. Future larger studies are needed to confirm our findings.

PF64

DEVELOPMENT OF AN ASSAY FOR DETECTING α -2 MACROGLOBULIN

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Introduction: Diagnosis of kidney problem is usually made depending on clinical history, medical examination, X-ray and laboratory tests. One of these laboratory tests is the presence of blood (haematuria) and proteins (proteinuria) in urine. Proteins especially the one with large size, are reabsorbed in the urinary tract and don't present in urine of healthy people except when there primary or secondary kidney problems. We tried to develop an

ELISA assay for the measurement of α -2-macroglobulin in urine and evaluate its performance as marker for haematuria.

Method: Method was established using commercial reagents, equipment and appropriate standards. Standard prepared by adding 0.2mL plasma calibrator to 2 mL of 1% BSA/PBS to give concentration of 181.8 mg/L α -2-macroglobulin. Serial dilution of the

curve was done to give a range from 0.04 µg/L–874 µg/L. The assay was optimized using different reagents and conditions. Urine samples from patients with kidney problems were tested for α-2-macroglobulin. The results were compared to blood content measured using urine strips. All results were tested statistically using analyze-it.

Result: Standard curve constructed using serial measurement of the standard with optimum conjugated antibody concentration 0.049 µg/mL and 14.2 µg/mL for coating antibody. Ova-albumin was chosen as a blocker. Sensitivity of the assay was

0.05 µg/mL, analytical recovery 86–110%. Dilution experiments showed parallelism in standard curves. Intra and inter-assay were 1.9–5.1% and 9.9–20% respectively. α-2-macroglobulin measured using the assay correlated with haematuria ($p < 0.05$; Spearman correlation) and proteinuria ($p < 0.01$). α-2-macroglobulin correlated with proteinuria in those who were positive for haematuria ($p < 0.01$).

Conclusion: Positive correlation with proteinuria excludes α-2-macroglobulin from being used as a sole marker for haematuria.

PF65

SUBCLINICAL HYPOTHYROIDISM IN PATIENTS WITH CHRONIC KIDNEY DISEASES

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Background: Subclinical hypothyroidism is highly prevalent in the general population. The prevalence of subclinical hypothyroidism in patients with chronic kidney diseases (CKD) not requiring chronic dialysis is not well defined.

Material and Methods: Cross-sectional data from 498 adult outpatients, who were consecutively referred by general population for routine blood testing in the last year, were analyzed. Glomerular filtration rate was estimated by the abbreviated Modification of Diet in Renal Diseases equation (MDRD). The concentration of thyroid hormones, TSH, fT4, T4 and aTPO was determined with ELISA techniques in Human analyzer. The microglobulin and Cystatin C concentration was determined with DAKO test (immunonephelometry) on Cobas Integra plus analyzer.

Results: Among 498 participants, 212 (42.5%) has subclinical hypothyroidism and 147 (24.5%) had an estimated GFR < 60 mL/min/1.73 m². The prevalence of subclinical hypothyroidism increased from 5.2% at

an estimated GFR > 90 mL/min/1.73 m² to 15.2% at an estimated GFR < 60 mL/min/1.73 m² ($p < 0.001$). Regarding thyroid autoantibody positivity, the aTPO positive group had a higher urinary albuminuria than the negative group (38.2 mg/day, 16.9 mg/day respectively ($p < 0.001$)). In hypothyroid patients, urinary albuminuria was significantly higher than in hyperthyroid patients and control. In the patients with hypothyroidism Cystatin C concentration increased significantly after treatment

Conclusion: Subclinical hypothyroidism is more common in patients with CKD not requiring chronic dialysis compared with those with normal kidney function. Future clinical and experimental studies should explore potential causal mechanisms linking subclinical hypothyroidism and CKD. Serum Cystatin C might be proposed as a valid indicator of thyroid hormone peripheral action, but since thyroid dysfunction affects in serum concentration, it can't be used as a valid marker for assessing GFR in this category of patients.

PF66

HEPCIDIN LEVELS IN DIALYSIS PATIENTS (PRELIMINARY STUDY)

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Background: Hepcidin is a 25-aminoacid iron regulating peptide. Increased hepcidin concentrations lead to iron sequestration in macrophages. It plays major role in the pathogenesis of anemia of chronic disease whereas decreased hepcidin is observed in iron deficiency and primary iron overload diseases such as hereditary hemochromatosis. Hepcidin quantification in human blood or urine may provide further insights for the pathogenesis of disorders of iron homeostasis and might prove a valuable tool for clinicians for the differential diagnosis of anemia.

Methods: Serum hepcidin levels were measured, using monoclonal sandwich ELISA method in 40 healthy individuals. Including criteria were normal

liver and kidney function, no evidence of diabetes, without concomitant infection disease. Hepcidin levels were measured in 10 dialysis patients with iron deficiency anemia with iron treatment for the last two years. The kit uses recombinant human hepcidin as a standard.

Results: Measured serum hepcidin levels in our group healthy individuals were average of 12.68 µg/L (1.77 µg/L – 27.3 µg/L), SD 6.8. Hepcidin levels in dialysis patients were significantly increased – average 33.05 µg/L (22.3 µg/L – 60.98 µg/L); p<0.05.

Conclusions: The results from our preliminary study are showing that iron deficiency anemia in dialysis patients leads to increased hepcidin levels.

PF67

PLASMA INTERLEUKIN-18, INTERLEUKIN-1β AND TNF - α IN KIDNEY TRANSPLANT PATIENTS ON TACROLIMUS BASED IMMUNOSUPPRESSION

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Introduction: The long-term immunosuppressive treatment could lead to nephrotoxicity as well as acute impairment of renal function which can be immunologically mediated. Interleukin-18 (IL-18), interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) are mediators of acute inflammation processes as well as acute exacerbation of chronic processes. Thus, the aim of this research was to

determinate the plasma level of IL-18, IL-1β and TNF-α in kidney transplant patients considering time since transplantation.

Patients and methods: The study included 35 kidney transplant patients divided into three groups and 25 healthy individuals as controls. Patients in the first group (n=15) have had kidney transplanted

for more than 12 months, but less than 24 months, the second group (n=10) between 24 months and 48 months and the third group (n=10) included patients who have had kidney transplanted for more than 48 months. Plasma levels of IL-18, IL-1 β and TNF- α were measured using ELISA Kit.

Results: Plasma levels of TNF- α in the first group were statistically higher in comparison to other groups of patients as well as in the controls ($p<0.05$). Interleukin-1 β plasma levels in the third group were statistically lower than in the second

group ($p<0.01$) and in the controls ($p<0.01$). Plasma levels of IL-18 were not significantly different among kidney transplant patients as well as between patients and controls.

Conclusion: TNF- α should be investigated more detailed in the second year post – transplant as a possible marker of immunologically mediated processes. IL-1 β has a decreasing trend with respect to time after transplantation, which may be a positive effect of long-term tacrolimus treatment.

PF68

HEMOGLOBIN LEVELS IN RENAL TRANSPLANT PATIENTS

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Background: This analysis was performed to assess the prevalence and the factors associated with hemoglobin (Hb) variability during treatment with erythropoiesis-stimulating agents. Variability of Hb has been considered a factor that influences comorbidity and mortality among hemodialysis patients. The purpose of this analysis was to describe the phenomenon of Hb variability during ESA treatment, to study associated factors among kidney transplant patients, and to assess the impact on patient and graft survivals.

Patients and Methods: Hb variability (defined as fluctuations of $Hb \pm 15$ g/L) was assessed in 37 renal transplant patients treated with ESA for at least 3 months and with a minimum of 6 Hb measurements during 1 year.

Results: Fifty-eight percent of the patients experienced Hb variability during follow-up. Only 3% of patients maintained stable Hb levels within the tar-

get range (110–130 g/L), although 83% of patients maintained Hb levels >110 g/L. Multivariate analysis showed that the clinical factors associated with variability were changes in ESA dose (relative risk [RR]: 2.94; 94% confidence interval [CI]: 1.1–8.6; $P<0.05$), infectious events with hospitalization (RR: 1.95; 95% CI: 1.23–2.14; $P<0.05$), and the use of sirolimus (RR: 1.2; 94% CI: 1.0–3.5; $P<0.05$). When dose changes and hospitalization were excluded from the analysis, variability was an independent predictor of worsening graft function.

Conclusions: Hb variability is common in renal transplant patients treated with ESA. Only a few patients maintained Hb levels within the therapeutic range, although most had levels >110 g/L. Dose changes, inflammatory status, and worsening graft function are the determining factors of variability. Variability had no influence on patient survival, although it was a marker of worsening graft function.

PF69

EXAMINATION OF GLOMERULAR FILTRATION RATE AND PROTEINURIA IN PATIENTS WITH ENDEMIC NEPHROPATHY

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Introduction: Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial disease with vague beginning and a slow progression to end-stage renal failure. BEN is characterized by tubular atrophy and dysfunction, followed by proteinuria, microalbuminuria, urinary excretion of β_2 microglobulin and/or α_1 microglobulin, and a decrease in glomerular filtration rate (GFR).

The aim of this study was to investigate the relationship of GFR and the concentration of total protein, albumin and microglobulin in the urine of patients with endemic nephropathy.

Materials and Methods: The study included 45 patients, aged 48 to 80 years, with a diagnosis of endemic nephropathy, from endemic villages near Bijeljina, Bosnia and Herzegovina. Concentrations of total protein, microalbumin and α_1 -microglobulin were determined in the patients' urine. Glomerular filtration rate was calculated using the Modification of Diet in Renal Disease (MDRD) formula.

Results: In 45 patients with endemic nephropathy average value of proteinuria was 0.43 ± 0.32 mg/mmol creatinine, albumin concentration in urine 41.5 ± 25.68 mg/mmol creatinine and microglobulin in urine 21.10 ± 43.40 mg/mmol creatinine. In the same subjects the average value of GFR was 68.41 ± 28.74 mL/min/1.73 m². The analysis of the results showed a significant negative correlation between GFR and total protein concentrations ($r = -0.782$, $p < 0.01$) and between GFR and α_1 -microglobulin ($r = -0.589$, $p < 0.01$) in the urine, and absence of significant association of GFR with albumin in the urine ($p = 0.453$). There was also a significant positive correlation between proteinuria and microglobulinuria ($r = 0.639$, $p < 0.01$).

Conclusion: Examination of the relationship of GFR and proteinuria in patients with endemic nephropathy showed the presence of a significant negative correlation between GFR and the concentration of total protein and urine microglobulin.

PF70

NEFROTOXICITY OF NSAIDs

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The adverse effects of NSAIDs are mediated via inhibition of prostaglandin synthesis by non-specific blocking cyclooxygenase leading to vasoconstriction and reversible mild renal impairment in volume contracted states. When unopposed, this may lead

to acute renal failure. The standard metrics to follow the progression of ARF, like serum creatinine and blood urea levels, are inconvenient and depend on kidney injury. That's why we must use specific markers for early detection. We used colorimetric

method for determination of N-acetyl- β -D-glucosaminidase (NAG), kinetic assay for alanine aminopeptidase (AAP) and γ -glutamyl transferase (γ -GT) in urine, Jaffe's method for serum creatinine and kinetic method for urea serum. We used 100 patients (80 RA^{sero+} and 20 RA^{sero-}) treated with Naproxen, Indometacin, Diclofenac, and Piroxicam compared with 80 healthy individuals. The follow up was 3 time-intervals in the course of 12 months. There are no changes in the values of creatinine and urea in the serum. When compare with the specific

urine enzymes (NAG, AAP, γ -GT) we have mild elevation of the values in all groups of patients, especially in patients with RA^{sero+} which were treated with above mentioned NSAID, in comparison with the control group of examinees.

We found far greater sensitivity of the specific urine enzymes. We can't confirm which group of NSAIDs is more nephrotoxic, but, if we follow the elevation of the level of the specific biomarkers, we are able to use them as early signals for nephrotoxicity.

PF71

ACTIVITIES OF SUPEROXID DISMUTASE AND GLUTATHION PEROXIDASE IN PLASMA OF PATIENTS WITH BALKAN ENDEMIC NEPHROPATHY

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Introduction: Balkan endemic nephropathy (BEN) is a familial chronic tubulointerstitial disease with insidious onset and slow progression to terminal renal failure. The aim of this study was to investigate the influence of oxidative stress on BEN by measuring the activities of antioxidative enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Methods: Activities of plasma GPX and SOD were determined spectrophotometrically in 30 BEN patients and 31 controls with nephrolithiasis.

Results: The activity of GPX was significantly lower in patients with BEN compared to the control group (368.1 $\pm$ 133.8, 425.7 $\pm$ 82.5, respectively, $p < 0.05$). When the BEN patients were divided according to eGFR, in patients with eGFR >90 mL/min/1.73m²

GPX activity was significantly higher compared to those with eGFR <60 mL/min/1.73m². Moreover, significant negative correlation was observed between GPX activity and creatinine, urea and proteinuria ($r = -0.530$, $p < 0.05$; $r = -0.704$, $p < 0.001$; $r = -0.475$, $p < 0.05$; respectively). However, SOD activity was about 10% higher in plasma of BEN patients in comparison with controls, but without statistical significance (48.7 $\pm$ 9.6, 44.2 $\pm$ 10.8, respectively, $p > 0.05$).

Conclusions: The reduced GPX activity in patients with BEN might be a consequence of the atrophy of the kidneys present in these patients. As a result of the reduced enzyme antioxidant capacity, it may be assumed that the increased production of free radicals may be an important factor in the progression of BEN.

PF20

EARLY MARKERS OF IRON STATUS IN HEMODIALYSIS PATIENTS

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Secondary lack of iron in hemodialysis patients is the main cause of inadequate response to treatment with recombinant human erythropoietin (rHuEPO). The aim of our study was to determine the value of hemoglobin content in reticulocytes as predictor of functional iron deficiency in hemodialysis patients treated with erythropoietin (rHuEPO), and then to evaluate the efficiency of using the value of hemoglobin content in reticulocytes in the administration of iron in HD (hemodialysis patients).

This is a prospective study which included 53 patients in chronic hemodialysis (HD) and continuing ambulatory peritoneal dialysis (CAPD). Additional iron therapy was administered intravenously (i.v.) to all patients in order to keep the level of ferritin

between 300 µg/L and 500 µg/L and transferrin saturation over 20%. The study was conducted from August to December 2008 at the University Clinical Centar Tuzla, and included the patients in chronic HD programme with additional intravenous treatment with rhEPO and iron, the patients in CAPD also treated with rhEPO and iron i.v., and the patients in chronic HD treated additionally with i.v. iron without rhEPO. If criteria such as transferrin saturation and the level of ferritin are used as a reference standard, there would be 26/53 (49.1%) patients with iron deficiency in the whole sample. The above hematological and biochemical parameters in examined HD patients provide with essential information for planning and leading an adequate erythropoietin therapy.

PF21

PRIMARY HYPEROXALURIA - CLINICAL METABOLIC AND GENETIC FEATURES OF MACEDONIAN PATIENTS

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Background: Primary hyperoxalurias are inborn errors in the metabolism of glyoxylate and oxalate. The clinical presentation is recurrent nephrolithiasis and nephrocalcinosis resulting in progressive renal damage, oxalate accumulation and systemic oxalosis. Diagnosis is often delayed and the disease is not recognized until signs of systemic oxalosis ensue. The treatment in this stage is combined liver kidney transplantation. Therefore urinary oxalate measurement is mandatory at the early stage when conservative measurement may prevent development of end stage renal disease and systemic oxalosis. In this work we present Macedonian experience with primary hyperoxalurias.

Methods: Urinary oxalate measurement was performed preferably in 24-hour urine collections. Primary hyperoxaluria was considered if the urinary levels were $>1.0 \text{ mmol/d/1.73 m}^2$. Secondary hyperoxaluria was excluded on the basis of dietary history, physical examination and laboratory tests. Urinary excretion of glycolate and glycerate was determined within the same samples. Pyridoxine sensitivity testing was performed using 24 hour urine collections; in anuric patients plasma oxalate concentration were monitored before and after pyridoxine treatment. Mutational analysis of the respective genes for primary hyperoxaluria type 1, 2 and 3 was performed.

Results: In the period 1995–2012 there were 8 patients with primary hyperoxaluria. In 5 patients diagnosis of PH1 was confirmed with urinary glycolate measurement and mutational analysis of the AGXT gene. There were no patients with PH type 2 and 3. The youngest patient at diagnosis was 4 months old and the oldest 52 years. Four patients progressed to terminal renal failure and two of them died. Three patients were sensitive to pyridoxine. One patient had nephrolithiasis which was initially considered as a consequence of ureteropelvic junction obstruction, but metabolic investigation revealed hyperoxaluria, hyperglycolaturia and normalization of urinary oxalate excretion under pyridoxine treatment. In one adult patient PH was diagnosed late due to the recurrence of the disease in the kidney

graft and confirmed with mutational analysis of the AGXT gene. This patient was anuric and pyridoxine challenge did not result in significant plasma oxalate reduction.

Conclusions: Macedonian PH patients presented with various clinical features and the diagnosis in majority was established late when progressive renal damage ensued. Mutational analysis confirmed the biochemical diagnosis of PH1. No cases of PH2 and PH3 were detected in our series. It seems that yet unidentified genes are responsible for PH in Macedonian patients. Implementation of urinary oxalate screening in all patients with urolithiasis/nephrocalcinosis and aggressive conservative treatment is mandatory in order to prevent systemic oxalosis.

PF72

CORRELATION BETWEEN INDICATORS OF GLOMERULAR FILTRATION RATE – SERUM CREATININE, CYSTATIN C, AND RETINOL BINDING PROTEIN

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Introduction: Glomerular filtration rate (GFR) is an important index of kidney function. Normal GFR varies according to age, sex, and body size, and declines with age. Estimates of GFR that are based on serum Creatinine are routinely used even though it is well known that serum Creatinine concentrations are highly dependent on age, muscle mass and gender. Cystatin C has been reported to be a more accurate renal marker and better at estimating small changes in GFR. Two meta-analysis have reported the superiority of Cystatin C as a renal marker compared with serum Creatinine. Retinol-Binding Protein (RBP) is elevated in patients with chronic kidney disease and has been discussed as marker of kidney function.

Material and methods: The study group consisted of 120 individuals (64 of males and 56 of females). The study was conducted in Clinical Center of Montenegro. Serum Cystatin C and serum RBP concentrations were measured using a nephelometric immunoassay on the Dade Behring BN II Nephelometer (Siemens Diagnostics, Germany). Serum Creatinine was analysed on Architect c8000 (Abbott Diagnostics, USA), using a spectrophotometric method. A GFR was calculated according to National Kidney Foundation's clinical practice guidelines which recommend the MDRD (Modification of Diet in Renal Diseases) Study equation to estimate GFR

$$\text{GFR} = (\text{mL/min/1.73 m}^2) = 175 \times (\text{S}_{\text{cr}})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$$

Other formulas used in this study were:

$$\text{Rule1 et al } 66.8 \times \text{Cystatin C (mg/L)}^{-1.3}$$

Poster Presentations

Rule2 et al $76.6 \times \text{Cystatin C (mg/L)}^{-1.16}$

Le Bricon et al $78/\text{Cystatin C (mg/L)} + 4$

Hoek et al $80.35/\text{Cystatin C (mg/L)} - 4.32$

Larsson et al $77.239 \times \text{Cystatin C (mg/L)}^{-1.2623}$

MacIsaac et al $86.7/\text{Cystatin C (mg/L)} - 4.2$

Stevens1 et al $76.7 \times \text{Cystatin C (mg/L)}^{-1.19}$

Stevens 2 et al $127.7 \times \text{Cystatin C (mg/L)}^{-1.17} \times \text{age}^{-0.13} \times 0.91$ if female

Stevens 3 et al $177.6 \times \text{serum Creatinine /} 88.4 (\mu\text{mol/L})^{-0.65} \times \text{Cystatin C (mg/L)}^{-0.57} \times \text{age}^{-0.20} \times 0.82$ if female

All data were expressed as median and standard deviation.

Spearman's coefficient of rank correlation and Kruskal-Wallis test were used. $P < 0.05$ was considered statistically significant.

Results: In the study group the value of serum Creatinine was $85.5 \pm 118.66 \mu\text{mol/L}$ (97.5 ± 143.9 for males and 70 ± 71.68 for females); Cystatin C $1.04 \pm 1.0 \text{ mg/L}$ and RBP $0.04 \pm 0.02 \text{ g/L}$. RBP correlates well with Cystatin C ($p < 0.001$) but not with serum Creatinine level. There was good correlation between Cystatin C and Creatinine Clearance, $p < 0.001$; as well as between RBP and Creatinine Clearance, $p < 0.0018$. A MDRD formula correlates well with all other used formulas for GFR calculation that include Cystatin C ($p < 0.001$). Cystatin C and RBP also correlate well with MDRD, $p < 0.001$. There is no correlation between RBP and all formulas for GFR determination which are based on Cystatin C concentration only (Rule1, Stevens1, Le Bricon,

Hoek, Larsson, MacIsaac, Rule2), Cystatin C and age (Stevens2) and also there is no correlation between RBP concentration and Stevens 3 GFR formula based on age and serum levels of Creatinine and Cystatin C. Kruskal-Wallis test shows dependence between RBP, body weight and gender ($p < 0.001$, $p < 0.001$)

Conclusion: The results indicate that formulas for GFR calculation based on Cystatin C could be used as alternative to the standard MDRD formula, which can be helpful in everyday clinical practice. RBP has been discussed as a marker of kidney function, but according to our data, RBP alone is not a good analyte for the estimation of GFR. The study results suggest that RBP serum concentration correlate with body weight and gender, therefore it is possible that RBP, combined with these two parameters, could be considered as a marker of GFR estimation.

Technology, instrumentation and method evaluation

PF25

ANALYTIC PERFORMANCE OF THE AUTION HYBRID AU-4050 AUTOMATED URINE ANALYSER AND COMPARASION WITH MANUAL TECHNIQUES

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Introduction: Routine examination of visual, chemical, and microscopic means has become an essential part of many diagnostic evaluations. We evaluated the performance of the Aution Hybrid AU-4050 automated urine analyzer. Automation is necessary in laboratory system, it enables reduction of

time required for sample analysis, as well as standardization of methods. Aim of this study is to compare the diagnostic performance of Aution Hybrid AU-4050 analyzer and determine the convenience of use in the laboratory.

Methods: A total of 368 consecutive fresh urine samples were analyzed by microscopy and automated analyzers. Certain elements of urine sediment separated by manual microscopy were compared to those analyzed by the Aution Hybrid AU-4050 system; erythrocytes, leukocytes, crystals, cylinders and bacteria. Precision of the system was tested using two commercial control urine materials. All elements of chemical examination were compared, such as: glucose, proteins, bilirubin, urobilinogen, pH, ketones, nitrites, specific gravity and hemoglobin.

Results: Within-run imprecision of cell counts of the analyser expressed as CV (mean cell count/HPF) was 5.2% for erythrocytes; for white blood cells (WBC) 1.9%; for squamous epithelial cell (SEC), 8.7% and cast 7.4%. Between-run imprecision of cell counts of the analyser was 8.8% for erythrocytes; for white blood cells (WBC) 6.9%; for squamous epithelial cell

(SEC), 12.0% and cast 16.8%. There were discrepancies between sediment counts, with 11 for red blood cells, 8 for white blood cells. Arbitration of these discrepancies with manual microscopy favored Aution Hybrid AU-4050. Except for urobilinogen, by the automated system AU-4050 some more positive findings were detected, although insignificantly, than by using Multistix stick. Testing pH value provides remarkable ($r=0.83$, $p<0.001$), as well as testing specific gravity, but to a somewhat lower degree ($r=0.62$, $p<0.01$).

Conclusion: Automated urinalysis Aution hybrid AU-4050 systems can save labor and time, and are valuable in high-volume laboratories. The sensitivity and specificity of Aution Hybrid AU-4050 would remove the need for manual microscopy for most specimens. The chemical urinalysis system showed excellent agreement.

PF26

EVALUATION OF IRIS-IQ200 SYSTEM TO IDENTIFY THE NEED OF FURTHER URINE CULTURE TESTING

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Introduction: Urine culture is the gold standard in urinary tract infection (UTI) diagnosis, but at least 24–48 h is required for results. Although the diagnosis and treatment of UTI's is quite simple, waiting for culture results leads to increased cost and antibiotics resistance. Our aim was to investigate the performance of the urine auto-analyzers to determine the need for urine culture in UTI diagnosis.

Methods: We evaluated the sensitivity, specificity, negative and positive predictive value of automated IRIS IQ200 analyzer combined with IRIS-ICHEM VELOCITY (BioDPC, USA) in patient with suspected UTI (n=300). For each urine sample culture was also performed. A special software program was used to identify the set of variables that best predict positive culture results. Three microscopic (leukocyte,

bacteria, and total small particule numbers) and two chemical (leukocyte esterase and nitrite) variables and special cut-off values were used to determination.

Results: Urine culture was positive in 67 patients, with an overall UTI prevalence of 22%. The software program showed significantly greater sensitivity (97%) and negative predictive value (98.6%) than the specificity and positive predictive value (62% and 42%, respectively). False negative rate was found as 0.8%.

Conclusions: We suggest that this software could improve the management UTI by identify the need for urine culture. Prospective studies are needed to clarify the usefulness of this software program.

PF27

LYME BORRELIOSIS – CHALLENGES REGARDING LABORATORY DIAGNOSTIC

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Introduction: Lyme disease (or Lyme borreliosis) is a multisystemic inflammatory disorder caused by the spirochete *Borrelia burgdorferi sensu lato*, which are transmitted by the hard ticks of the *Ixodes ricinus* species complex. Predominant clinical symptoms of the disease in its early stages are pathological skin lesions, namely erythema chronicum migrans. In the late stages are involved joint malfunctions and neurological or cardiac disorders which may dramatically decrease the quality of life. Lyme disease, in all its stages, may be successfully treated with antibiotics.

Material and method: The discovery of the causative pathogen *Borrelia burgdorferi* in 1982 opened the way for the firm diagnosis of diseases in several clinical disciplines and for causal antibiotic therapy. Different laboratory diagnostic methods were developed and, in the same time it was underlined the

necessity of correlation between clinical and paraclinical diagnostic.

Results: Laboratory diagnostics in Lyme disease is based on two kind of methods: direct methods for detecting the presence of the antigen (which include microscopy, culture using specific media, PCR) and indirect methods for detecting the presence of the specific antibodies (IFA, ELISA, Western-Blot, LIPS).

Conclusions: Lyme borreliosis remains the most common arthropod-borne disease in temperate regions of the northern hemisphere. That is why uniform, European-wide surveillance programs (particularly on a local scale) and standardization of diagnostic tests and treatments are still urgently needed, especially in the light of climate change scenarios and land-use and socio-economic changes.

PF28

INFLUENCE OF TYPE OF BIOLOGICAL MATERIALS ON MEASUREMENT OF tHcy

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As a biological material for determination of total homocysteine (tHcy) manufacturer recommended heparinized and EDTA plasma as a samples of choice, but serum is also suitable for use. Blood collection tubes from different types may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators or anticoagulants.

We aimed to compare levels of tHcy measured in K₂EDTA plasma and serum using Becton Dickinson tubes.

The study included 46 paired samples analyzed in parallel. All patients, with average age 48.74±11.18 years old (20–78), were randomly selected and were with normal renal function - mean creatinine – 69.85±14.73 µmol/L (32-104). In 10.9% of individu-

als, only females, anemia was established – hemoglobin under 120 g/L.

Blood was collected by standard venipuncture after fasting overnight. K₂EDTA tubes, and gel separated tubes were used. After blood taking both plasma and serum were immediately centrifuged (for 15 min at room temperature at 15,000 x g) and examined by competitive immunoassay with direct chemiluminescence detection on immunoanalyzer IMMULITE®1000, Siemens.

Levels of tHcy in K₂EDTA plasma and serum were statistically different ($p=0.005$) and higher in serum

$x=14.35\pm4.41$ $\mu\text{mol/L}$ (8.13–31.00) than in plasma $x=13.39\pm3.98$ $\mu\text{mol/L}$ (6.93–27.00), but we established a high correlation between them – Pearson coefficient=0.86. Under 15.00 $\mu\text{mol/L}$ tHcy were 69.9% of plasma samples and 60.9% of serum ones.

The values of tHcy correlated well depending on the type of biological material, but in examination and interpretation of tHcy it is recommended each laboratory to estimate its own pre-analytical rules and reference limits.

PF29

CLINICAL ASSESSMENT OF ERYTHROCYTES AND LEUKOCYTES IN URINE: MANUALLY VERSUS AUTOMATIC EVALUATION

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Introduction: Urinalysis is one of the most commonly performed tests in the clinical laboratory. Although manual urinalysis is accepted “gold standard”, it has been increasingly replaced with standardized automation equivalents. The aim of this study is to show correlation between the manual and automated system of blood (RBC) and leukocytes (WBC) count in urine. Two comparisons were done for each specimen: chemical analysis for these two parameters between test strip analyzer and automated urine system and RBC and WBC counts in urine sediments calculated manually versus urine analyzer.

Methods: Fresh urine specimens of 293 patients were analyzed by Automated Urine System Iri-cell2000-Iris Diagnostics, Chatworth, CA and manually. Manual examination was performed by test strip analyzer URISCAN PRO II YD (Electronics, Korea) and manual microscopy by well educated laboratory technicians. Results were statistically calculated

by the regression analysis, where x represents manual and y automated count of RBC and WBC in urine specimens.

Results: We found strong statistical correlation between test strips results of Uriscan and Iris: for blood and WBC

$y=0.069+0.981x$, $r=0.947$; $y=-0.036+0.852x$, $r=0.869$, respectively. The correlation between microscopic and automated counts of RBC and WBC was: $y=-3.769+2.065x$, $r=0.953$; $y=-10.879+2.215x$, $r=0.959$, respectively. For concordant results obtained by test strips and automated sediment urinalysis we found 1.36% (RBC); 2.04% (WBC) false negative and 0.68% (RBC); 1.02% (WBC) false positive results.

Conclusions: RBC and WBC urine results obtained by skilled observer were not different from time-consuming automation system but a fair number of manual microscopic examinations is required.

PF30

DETERMINATION OF PARACETAMOL BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Background: Paracetamol, also known as acetaminophen, has been used as an analgesic for approximately 50 years.

Objectives: The aim of this work was to develop a simple, fast and accurate tandem mass method for determination and quantification of paracetamol. This method is designed for high sample throughput of only 10 µL of human plasma and serum.

Methods: Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an electrospray ion source (ESI) operating in positive mode.

Results: The intra-assay CV for serum paracetamol were 4.93, 13.94, 13.08% for 12.5, 50, 120 µg/mL concentrations. The inter-assay CV for serum para-

cetamol were 13.2, 10.8, 11.1% for 12.5, 50, 120 µg/mL concentrations. %Bias were calculated as 8.96, 1.84, 19.4 % for 12.5, 50, 120 µg/mL concentrations. The recovery ranged between 95-125% for serum paracetamol. The linearity of calibration curves in plasma was estimated by the coefficients of correlation (r^2), which ranged from 0.987 to 0.999. The standard curves for paracetamol was linear within the range of 1–200 µg/mL for plasma. The equation for calibration was $y=0.943x+7.469$ and $R^2=0.992$. Total run time was 2 minutes.

Conclusions: Data from calibration curves and method validation reveal that the method is accurate and precise. The short and fast run time, the feasibility of high sample throughput and the small amount of sample required make this method very suitable for routine analysis in the clinical setting.

PF31

DETERMINATION OF 25-HYDROXY VITAMIN D₃ BY TANDEM MASS SPECTROMETRY

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Introduction: Although radioimmunoassay (RIA) system was considered as a gold standard for vitamin D analysis historically, liquid-chromatography tandem mass spectrometry (LC-MS/MS) system has an increasing popularity and became a preferred choice for vitamin D determination in clinical laboratories. Our aim was to evaluate the method performance of LC-MS/MS system.

Material and Methods: Blood samples were collected from 214 random samples obtained for rou-

tine analysis. 100 µL internal standard and 1000 µL acetonitrile was added to 250 µL of plasma, calibrator, control for protein precipitation, vortexed for a minute and centrifuged at 13,000 rpm for 10 minutes. 40 µL of supernatant was injected into HPLC analytical column for chromatography. Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an atmospheric pressure

chemical ionisation (APCI) operating in positive mode.

Results: The intra-assay CV for plasma Vitamin D were 9.35, 3.81, 2.29% for 10, 32, 150 µg/L concentrations. The inter-assay CV for plasma Vitamin D were 5.6, 8.8, 7.4 % for 10, 32, 150 µg/L concentrations. %Bias were calculated as 8.96, 1.84, 19.4% for

12.5, 50, 120 µg/mL concentrations. The recovery ranged between 95–125% for plasma vitamin D.

Conclusions: Due to interferences in immunoassay systems, LC-MS/MS is considered as a better alternative technology. According to our study, 25-OH vitamin D can be measured accurately by tandem mass spectrometry.

PF32

COMPARISON OF THE RESULTS FOR THE MOST FREQUENTLY APPOINTED BIOCHEMICAL PARAMETERS OBTAINED WITH MANUAL AND AUTOMATED ASSAY PROCEDURES

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Introduction: For the requirements of laboratory accreditation according to the ISO/IEC 17025:2006 standard we conducted parallel determination of the concentration of the 10 most frequently appointed biochemical parameters, by manual and by automated assay procedures. The objective of the study was to make a comparison between both assay procedures, using the same reagents, with the aim to evaluate manual assay procedure as a back up of automated analyzer.

Methods: We analysed 10 parameters in serum: activity of aminotransferases AST and ALT, creatinine, urea, glucose, iron, albumin, total protein, cholesterol and triglycerides, using Giese diagnostics and Human reagents. All parameters were

measured for 10 days, manually on Minitco photometer and automatically on ChemWell biochemical analyzer.

Results: We found statistically significant correlation between the results of manual and automated assay procedure. The results were: for glucose $r=0.97$, $p<0.0001$; creatinine $r=0.75$, $p=0.005$; urea $r=0.79$, $p=0.0022$; triglycerides $r=0.97$, $p<0.0001$; cholesterol $r=0.9$, $p<0.0001$; ALT $r=0.94$, $p<0.0001$; AST $r=0.63$, $p=0.0281$; albumin $r=0.65$, $p=0.0221$; total protein $r=0.52$, $p=0.0831$; iron $r=0.93$, $p<0.0001$.

Conclusion: The results showed that manual assay procedure can be a good back up of automated assay procedure in consecutive laboratory work.

PF33

BENEFITS OF SYSTEMATIC AUTOMATED SAMPLE INTERFERENCES TESTING – OUR EXPERIENCE

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Background: Traditionally, presence of sample interferences such as hemolysis, lipemia and icterus is visually estimated. That method is unreliable. Obtained results are qualitative and it is difficult to estimate their impact on patient's results. Modern laboratory analyzers offer automated detecting of interferences present in sample and their semi-quantification.

Aim: Aim of this paper is to evaluate advantages of automated sample interference testing in our laboratory.

Method: Comparison of working process before and after hemolysis, icterus and lipemia (HIL) testing implementation.

Results: During October 2012 we applied sample interferences indices saline protocol on our clinical chemistry analyzer Architect c16000. Furthermore,

we defined in LIS which analytes require testing for one or more of these interferences, so that operators don't have to make orders for HIL. Results from the analyzer are sent to LIS and are available to clinical chemistry specialist for evaluation and decision making. From 01.11.2012. to 31.05.2013. 19586 samples were tested for hemolysis, 20740 for lipemia and 15960 for icterus. Hemolysis was detected in 2.84%, lipemia in 1.70% and icterus in 1.81% of tested samples. In all that cases, tables provided by manufacturer were applied to decide if the patient's result is acceptable or not.

Conclusions: There are at least three benefits our laboratory experienced with automated HIL testing: more reliable discovering of sample interferences present, less time consumption, more precise data for decision making during validation results in LIS. Adopting of this protocol is one of the steps to optimization of laboratory process.

PF34

SOLUBLE TRANSFERIN RECEPTOR – A NEW ROUTINE PARAMETER IN THE LARGE CLINICAL LABORATORY

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Background: Researches from the last two decades on the iron metabolism explore the great role of the receptor for transferrin, expressed on the surface of cells, which needs Iron. The soluble part of this receptor, measured in blood serum or heparinized plasma is called soluble transferrine receptor (sTfR). Now its determination is possible to be run on sen-

sitive automated apparatus as a part of the everyday routine of large laboratories.

Aim: Goal of the investigation is to present the analytical reliability of the determination of sTfR and our first experiences from its application by patients with anemias.

Material and methods: sTfR is determined on a Beckman – Coulter – analyzer Access2 using reagents and controls of the same producer (ref Nr 32494, 62495). Reference rang 0.9–2.1 mg/L. We present data from 50 determinations for control

purpose, as well in serum samples of patients with anemias and of healthy persons.

Results and discussion: We prove good analytical reliability of the parameter sTfR.

Precision data	n	mean	SD	CV(%)	min	max	d%
Serial (patient serum)	10	1.15	0.01764	1.53	1.13	1.18	-
Day to day (1month)	10	1.136	0.02633	2.32	1.10	1.18	-
Accuracy (6 months, 4 calibr)	10	0.622	0.0382	6.14	0.56	0.67	-9.8

Conclusion: sTfR is a new routine parameter for our laboratory. We prove that its analytical reliability parameters are good. The use of sTfR together with other parameters as serum transferrin, ferritin and

indices, generated with their values, characterize better the status of anemic patients and can be used for follow up their therapies.

PF35

WHAT SHOULD WE DO WITH LIPEMIC SAMPLES IN EVERYDAY LABORATORY PRACTICE?

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Background: According to the Clinical and Laboratory Standards Institute (CLSI) recommendations, ultracentrifugation should be considered as preferred approach. This kind of equipment isn't available in most laboratories. Therefore, each laboratory should establish its own recommended method for lipemia removal. The aim of this study was to evaluate which method, diethyl-ether or magnesium-chloride/heparin lipemia clearing reagent (Lipoklir), has got better performance characteristics for everyday laboratory practice.

Materials and methods: In 12 different non-lipemic serum samples the following parameters were determined: glucose, urea, creatinine, uric acid, total and direct bilirubin, AST, ALT, ALP, GGT, LD, CK, lipase, amylase, total protein, albumin, CRP, troponin I, sodium, potassium, calcium, and phosphates. All measurements were performed in duplicates, before and after removing lipoproteins, on Architect c8000 analyzer (Abbott, Germany), except troponin I on Architect i2000 analyzer (Abbott, Germany).

Results: Each parameter was compared before and after treatment with diethyl-ether and Lipoklir (multiplied with 1.1 for dilution correction). T-test for Dependent Samples showed that there was no statistically significant difference ($p > 0.005$), for all measured parameters, except for sodium ($p < 0.05$) in samples treated with diethyl-ether, and creatinine, direct bilirubin, sodium, potassium, calcium and phosphates ($p < 0.05$) in samples treated with Lipoklir.

Conclusion: According to our results, both methods are acceptable for all measured parameters except for sodium in samples treated with diethyl-ether, and creatinine, direct bilirubin, sodium, potassium, calcium and phosphates in samples treated with Lipoklir, but method with diethyl-ether showed less interferences in analytical phase than Lipoklir and better performance characteristics in this study.

PF36

SPECIMEN TYPE COMPARISON FOR DETERMINATION OF 25-HYDROXY VITAMIN D₃ BY TANDEM MASS SPECTROMETRY

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Introduction: 25-hydroxy vitamin D's lipophilic nature renders it especially vulnerable to the matrix effects. Something present in the sample assay tubes that is not present in the standard assay tubes can cause matrix effects. They could be anything contained in the serum or plasma sample. It has been reported that endogenous 25(OH)D and are extremely stable in serum or plasma. In this study, our aim was to compare serum and plasma 25-OH vitamin D results by LC-MS/MS system.

Method: Blood samples were collected from 34 random samples obtained for routine analysis. 100 µL internal standard and 1000 µL acetonitrile was added to 250 µL of plasma, calibrator, control for protein precipitation, vortexed for a minute and centrifuged at 13,000 rpm for 10 minutes. 40 µL of supernatant was injected into HPLC analytical column for chromatography. Mass spectrometric ana-

lyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an atmospheric pressure chemical ionisation (APCI) operating in positive mode. Kolmogorov-Smirnov test was performed to check the distribution of values. Paired sample t-test was used to compare the results.

Results: According to Kolmogorov-Smirnov test, the values distribution was normal. By using paired sample t-test, it was found that there was no statistically significant difference between serum and plasma 25-OH vitamin D results ($p=0.698$).

Conclusions: LC-MS/MS is considered as gold standard for measuring 25-OH vitamin D. According to our study, 25-OH vitamin D can be measured accurately by tandem mass spectrometry in serum or plasma sample.

Toxicology and therapeutic drug monitoring

PF37

SIMPLE HPLC METHOD FOR ROUTINE SINGLE PLASMA LEVODOPA DETERMINATION

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Background: In Parkinson's disease (PD), levodopa (3,4-dihydroxyphenylalanine) is widely used to prevent negative symptoms of the disease. There are very few data about levodopa concentration in

plasma, and its potential influence on the efficiency on the therapy. The aim of this study was to evaluate the potential simple HPLC method for routine levodopa determination in plasma. It was de-

terminated in plasma samples withdrawn from patients, whose were administrated levodopa under controlled conditions in Clinic of Neurology, Clinical Centre of Serbia.

Method: Levodopa was determined in lithium-heparin-plasma with 1mg/mL sodiummetabisulfite. Levodopa and catecholamines was extracted from 500µL plasma, by original solid phase extraction method given by "Chromsystems". HPLC separation has been done on isocratic HPLC line, consisted of: HPLC pump "Waters 1515", autosampler "Waters 2707", and electrochemical detector, "Waters 2465". All chromatographic data were evaluated by "Empower 2 Waters" program. All samples were spiked with 3,4 dihydroxybenzylamin solution, as internal standard. Isocratic HPLC separation has been done with original mobile phase (flow rate 1.2 mL/min), and column for determination catecholamines in plasma, delivered by "Chromsystems". Electrochemical detection has been done on 0.8V.

Results: Quantitative analysis has been performed on assessment of peak heights. The method was validated as linear in concentration range between 0.3 and 35 µg/L. Within run precision for "low" sample concentration, as 1.5 mg/L was 4.4%, and for "high" sample concentration, as 30 µg/L, was 3.9%. Between run precision, for same concentration were 6.5% and 7.1%, respectively. Analytical and absolute recovery was 91% and 99%, respectively.

Conclusion: An accurate determination of levodopa concentration in plasma samples can be obtained, using this simple extraction and separation technique in biochemical laboratories which performed HPLC technique for different analytical purposes. This modification can be easily performed as, fast, reliable and cheap manner especial in laboratory which still performed catecholamines determination.

PF38

THERAPY-RELATED MYELOID NEOPLASMS FOLLOWING FLUDARABINE, CYCLOPHOSPHAMINE AND RITUXIMAB (FCR) TREATMENT IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LUMPHOCYTIC LYMPHOMA PATIENT

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This case is focused on therapy related myeloid neoplasm after the most promising frontline FCR (fludarabine, cyclophosphamide, rituximab) therapy in a patient diagnosed with chronic lymphocytic leukemia/small lymphocytic lymphoma. We are presenting the case of an Albanian 50 years old patient diagnosed with B cell chronic lymphocytic leukemia/small lymphocytic lymphoma who received 8 cycles of fludarabine, cyclophosphamide and rituximab which led to complete remission. He underwent hematological consultations every 3 months. After 6 years of complete remission the patient was hospitalized because of a febrile state. The patient presented pancytopenia with WBC 3600/mm³, RBC 3,240,000/mm³, HGB 10.2 g/dL and

PLT 138,000 mm³. The patient underwent bone marrow aspiration and 23% blast was found with extreme signs of trilineage myelodysplasia. On flow cytometry of the bone marrow aspiration 30% of an abnormal population with CD 34 (positive), CD 117 (positive), CD33 (positive), CD13 (positive), and CD38 (positive) were found. According to WHO the patient was diagnosed with Acute Myeloid Leukemia. No residual B cell chronic lymphocytic leukemia/small lymphocytic lymphoma was found. The patient underwent AML chemotherapy regimen protocol with cytarabine and anthracycline base. Although myelodysplasia and secondary acute myeloid leukemia are rare following fludarabine monotherapy the risk of these diseases may poten-

tially be increased when fludarabine is combined with cyclophosphamide due to synergistic effects

on the inhibition of DNA repair.

PF39

EFFECTS OF MORPHINE ON SOME ANTIOXIDATIVE SYSTEMS IN STOMACH MUCOSA OF RATS EXPOSED TO COLD RESTRAINT STRESS

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Introduction: Stress ulcer represent acute lesion of gastric mucosa resulting from influence of various factors: burns, surgical operation, trauma, shock, severe metabolic disorders, drug administration, etc. Effects of reactive oxygen species (ROS) on gastric mucosa in various experimental models of stress-induced mucosal injury was proven. The role of oxygen derived free radicals in the generation of gastric injury is also well known. Pretreatment with different drugs could effectively prevent gastric mucosa from the development of erosions and ulceration. Previous studies demonstrated that the damaging action of cold-restraint stress (CRS) could be attributed to the enhancement in the ROS and the ROS-dependent of antioxidative enzyme activity contributed to morphine.

The aim of this study: was to investigate effects of opioid analgesic morphine on content of glutathion

(GSH) and activity of catalase (CAT), peroxidase (Px), glutathion peroxidase (GSHPx) and glutathion reductase (GSHR) in stomach mucosa of rats exposed to cold-restraint stress (CRS).

Material and methods: Research was performed on adult, male Wistar rats weighting 220-230 g. Animals were pretreated with morphine hydrochloride (Stellorphanad 10®) in the doses of 10 mg/kg/1mL, injected intraperitoneally, just before cold-restraint stress. Content of GSH and activity of CAT, Px, GSHPx i GSHR were determinated in stomach mucosa.

Results: Pretreatment with morphine was significantly ($p < 0.001$) enhanced content of GSH and activity of GSHR, reduced activity of CAT ($p < 0.01$) and Px ($p < 0.05$), but was not changed activity of GSHPx.

CHANGES IN BIOCHEMICAL PARAMETERS AFTER THE TREATMENT WITH NIACIN

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Niacin (nicotinic acid) is well known lipid lowering drug. However the use of niacin has been limited due to side effects and changes in biochemical parameters especially elevation of uric acid and homocysteine levels. Thus the objective of this study was to examine the side biochemical effects of niacin in rats treated for 15 weeks with niacin dissolved in water.

Male Sprague-Dawley rats, weighing 250-290 g were used for the study. They were housed in special cages in well ventilated room on controlled temperature and on a 12 h light-dark cycle. All animals had free access to water and pellet diet. They were divided into two groups (control and experimental group) of five each. Niacin 500 from GNC – General Nutrition Corporation, which was dissolved in 1000 mL of water, was given to the rats in experimental group for 15 weeks. The dose levels of niacin were close to those consumed by humans on

a body weight basis. After a run in period, the rats were killed; blood samples were collected and analyzed for total proteins, creatinine, ALP, ALT, AST, γ GT, homocysteine, B₁₂ and folate levels. The livers were removed and weighed.

During the run-in period animals drink about 40 mL of water each per day. In experimental group a significant increase in body (385 g in control and 429 g in experimental group) and liver weight (10.68 g and 14.32 g respectively) was achieved. Total protein concentrations were elevated in experimental group (71 g/L $\pm$ 4.883 and 87 g/L $\pm$ 5.918) together with creatinine concentrations 43 μ mol/L versus 57 μ mol/L. The higher increase in enzyme activity was achieved for γ GT. Homocysteine and B₁₂ levels were not affected by niacin.

Despite the fact that in many studies niacin had increased homocysteine concentration it was not proven in our study.

PT55

TRACE ELEMENTS DYSHOMEOSTASIS INDUCED BY EXPOSURE OF MICE TO GASOLINE VAPOR AND STRESS

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To investigate the effects of air pollution related with gasoline/petrochemical industry the expression of metallothionein I (MT-I) mRNA and tissue metals were analyzed in organs of mice, exposed to the gasoline (G) vapor in laboratory conditions. Control groups consisted of intact mice and of those exposed in the metabolic chamber to fresh air. The data obtained by RT-PCR and inductively coupled plasma spectrometry have shown that exposure to G vapor leads to upregulation of MT-I mRNA in or-

gans that receive a strong respiratory and olfactory input or participate in gasoline degradation and elimination (lungs, brain, kidney and liver). Simultaneously, the tissue content of Zn²⁺ decreased in the brain and the concentration of Cu²⁺ and Mg²⁺ in the brain, lungs, kidney and liver (p<0.001). Some of these changes were found also in mice closed in the metabolic chamber, pointing to the involvement of stress-induced mechanisms in the transcriptional regulation of MTs.

PT56

LEAD CONTENT IN CHILDREN'S BLOOD IN PODGORICA, MONTENEGRO

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Children are subpopulation of concern to environmental lead exposure due to their behaviors such as playing on the floor or outdoors, sucking the objects and hand-to-mouth activity. Lead is neurotoxic and numerous studies indicate that blood lead concentrations above 10 µg per deciliter (0.483 µmol per liter) are associated with adverse outcomes on measures of intellectual functioning and social-behavioral conduct. Recent analyses suggest that health effects may become apparent at concentrations of <5 µg/dL and, indeed, that no evidence exists for a threshold, even at 1 µg/dL. Estimates published recently suggest that the theoretical-minimum-risk of health effects may occur at blood-lead concentrations as low as 0–1 µg/dL.

The aim of this study was to determine lead content in children's blood samples. Forty whole blood samples were taken in June 2013 from six year old children from one kindergarten in Podgorica. Sampling was conducted at Health Center of Podgorica. Blood samples were prepared by microwave digestion according to the manufacture instruction of instrument (ETHOS 1). 1.5 mL of whole blood sample were digested with 8 mL of HNO₃ and 2 mL of H₂O₂ and diluted to 25 mL volume with deionized water. Lead content in digested blood samples were determined by GFAAS (graphite furnace atomic absorption spectrometry) technique (Agilent).

The obtained results showed that there was no lead contamination in analyzed children's blood. Lead

concentration was below LoD (limit of detection) which is 1 µg/dL.

This study was a part of scientific project and this data are not enough to get a whole picture of child-

ren health in Podgorica. It is recommended to continue the research and collect more blood samples to evaluate children's health and lead exposure.

Varia

PF41

EPIDEMIOLOGY OF HIV/AIDS AND SYPHILITIC INFECTIONS AS STDs IN THE UHC, TIRANA

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Introduction: Worldwide, HIV and syphilis are transmitted primarily through unprotected sexual route. The infected person is often unaware of the disease and unknowingly passes it on to his or her sexual partner. Individuals who are infected with STDs are at least two to five times more likely than uninfected individuals to acquire HIV infection if they are exposed to the virus through sexual contact.

Method: During the year 2012 a total of 393 sera were tested for HIV 1, 2 Ag/Ab using the AXSYM system (Abbott, Wiesbaden, Germany) a micro particle enzyme immunoassay. 47 serum samples were tested at the same time for RPR, an agglutination testing (Inter Medical, Italy) and for T. pallidum IgM, an enzyme immunoassay (DIA. PRO, Milan, Italy).

Results: 24 patients samples (6%) resulted HIV 1, 2 Ag/Ab positive, 5 (21%) children and 19 (79%) adults and female male ratio was 1:1.7. HIV Ag/Ab ODRs varied from (min; max) 6.31 to 57.76, (mean±sd) 38.06±10.78, median 38.04. 9 sera (18%) resulted positive for syphilitic infection (positive T. pallidum IgM and RPR). T. pallidum IgM ODRs varied from 10.06 to >12.09, (mean±sd) 11.87±0.93, median 11.7 while female male ratio was 1:3.5.

Conclusions: No HIV/syphilitic co-infection was detected during serum samples testing. Albania remains a country with relatively a low HIV infection rate, however it is seen a trend in rise year after year regarding HIV/AIDS infection. Higher rates are seen in ages from 35-45 years old and the most common route of transmission is the sexual one (82% hetero and 10% homo and bisexual, ref PHI).

FRUCTOSE AND CORRECTED FRUCTOSE IMPORTANCE IN DETERMINING THE QUALITY OF SEMEN

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Vesica seminalis or semen bags (SB) are lined with epithelium, which secretes mucous secretion. This secretion contains a lot of fructose (F), which is synthesized by androgen hormones. Including F, prostaglandins, fibrinogen, citrate, the elements K, Ca, Zn, Cl, Na and many enzymes are also components of seminal plasma (SP). The concentration of F is determined as an indicator of the SB function or potential agenesis of ductus deferens and seminal vesicles.

The aim is to examine the relationship between the concentration of F and corrected fructose ($CF = \log(\text{sperm number})_{\text{mL SP}} \times cF$) with sperm concentration (Cs), seminal plasma volume (SPV), sperm motility index (SMI), the concentration of functional sperm (FSH) and percentage of actively motile sperm (APS).

We analyzed the semen of 41 men, average age 34.5 y. (18–51 y.) with Cs estimate by $26.4 \times 10^6/\text{mL SP}$ (1.2–100), without a defined diagnostic issues. The sample was obtained after 2–5 days of abstinence (coitus interrupts) and analyzed 5 min after collection. Patients were divided according to different criteria: group I (SPV criteria, subgroup A < 2.0 mL, subgroup B > 2.0 mL), group II (SMI criteria, subgroup A < 100, subgroup B > 100), group III (cS criteria, subgroup A < $20 \times 10^6/\text{mL SP}$, subgroup B > $20 \times 10^6/\text{mL SP}$), group IV (FSH criteria, subgroup A < $10 \times 10^6/\text{mL}$, subgroup B > $10 \times 10^6/\text{mL SP}$). SP was analyzed on the device SQA II CP. The principle of this method is conversion of light into electrical signals which are digitized in the result. BioSystems reagents are used in measuring fructose, which is converted by isomerase in glucose that can be measured by spectrophotometry.

We performed a correlation between F and CF with tested parameters. The level of correlation is significantly higher with CF, except in relation to the volume of SP, in this order: F i CF/SPV ($r=0.25$; 0.13), F i CF/SMI ($r=0.42$; 0.71), F i CF/cS ($r=0.43$; 0.71), F i CF/APS ($r=0.35$; 0.66), F i CF/FSH ($r=0.51$; 0.74). Student's t-test shows that in subgroups I-A and I-B ($t=8.66$, $p<0.001$) within the given group, concentrations of F and CF did not differ significantly ($t=0.43$, $p>0.05$; $t=0.49$, $p>0.05$). Between subgroups II-A and II-B ($t=7.36$, $p<0.001$), the concentration of F has no statistical significance ($t=1.15$, $p>0.05$), while KF is significantly different ($t=2.29$, $p<0.05$). Within the third group ($t=6.93$, $p<0.001$) between the concentration of F subgroup III-A and III-B did not differ ($t=2.29$, $p>0.05$), while CF is significantly different ($t=3.21$, $p<0.01$). According to the criteria IV, subgroups A and B differ significantly ($t=4.47$, $p<0.01$), F concentrations did not differ ($t=0.72$, $p>0.05$), while CF significantly differ between the two groups ($t=3.26$, $p<0.01$).

We conclude that CF is more useful indicator of quality SP, does not depend on SPV, directly is related to the number and mobility of sperms (S). In addition to the existing knowledge that F indicates the existence of obstacles in the seminal ducts, it's the main energy material of sperm mobility, deficiency may indicate a problem in SB synthesis and androgenic effects or it's metabolism in the S, which is associated with a functional concentration, that is dependent on the speed S and morphology. Lack of aldose reductase leads to an inability to transform fructose into glucose and accumulation of sorbitol. Sorbitol as an osmotic active alcohol disrupts the morphology of S and slows movement, which is the reason of asthenozoospermia.

PF43

POTENTIAL IMMUNOREGULATORY EFFECTS OF BIRCH (*Betula pendula* Roth.) AND RED RASPBERRY (*Rubus idaeus* L.) DRIED LEAF EXTRACTS IN ALLOXAN INDUCED DIABETIC RATS

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Objective: Evaluation of the immunoregulatory potential of birch and red raspberry leaf ethanolic dried extracts in alloxan induced diabetic rats.

Methods: 200 mg/kg extracts, alone or combined with metformin (100 mg/kg), were given orally, to 72 Wistar diabetic rats (130 mg/kg alloxan i.p), divided in 6 groups of 12 each: group I (diabetic control), group II (metformin), group III (birch extract), group IV (birch extract + metformin), group V (red raspberry extract), group VI (red raspberry extract + metformin). Immunoturbidimetric assays were used for C₃, C₄ and C-reactive protein (CRP) quantitative analysis.

Results: Groups I (26.92±4.42; 19.03±5.18; 0.46±0.19) and II (39.54±5.11; 20.81±3.98; 0.52±0.35) had high values of C₃, C₄ and CRP. Significantly differences (p<0.05, IC=95%) were found for groups III (23.82±2.84; 12.93±2.03; 0.14±0.09) and V (21.93±1.98; 10.32±2.07; 0.09±0.09), in comparison with I and for IV (16.32±3.01; 13.06±2.77; 0.16±0.14) and VI (27.01±2.01; 7.78±1.85; 0.12±0.11), as compared to II.

Conclusion: Extracts showed strong immunoregulatory activity, influencing both classic and alternative pathways of complement activation.

PF44

BLOOD OXIDATIVE STRESS MARKERS AND ANTIOXIDATIVE ENZYMES LEVELS IN AGE-RELATED CATARACT PATIENTS

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Introduction/Aim: The pathogenesis of cataract is known to be influenced by a number of factors including oxidative stress. Our study was aimed to investigate the levels of conjugated diene (CD), total hydroperoxides and malondialdehyde (MDA) as lipid peroxidation markers, total protein SH-groups as a marker of protein oxidative damage, and activi-

ties of GSH-dependent antioxidative enzymes in blood specimens from patients with age-related cataracts.

Material and methods: Hundred patients with age-related cataract and forty-five noncataract controls of matched age and gender were included in this study. Plasma total sulfhydryl groups were meas-

Poster Presentations

ured with Ellmans's reagent. Glutathione peroxidase (GPx) activity was measured by the colorimetric method using cumene hydroperoxide as a substrate. Glutathione S-transferase (GST) activity in the plasma was measured with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate.

Results: The concentration of CD (460.7 ± 99.1 versus 300.7 ± 67.4 $\mu\text{mol/L}$; $p < 0.001$), hydroperoxides (7.2 ± 1.5 versus 6.2 ± 1.7 $\mu\text{mol/L}$; $p < 0.05$) and MDA (4.5 ± 1.1 versus 3.4 ± 0.9 $\mu\text{mol/L}$; $p < 0.001$) significantly increased in patients plasma compared with control. Cataract patients had significantly lower

total plasma SH-groups compared with healthy subjects (343.8 ± 71.3 versus 546.7 ± 105.3 ; $p < 0.001$). The activities of GPx ($p < 0.001$) and GR ($p < 0.001$) in cataract group were lower than those in the control group. In comparison to the control values, serum GST activity was significantly higher in cataract groups ($p < 0.001$).

Conclusion: These results suggest that the development of age-related cataract associated with systemic oxidative stress in which the decreased activities of GSH-dependent antioxidants may be involved.

PF45

GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) AND PHYSIOLOGICAL ACTIVATION OF SPERM MOTILITY

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Background: GM-CSF is 23 kD glycoprotein, and after secretion is associated with the special α and β membrane receptors. It is scientifically proven that GM-CSF prevents apoptosis, improves cell proliferation and metabolism. Expression of GM-CSF receptors occurs in the prostate, testes, as well as in germ cells (sperm) and improves their motility. The aim of this study was to determine the influence of GM-CSF on sperm motility, with special emphasis on TESE sperm.

Methods: For this research, collected semen samples from 10 patients (olygoasthenospermia (OAS), and asoospermia), were processed by Density gradient or just by washing. After that, each processed sample was divided into two equal parts. One part

was used as a control sample, while in the second part it was added 50 μl of GM-CSF. After that, sperm motility was examined by comparing control samples and samples with GM-CSF, in different time intervals.

Results: By comparing control vs GM-CSF samples from 10 OAS and asoospermic patients, it was shown that number of progressive and vibrating sperm (P+V) increased almost double. From 47% in control samples, to 90% in samples with GM-CSF.

Conclusion: The data obtained in this study showed, that by using media with GM-CSF it can be perform: physiological activation of sperm motility, higher number of progressive motile sperm, selection and collection of best motile sperm, for a short time.

TOXOPLASMA GONDII SUSCEPTIBILITY IN WOMEN OF CHILDBEARING AGE

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Objective: Toxoplasmosis is disease caused by the *Toxoplasma gondii* parasite which infects warm blooded animals including humans. In healthy uncompromised adults the parasite may give occasionally flu-like symptoms and in most cases no symptoms at all. On the other hand, if the mother comes in contact for the first time with the parasite just before or during pregnancy it may result in congenital toxoplasmosis which is a rare but potentially serious infection with severe consequences for the developing fetus.

Toxoplasma gondii parasite can be contracted while handling unwashed fruits, undercooked meat or contaminated water with *T. gondii* oocysts. On the other hand transplacental transmission from mother to child causes congenital infection.

The aim of this study is to evaluate *Toxoplasma gondii* susceptibility in women of childbearing age during the last 2 years at our hospital.

Methods: The study included a total of 705 women of childbearing age. The sera from 705 patients were analyzed for both *Toxoplasma gondii*-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) using electrochemiluminescence immunoassay (ECLIA).

Results: Among 705 women of childbearing age analysed, *Toxoplasma* IgG positive rate was 24.1% or 170 women out of 705, while *Toxoplasma* IgM positive rate was found to be 2.12% or 15 women out of 705 analysed. Furthermore, IgG avidity was recommended to 5 pregnant women who were seropositive for both *Toxoplasma* IgG and IgM. Whereas, 3 pregnant women were seropositive only for *Toxoplasma* IgM antibodies, indicating acute infection.

Conclusion: *Toxoplasma* IgG antibody seroprevalence rates of 24.1% indicate that 75.9% of women of childbearing age are susceptible to *Toxoplasma* infection, whereas the rate of acute infection is 2.12%.

The above data suggest that in order to prevent congenital infection with *T. gondii* prenatal screening is of utmost importance especially among women with negative *Toxoplasma* IgG antibodies who should be screened monthly for both *Toxoplasma* IgG and *Toxoplasma* IgM antibodies. Furthermore, continuous education of pregnant women regarding preventive measures such as hygienic conditions and healthy eating must be implemented in order to prevent *T. gondii* infection.

PF47

PREVALENCE OF HCV COINFECTION AMONG HIV POSITIVE PATIENTS

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Background: The cirrhosis possibility in patients with chronic hepatitis C, in an interval of 20 years, is 2–20%. The HIV coinfection worsens the prognosis and increase the mortality of these patients.

Objectives: The aim of this retrospective study was the determination of HIV/HCV coinfection's prevalence in the Second Internal Unit Department and Infection Unit, Papadimitriou –Sismanoglio Hospital of Athens.

Materials and methods: We have studied 118 HIV seropositive patients during a 2 years period (2010–2012). Anti-HCV was determined with MEIA (Micro-particle Enzyme Immunoassay). In these patients CD4 cells count was performed with flow cytometry and HIV viral load were also measured with real time PCR.

Results: Five out of 118 (4.2%) HIV seropositive patients, were anti-HCV positive. Three (60%) out of 5 patients with HIV/HCV coinfection, were Greek and 2 (40%) out of 5 patients were foreigners (mean age 46 years). Intravenous drug users were 2 of the patients with HIV/ HCV co-infection. In coinfecting patients the mean CD4 count was 402 cells/ μ L and the HIV viral load varies between 50–1587 copies/mL.

Conclusions: The HIV/HCV coinfection prevalence was 4.2%. The HAART application in HIV infected patients improves the prognosis of this disease. The coinfection with HCV increase the complications and the mortality. Consequently, the HAART therapy should be carefully applied in coinfecting patients because of the possibility of hepatotoxicity.

PF48

THE SYSTEMIC METABOLIC SHIFTS DUE TO STATES OF MITOCHONDRIAL AND ENDOTHELIAL DYSFUNCTION

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Mitochondrial and endothelial (MD, ED) dysfunction mediate the development of an unfavorable outcome in the progression of cancer, neurological and cardiovascular diseases.

The results of testing patients plasma (n=35, from 50 to 83 years) with D-dimer level above 500 ng/mL and lactic acid above 2.2 mmol/L were compared

with those of healthy donors (n=20, from 55 to 61 years). Total homocysteine (tHcy), cytochrome C (CytC), sum of nitrites and nitrates (NOX), S-nitrosothiols (NT) and amino acids related to mitochondrial routes were determined.

Patient levels NOX 43(26–55) and tHcy 13(9–19) μ mol/L were significantly higher than in donors

11(8–12) and 21(17–25) $\mu\text{mol/L}$, and NT rates in patients 562(288–943) were lower than in donors 958(672–2663) nmol/L . The positive relationships between CytC and Ile(1), Leu(2), Gly(3) rates in the patient group ($r_1=0.54$, $r_2=0.40$ and $r_3=0.50$; $p<0.05$) were observed. CytC levels positively correlate with tHcy and Arg ($r=0.46$ and $r=0.50$, respec-

tively, $p<0.05$), but indicated characteristics of MD were observed in a subgroup of patients with normal rates tHcy too ($n=14$).

Thus, within creasing values of ED indicators were intensified signs of MD. In a part of patients with disorders of coagulation and fibrinolysis MD may occur without changes of ED indicators.

PF49

CLINICAL-MYCOLOGICAL PROFILE OF SUPERFICIAL MYCOSIS IN PAEDIATRIC AGE GROUP

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Introduction: Infants and children are susceptible to fungal infections because of their immature immune system and enhanced exposure to the sub-clinical infection carriers in school, unneffective fungistatic activity of sebum till puberty, sometimes in close contacts with animal and pets.

Materials and Methods: 70 patients including infants, preschool and school going children up to the age of 12 years clinically diagnosed as having superficial dermatomycosis were enrolled, from May 2010 to September 2012. The specimen: skin scrapings, hair and nail clippings were taken for mycological examination, microscopic with KOH sol. and cultivated on Sabouraud dextrose agar with cycloheximide and chloramphenicol.

Results: Of the 70 children, infants and school going children were almost in equal numbers 30 (42.86%) and 33 (42.14%), 7 (10%) were preschool age group. Affected male to female ratio was 2.5/1. Among the school age group 30 (99.9%) were suffering of dermatophytosis and 3 of candidiasis (9.01%). Among the preschool age group 6 (85.7%) were suffering of dermatophytosis and 1 (14.3%) of candidiasis. Of the infants, 24 (80%) had candidiasis, 5 pityriasis and 1 dermatophytosis. Tinea capitis was the com-

monest of the dermatophytosis (32.43%), followed by tinea corporis (27.03%).

Intertrigo was the commonest of the candidiasis (42.86%) followed by diaper dermatitis (32.14%). Direct microscopy was positive in 54 (77.14%). Culture was done in 65 cases except of pityriasis versicolor of which 20 had no growth. Of the 28 cases of candidiasis, culture was positive in 21 cases and all of them were *C. albicans*. 3 of 37 cases of dermatophytosis were culture negative. *Trichophyton rubrum* was isolated in 10 cases, all of them had tinea corporis.

Discussion: The susceptibility of school going children to dermatophytosis may be explained by their excessive intermixing with other children facilitating transfer of infection. The susceptibility of infants to candidiasis may be due to developing immune system, which makes them vulnerable to opportunistic infections.

Almost half of the cases presented during the months of June to September. This seasonal variation indicate the role of high relative humidity and temperature in causation and exacerbation of superficial fungal infection.

PF50

THE RATIO OF THE CSF GLUCOSE WITH SERUM GLUCOSE AS AN INDEX VALUE TO HELP FOR THE DIFFERENTIAL DIAGNOSES OF TUBERCULAR MENINGITIS FROM VIRAL ONE

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Purpose of the study: Comparison of the ratio of glucose CSF/serum in patients with tubercular meningitis and viral one.

Material and method: We studied 49 patients over 18 years of age presented at the emergency service of Infectious Diseases Hospital in the period May 2006–May 2010 in the clinical context of an acute encephalopathy and divided in two groups according to final diagnosis (13 patients with tubercular meningitis and 36 patients with viral one). To all patients was analyzed serum glucose (derived from blood collected 2 hours before lumbar puncture) and CSF glucose. Determination of glucose and serum in CSF was made in Olympus AU 640 analyzer with enzymatic method. The ratio CSF/serum glucose was calculated. The ratio <0.4 was estimated as low. For data analyses was used SPSS 17.0 statistical package.

Results: The ratio of glucose was found low in 76.9% of patients with tubercular meningitis and in 8.3% of patients with viral one. The average value of the glucose ratio in tubercular meningitis was 0.34 (DS0, 09), the minimum value 0.17, while the maximum value was 0.52. The average value of the glucose ratio in viral meningitis was 0.53 (DS0, 1), the minimum value 0.32, while the maximum value was 0.78. We found that there is statistically significant difference between the values of the ratio of glucose of patients with tubercular meningitis against those with viral meningitis ($p < 0.05$).

Conclusion: The ratio of the CSF glucose with serum glucose is an important index to help the differential diagnosis of tubercular meningitis from viral one.

PF51

LABORATORY DIAGNOSIS OF MENINGITIS IN KOSOVO

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Introduction: Meningitis still remains a life threatening problem worldwide (especially for developing countries). In Kosovo, meningitis is still frequent, but special tests are still unavailable. Aim of this study is to evaluate the diagnostic importance some

of biochemical parameters, glucose, proteins, C-reactive protein, lactate dehydrogenase, in cerebrospinal fluid (CSF) and serum and determining of ratios CSF/serum glucose, CSF/serum LDH like early indicators for the diagnosis of acute meningitis.

Methods: In this study were examined 50 CSF and serum samples of patients with meningitis (1–75 years of age), which were hospitalized and diagnosed in Clinic of Infectious Diseases of UCCK Pristina, during period October 2010–February 2011. Patients are divided into 3 groups: BM-bacterial meningitis (n=20), AM-aseptic meningitis (n=17), and control group (n=13), who presented with fever, dehydration or electrolyte disturbances and had undergone lumbar puncture, to exclude the presence of meningitis. The results have been elaborated with the statistical program VassarStats.

Results: CSF glucose values were significantly decreased ($p<0.001$) in patients with BM (1.64) compared to AM (3.21 mmol/L) and control group (3.25 mmol/L). Mean value of CSF/serum glucose ratio (0.33) in patients with BM was significantly

lower ($p<0.001$) than patients with AM (0.61) and patients without meningitis (0.64). Mean concentration of CSF protein in group with BM (1.58) was increased significantly ($p<0.001$) compared to the group with AM (0.72 g/L) and non meningitis group (0.24 g/L). LDH activity in CSF was significantly higher ($p<0.001$) in the BM group (146 U/L) compared with the AM (45 U/L) and control group (14 U/L) and mean value of CSF/serum LDH ratio was significantly higher ($p<0.001$) in patients with BM (0.4) compared with AM group (0.13) and control group (0.04).

Conclusions: Simultaneous determination of glucose, proteins, CRP, LDH in CSF and serum and their ratios will increase diagnostic efficacy and could be utilized to distinguish between BM and AM.

PF52

STUDY THE EFFECT OF DIFFERENT CONCENTRATIONS OF DMSO ON THE ANIMAL CELL CULTURE IN THE DRUG DISCOVERY PROCESS

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Introduction: Cell culture is in use for the prediction and testing of the biological effects of different therapeutic drug and bioactive compounds. In vitro studies often use a variety of solvents, but they have some toxic effects. One of them is dimethyl sulfoxide (DMSO). DMSO is a small amphipathic molecule that is widely used as a solvent due to its abilities to dissolve both polar and nonpolar compounds. The purpose of this study was to evaluate the cytotoxic effects of different concentrations of DMSO and determinate the best concentrations usable for the future work in conducting research in the test animal cell cultures.

Materials and Methods: The assessment of the cytotoxic effects of DMSO was carried out using two biological systems cell culture: VEPK and SK-6. We

determined influence the different concentration of DMSO on monolayer integrity of cells.

Results: Thus, the performed research demonstrated the DMSO presence even in low concentrations media as causing a toxic effect on cells. The DMSO concentrations of 0.04–1.89% of DMSO were established as the optimal ones, which has not suppressive effect on the VEPK cells. Cell culture SK-6 was more sensitive to the effects of DMSO and its maximum possible permissible concentration was – 1.25%, which preserved the ability of the cells to proliferate and differentiate.

Conclusion: DMSO may be used at concentrations up to 1.89% in VEPK cells and up to 1.25% in SK-6 cells without any significant cell damage.

PF53

PREVALENCE OF HBV COINFECTION AMONG HIV POSITIVE PATIENTS

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Background: Chronic active hepatitis B can lead to cirrhosis or to hepatocellular cancer. As the HAART therapy improve the HIV infection progress, it is important to determine the influence of HBV on HIV infected patients receiving HAART.

Objective: The aim of this retrospective study was the determination of HIV/HBV co-infection's prevalence in the Second Internal Unit Department and Infection Unit, Papadimitriou-Sismanoglio Hospital of Athens.

Materials and methods: We have studied 118 HIV seropositive patients during a 2 years period (2010–2012). HBsAg, anti-HBs and anti-HBc antibodies were determined with MEIA (Microparticle Enzyme Immunoassay). In these patients CD4 cells count and HIV viral load were also measured with flow cytometry and real time PCR respectively.

Results: Anti-HBs and/or anti-HBc positive (previous infection) were observed in 32/118 (27.1%) patients. Anti-HBs positive (previous vaccination) were observed in 20/118 (16.9%) patients. Eleven out of 118 (9.3%) HIV positive patients were also HBsAg positive. Nine (9/11, 81.8%) of these patients were Greek and 2 of them (2/11, 18.2%) were foreigners. In HIV/HBV coinfectd patients the mean CD4 count was 390cells/mL and the HIV viral load varies between 36–1275 copies/mL. Four out of 11 (36%) coinfectd patients had increased transaminase values and one patient has received interferon for HBV infection treatment.

Conclusions: The follow-up of coinfectd patients, in order to avoid the HBV reactivation, and the rational HAART therapy application could help in better treatment of HIV/HBV coinfectd patients.

PF54

THE STATUS OF THE MEDICAL BIOCHEMISTRY AND LABORATORY MEDICINE AS A SPECIALITY IN THE REPUBLIC OF MACEDONIA

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The medical biochemistry as a profession in the Republic of Macedonia is an integral part of the System for Health Care, and the specialization in this field exists for more than 50 years. The specialization in medical (clinical biochemistry) is performed at the Faculty of Medicine in Skopje, according to a 4-year postgraduate curriculum which

was recently revised and upgraded with a purpose of approaching to the standards and criteria required by EC4 and the European syllabus. However, in the Republic of Macedonia a law which regulates the medical biochemistry as a profession still does not exist, and the changes of the right for specializing in this field throughout the years, result with a

decrease of the interest for this deficient profession which becomes more and more important in the world. This represents a disadvantage for a country which tends to integrate into the great European society, and that is why examining the opportunities for implementing the EU regulations and undertaking actions for reaching equivalence of the standards with those of the European Syllabus and the

European Register, should represent an imperative for all health workers in this field. It is essential to build one common identity which will serve for unifying the professional characteristics of all past and future specialists, which on the other hand will lead to raising the quality of the services and standards of this profession, and as well as to its promoting.

PF55

THE IMPACT OF HELICOBACTER PYLORI INFECTION ON IRON STATUS IN CHRONIC IDIOPATHIC URTICARIA PATIENTS

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Objective: The aim of this study was to investigate the impact of *Helicobacter pylori* (HP) infection on iron status parameters in chronic idiopathic urticaria patients (CIU).

Materials and Methods: We analyzed 79 (CIU) adult patients: 42 cases with positive anti-HP antibodies and 37 cases with negative anti-HP antibodies. Iron status was assessed by: hemoglobin, sideremia, transferrin saturation coefficient (TFS), total iron binding capacity (TIBC), ferritin and soluble receptors for transferrin (sTfR).

Results: Out of 32 CIU HP-negative patients, 78.4% had iron sufficiency store and 21.6% had iron deficiency anemia.

Out of 42 CIU HP-positive patients, 57.1% had iron sufficiency store, 30.9% had iron deficiency anemia

and 12% had anemia of chronic disease. Iron deficiency anemia was associated with decreased sideremia/TIBC ratio, increased sTfR/ferritin ratio and positive relationship between sTfR and TIBC ($r=0.419$, $p<0.05$) and negative relationship between sideremia and sTfR ($r=0.506$, $p<0.05$).

Anemia of chronic disease was associated with decreased sTfR/ferritin ratio and increased sideremia/TIBC ratio.

Conclusions: HP infection induces many alterations in CIU patients, resulting in iron deficiency anemia and anemia of chronic disease. HP infection treatment could be a condition for the efficiency of the antihistamine treatment.

PF56

RISK ASSESSMENT FOR TORCH COMPLEX INFECTION IN WOMEN OF CHILDBEARING IN TIMISOARA, ROMANIA

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Introduction: The objective was to present the percentage of gravidas in our area with the risk of contacting primary infections with TORCH-agents during pregnancy.

Material and Methods Retrospective study based on 2041 pregnant women that were tested in our hospital during the first trimester of pregnancy in order to detect anti-Toxoplasma Gondii, anti-cytomegalovirus (CMV) and anti-rubella IgG/IgM-antibodies. The pregnant women were divided according to age (one-year intervals). The percentage of immunized gravidas was calculated for each group.

Results: Out of 2041 tested gravidas for IgG-anti-Toxoplasma Gondii, 894 were positive, 10 inconclusive and 1137 negative, for IgG-anti-CMV 1905 were

positive and 136 negative and for IgG-anti-rubella, 1903 were positive, 14 inconclusive and 124 negative. Among the tested patients, 27 were positive for IgM-anti-Toxoplasma Gondii, 3 for IgM-anti-CMV and 8 for IgM-anti-Rubella. The age of the gravidas varies between 14 and 46.

Conclusions: About 56% of the gravidas were not immunized before pregnancy for Toxoplasma Gondii, 7% were not immunized for CMV or Rubella and they could potentially be infected during the first trimester of pregnancy. Due to the fetal malformation risks caused primarily by the infection with TORCH agents during pregnancy, it is strongly recommended to perform TORCH screening tests for all pregnant women, no matter what area they come from (urban/rural) or their age.

PF57

CLINICAL AND SEROLOGICALLY CONFIRMED CASES OF MARSEILLES FEVER IN SOME ENDEMIC REGIONS IN BULGARIA DURING THE PERIOD 2007-2013

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Introduction: The Mediterranean region and Southern Europe, including Bulgaria was an endemic area for tick-borne diseases. Marseilles fever was one of this and caused by etiological agent Rickettsia conorii, which was transmitted through the bite of the brown dog tick Rhipicephalus sanguineus. The

commonest symptoms were high fever, a maculopapular rash, myalgia, headache, arthralgia and/or a unique eschar (the "tache noire") at the tick bite site. The aim of our retrospective study was to evaluate the symptoms and seroprevalence of R.

conorii infection in patients from different regions of Bulgaria during the period 2007–2013.

Methods: The study was conducted at the Laboratory of Rickettsiae and Tissue cultures at National Centre of Infectious and Parasitic Diseases – Sofia (Bulgaria). Serum samples were collected from 554 patients of both sexes with clinical evidence of Marseilles fever aged 12 to 80 years. All the patients had a high fever and a maculopapular rash (may include palms or soles) and treated with doxycycline. Presence of anti-R. conorii IgM and IgG antibodies in the samples were tested by enzyme-linked immunoassay (ELISA).

Results: Recent infection was indicated in 246 persons (44.4%). Most of the patients (76.8%) were

farmers, living in rural areas of five cities (Nova Zagora, Shumen, Pleven, Pazardjik, Kustendil). All had contact with dogs but 60 patients remembered being bitten by a tick. 150 (61%) of the patients were over the age of 60. The prevalence of anti-R. conorii IgG antibodies were 83% (204 of 246). Seasonal distribution was noted. All the patients were treated with doxycycline (200mg/day, 10 days). No treatment failure or relapse occurred.

Conclusions: Confirmed diagnosis had the most patients in Nova Zagora (41.5%), Shumen (20.7%) and Kustendil (13.8%). The region around South Bulgaria (Trakya) was an endemic for Marseilles fever and further clinical studies are needed to isolate and characterize the agent by molecular methods.

PF58

HPV INFECTION AND GENOTYPES' DISTRIBUTION IN CERVICAL SAMPLES IN MONTENEGRIAN WOMEN

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Background/Aim: Information on distribution of HPV genotypes in women is important for the selection of the appropriate screening test for detection of HPV infection. Therefore, the purpose of this study was to determine the range and frequency of HPV genotypes in studied group of women in Montenegro.

Methods: HPV genotypes were determined using the method of enzyme restriction of PCR products amplified with group-specific primers MY09/MY11 and restricted with seven different restriction end nucleases. In all studied women cervical smears were taken during a routine gynecological examination at the Clinical Center in Podgorica.

Results: Out of the total number of 189 women HPV infection was found in 1/5 of participants (20%). We found the presence of 16 different HPV genotypes. Genotyping performed in 38 HPV DNA positive women shows that the HPV genotype 16 is

dominant and present in more than 1/3 of the participants (36.8%). The second most frequent HPV infection is with HPV genotype 58 and it is found in 10.5% of participants. HPV 31 and HPV 6 infections are present in 7.9% of women, while infections with other genotypes were demonstrated individually by 2.6%. Analyzing 16 detected HPV genotypes, in 4/5 patients (81.6%) the presence of high-risk HPV genotypes was confirmed. Multiple HPV infection was demonstrated in 18.4%. Also, in our group of participants it was found that multiple infections, with more than one HPV genotype are dominant in younger women (aged 25 to 30 years) and with at least one high-risk HPV genotype.

Conclusions: According to the results of our research we believe that in active search of women, who are more likely to develop cervical cancer, it necessary to do the tests that are able to detect broader spectrum of high-risk HPV genotypes. Sen-

sitive detection of HPV genotypes in patients is, also, especially important for the determination of

persistence of infection and cervical cancer prevention.

PF59

SEROPREVALENCE OF HEPATITIS B VIRUS INFECTION IN PREGNANT WOMEN IN TIMISOARA, ROMANIA

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Introduction: The objective of the presentation is to evaluate the percentage of gravidas in our area which present an Antigen Hbs (AgHbs) positive reactivity in serum.

Material and methods: There were 2510 sera from gravida tested by chemiluminescence technique during the first trimester of pregnancy to detect antigen Hbs. The pregnant women were divided according to age (five-year intervals) and their provenience area (urban, rural and unknown). The percentage of AgHbs positive gravidas was calculated for each group.

Results: The 2510 gravidas were grouped according to age: Group A (120 gravidas/14y–20y), Group B (650 gravidas/21y–25y), Group C (1038 gravidas/26y–30y), Group D (548 gravidas/31y–35y), Group E (154 gravidas/ 36y–43y). The prevalence of AgHbs was 2.78% (overall), respectively 6.66% (group A), 3.54% (group B), 2.11% (group C), 2.19% (group D), and 3.24% (group E). Considering the

medium of provenience we found that the gravidas from the urban (U) and rural (R) areas show the following prevalence of AgHbs: A (U-6.52%; R-6.55%), B (U-3.41%; R-3.31%), C (U-2.05%; R-2.77%), D (U-1.99%; 2.5%) and E (U-3.7% ;R-0%).

Discussion: Chronic hepatitis B virus infection is a major cause of cirrhosis and hepatocellular carcinoma. Hepatitis B virus infection of gravida can lead to materno-fetal transmission of the virus, if the infection is not diagnosed in pregnancy and appropriate measures are not implemented at birth. In order to minimize the risk of materno-fetal transmission the AgHbs status of gravida must be determined. Our study shows a percentage of around 2.78% of AgHbs positive gravidas with the maximal prevalence in the age interval from 14y to 20y.

Conclusions: About 2.78 % of gravidas were AgHbs positive with the highest prevalence between 14–20y, with a similar distribution in the urban and rural area.

PF60

BIOCHEMICAL RESULTS AS AN INDICATOR OF QUALITY IN PHARMACOVIGILANCE PLASMA DERIVATIVES

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Pharmacovigilance is a set of activities related to the collection, detection, assessment, understanding and prevention of adverse reactions to the drug, as well as other problems associated with the drug. We distinguish adverse reaction-which means any harmful and unintended response to a drug that has emerged in the application of the usual dose in humans (for purposes of treatment, disease prevention, diagnosis, restoration, improvement or alteration of physiological function), or the application of any dose drug during clinical investigations. After, serious adverse reaction which is harmful and unintended response to a drug which has resulted in: death, life-threatening, or serious permanent damage, disability, hospitalization or prolongation of existing hospitalization, congenital anomalies or birth defects and other significant medical condition. In addition to these we also have an unexpected adverse reaction, adverse event, adverse experiences and serious adverse event.

Plasma derivatives (stable drugs) that are produced in the Institute for Blood Transfusion of Serbia (human albumin and specific immunoglobulin) are purified plasma proteins whose electrophoresis purity of over 95% and were obtained by fractionation of human plasma. Protein concentration using ultra filtration dress up during the process, with regular biochemical controls the protein content and the concentration of ethanol-which precipitating agents during plasma fractionation.

In case if there is detect of post-marketing reactions to any product produced in Institute of Blood Transfusion, it is necessary to reanalyze sample to all parameters, prescribed by the European pharmacopoeia (Ph. EUR), which can be a key for using traceability to find what could be the cause eventual reactions in patients.

PF61

EXAMINATION OF POTASSIUM P-NITROPHENYLPHOSPHATE ENZYME (K-pNPP) ACTIVITY IN HUMAN ERYTHROCYTE MEMBRANES

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Introduction: The membrane proteins perform the function of receptors, cation pumps; they are also carrying the polysaccharide blood group antigens as well, and many of them are involved in anion transport also. Due to the NaK-ATPase activity, the concentration of electrolytes in human erythrocyte is maintained in reference range.

Aim: Testing the kinetic activity of K-pNPPase regarding K^+ ion and the human erythrocyte membrane PNPP.

Materials/Methods: The activity of K-pNPPase was determined in purified erythrocyte membranes which were previously isolated from the blood of healthy male adults aged from 20 to 40 years.

Poster Presentations

Blood was collected in a test tube with 0.1 mL of EDTA (5mmol/L) and 0.05 mL of heparin solution (5000 IU). Human red blood cells were isolated by using the modification of Dodge and associates' method. The total protein concentration was determined by using the Bradford's method. A K-pNPPase activity determination method is based on free p-nitrophenyl concentration measurement in specimen. When K^+ ion present, this enzyme decomposes p-nitrophenylphosphate into p-nitrophenyl and inorganic phosphorus. A K-pNPP enzyme activity is calculated using the molar absorption coefficient. Specific activity is expressed as pNPP activity unit per total protein milligram. The K-pNPP activity increases after the K^+ ion concentration reaches the value of 5 mmol/L, soon after the enzyme activity plateau is seen. The second increase of enzyme activity follows the plateau, until the KCl concentration reaches the value of 50 mmol/L. Any further K^+ ion concentration increase appeared to reduce the K-pNPP enzyme activity. On the other hand, increasing of pNPP concentration has a stimulative effect on the K-pNPP enzyme activity.

Results: Maximum enzyme activity is achieved already by using 15 mmol/L of pNPP; there was no statistically significant difference between this one and the maximal used of pNPP of 25 mmol/L. The obtained values of kinetic parameters amounted to $V_{max}=3.61$ nmol pNPP/mg per protein/min and $K_m=4.02$ mmol/L pNPP. In the presence of KCl (40 mmol/L), maximum activity of K-pNPPase is achieved after adding only 10 mmol/L of pNPP. There was no statistically significant difference between enzyme activity either it was 10 or 25 mmol/L of pNPP added. The obtained values of the kinetic parameters amounted to $V_{max}=3.49$ nmol pNPP/mg per protein/min and $K_m=4.02$ mmol/L pNPP.

Conclusion: The results may be a confirmation of the assumption that the presence of potassium ions activates the binding places for sodium ions. According to generally accepted pattern of $Na^+ K^+$ ATPase activity, binding of $3Na^+$ ions for their substrate from the inside of the membrane cell favors the binding of ATP.

Vitamins and nutrition

PT31

NUTRITIONAL STATUS IN HEMODIALYSIS PATIENTS THROUGH ALBUMINE AND TRIGLICERIDE LEVELS

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Introduction: Low levels of albumins (Alb) and triglycerides (Trig) are one of the most important parameters in assessment of nutritional status. Malnutrition is common in haemodialysis in patients and it is closely related to morbidity and mortality in this group of patients.

Objective: The aim of this study is to examine Alb and Trig levels and to determine the correlation between those parameters in haemodialysis patients.

Design and methods: The research was conducted on 53 patients (M:F 25:28) who regularly go to haemodialysis treatment, the average age is 56.47 ± 11.79 years, average length of dialysis 5.33 ± 4.48 years and average KT/Vsp index 1.12 ± 0.15 . Albumin levels were measured by immunoturbidimetric test, while triglycerides were measured using enzymatic colorimetric method. Statistical analysis of the results was performed by Spearman rang correlation test.

Results: Our study showed that 15.09% of patients had hypoalbuminemia while 84.91% had albumin levels in reference range. In examined group 73.59% of patients had normal levels of Trig and 26.41% elevated levels. The mean value of Alb was 37.71 ± 3.05 g/L, while the mean value of Trig was 1.42 ± 0.60 mmol/L. We found highly significant cor-

relation between Alb and Trig levels for $p=0.001$ ($p<0.01$).

Conclusion: In our study, a significant number of patients showed low levels of Alb and Trig. The correlation between Alb and Trig can be observed as a common consequence of bad nutritive habits in haemodialysis patients.

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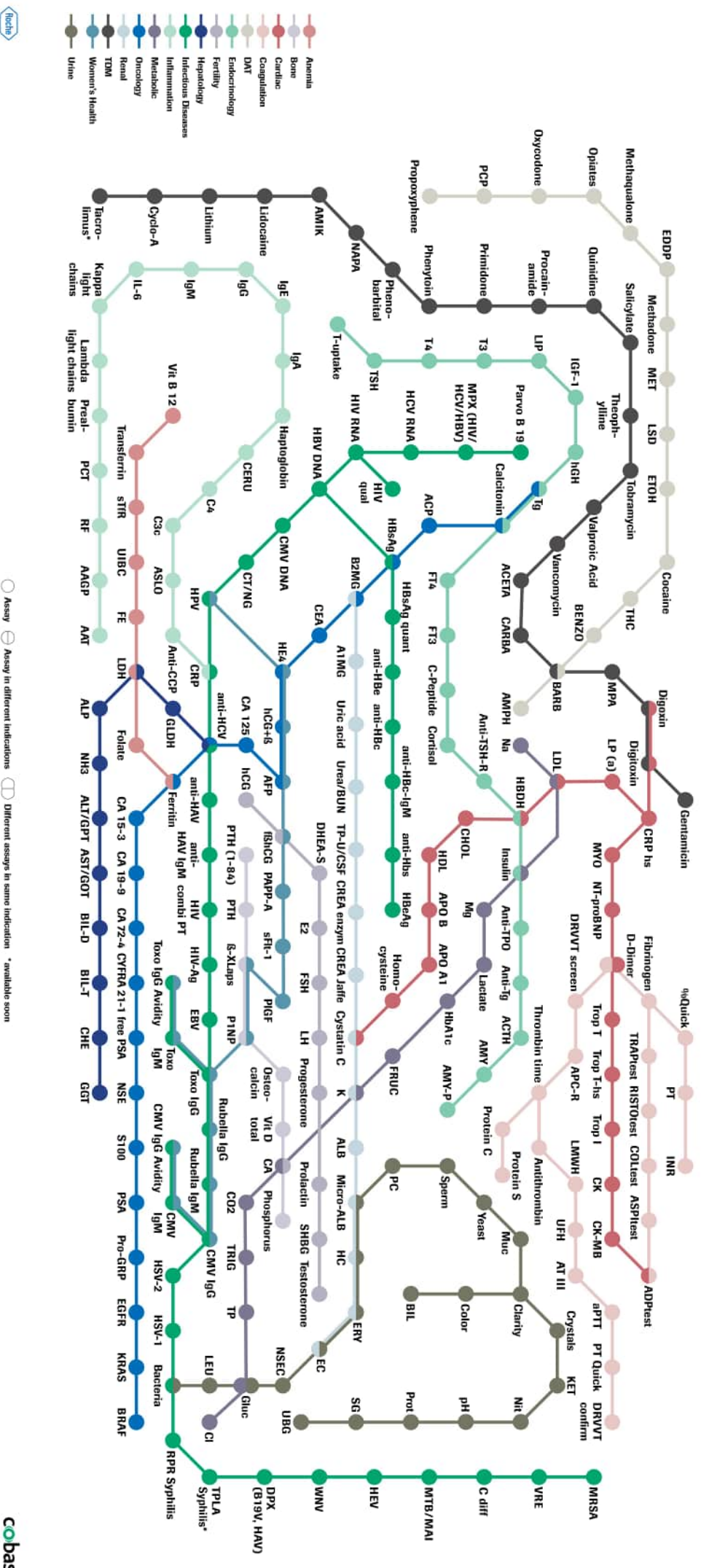
- CBC Results
- Differential Results (absolute and %)
- Platelet Results
- Reticulocyte Results (absolute and %)
- CSF assay results
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Helping Improve Clinical Outcomes

Manual ESR determination methods have the potential for errors at numerous stages in the analytical process.

Our new BD Sedi-40, in combination with the established tube technology of the BD Seditainer™, provides an automated solution to help improve clinical outcomes by standardising ESR determinations.

This results in more accurate, timely results, a more efficient workflow – and helps improve patient care.

Improved Efficiency

- 30 minute analysis time – half the time of a 1 hour modified Westergren
- Integrated barcode reader for fast, accurate sample ID entry
- Integrated sample mixing
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- Connectivity capabilities for automatic data transmission



Accuracy:

- Standardised procedures delivering greater accuracy for optimal patient management

Improved Efficiency

- 30 minute analysis time – half the time of a 1 hour modified Westergren
- Optional barcode reader for fast, accurate sample ID entry
- Optional printer for hard copy results



Accuracy:

- Standardised procedures delivering greater accuracy for optimal patient management



BD Sedi-20

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- Optional barcode reader for fast, accurate sample ID entry
- Optional printer for hard copy results



Accuracy:

- Standardised procedures delivering greater accuracy for optimal patient management



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- Clinical equivalence to the gold standard 'Westergren', incorporating temperature correction'
- On board QC management

Flexible

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Clinical equivalence:

- Clinical equivalence to the Westergren gold standard'



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