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Wednesday, 27.09.2023.			
Session 1: Bone and mineral metabolism	Emir Muzurovic	14:00	14:30
	Etienne Cavalier	14:30	15:00
	Aylin Sepici Dincel	15:00	15:30
	Nela Raseta Simovic	15:30	16:00
Session 2: Total testing process in clinical laboratory	Mario Plebani	16:00	16:30
	Khosrow Adeli	16:30	17:00
	Sverre Sandberg	17:00	17:30
	Commercial workshop 1	17:30	18:00
	Welcome addresses	18:30	19:00
Opening lecture	Tomris Ozben	19:00	19:45
	Welcome cocktail	19:45	21:00
Thursday, 28.09.2023.			
Lab-clinical case reports: From diagnostics to diagnosis	Petra Uljarevic	7:30	8:15
Lab-clinical case reports: From diagnostics to diagnosis	Aleksandra Antovic	8:15	9:00
	Coffee break	9:00	9:15
Session 3: Laboratory haematology	Sverre Sandberg	9:15	9:45
	Slavica Pavlovic Djuranovic	9:45	10:15
	Marko Trtica	10:15	10:30
	Miljan Savkovic	10:30	10:45
Plenary lecture	Gary Moore	10:45	11:30
Session 4: Thrombosis and haemostasis	Jovan Antovic	11:30	12:00
	Ivana Lapic	12:00	12:30
	Nikola Bakic	12:30	13:00
	Milena Velizarova	13:00	13:15
	Commercial workshop 2	13:15	13:45
	Lunch	13:45	15:00
	Poster presentations		
Session 5: Cardiovascular diseases and cardiovascular disease risk prediction	Aneta Boskovic	15:00	15:30
	Murat Cihan	15:30	15:45
	Vladimir Prelevic	15:45	16:15
	Ebru Sezer	16:15	16:45
	Muhammed Fevzi Kilinckaya	16:45	17:00
	Commercial workshop 3	17:00	17:30
	Coffee break	17:30	17:45
Session 6: Immunology / Allergology	Urska Bidovec Stojkovic	17:45	18:15
	Marija Saric Matutinovic	18:15	18:45
	Neda Milinkovic	18:45	19:00
	Verica Jakjimoska	19:00	19:15

Friday, 29.09.2023.			
Lab-clinical case reports: From diagnostics to diagnosis	Adie Viljoen	7:30	8:15
Lab-clinical case reports: From diagnostics to diagnosis	Marina Minic-Novcic	8:15	9:00
	Coffee break	9:00	9:15
Session 7: Biomarkers in neurobehavioral and psychiatric disorders	Ole A. Andreassen	9:15	9:45
	Sergej Djuranovic	9:45	10:15
	Srdjan Djurovic	10:15	10:45
	Milena Petrovic	10:45	11:00
Plenary lecture	Maurizio Ferrari	11:00	11:45
Session 8: Precision medicine technologies and molecular diagnostics	Ron van Schaik	11:45	12:15
	Maurizio Ferrari	12:15	12:45
	Dobrin Svinarov	12:45	13:15
	Commercial workshop 4	13:15	13:45
	Lunch	13:45	15:00
	Poster presentations		
Session 9: Advancing healthcare for transgender patients	Marta Bizic	15:00	15:30
	Jeroen Vervalcke	15:30	16:00
	Dusica Markovic Zigic	16:00	16:30
	Commercial workshop 5	16:30	17:00
	Coffee break	17:00	17:15
Session 10: Dyslipidemia, diabetes, adiposity	Khosrow Adeli	17:15	17:45
	Adie Viljoen	17:45	18:15
	Marina Minic-Novcic	18:15	18:45
	Marina Jaksic	18:45	19:00
	Aleksandra Atanasova-Boshku	19:00	19:15
Saturday, 30.09.2023.			
Session 11: Masterclass from Balkan region I	Katerina Tosheska-Trajkovska	8:00	8:30
	Christos Tsatsanis	8:30	9:00
	Irini D. Leimoni	9:00	9:30
	Ledina Mino	9:30	9:45
Selected oral presentation from Balkan region	Vjeroslava Slavic	9:45	10:00
Selected oral presentation from Balkan region	Ozlem Unay Demirel	10:00	10:15
Plenary lecture	Julieta Hristova	10:15	11:00
	Coffee break	11:00	11:15
Session 12: Masterclass from Balkan region II	Radivoj Jadric	11:15	11:45
	Sorin Giju	11:45	12:15
	Aleksandra Klisic	12:15	12:45
	Kenan Preljevic	12:45	13:00
	Closing remarks	13:00	13:15

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OPENING LECTURE

Implementation of Sustainable Practices in Medical Laboratories. Switching Clinical Laboratories to Green Labs.

***Tomris Ozben*[1,2,3]**

[1]*European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), President*
[2]*EFLM Task Force-Green and Sustainable Laboratories, Chair*
[3]*International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), President-Elect*

Laboratory medicine should contribute to a sustainable healthcare system ensuring that resources are used efficiently from ecological, social, and economical perspectives, while providing high-quality services to patients and physicians. It will be a challenge for clinical laboratories to achieve sustainable operations. Clinical laboratories use more energy and water than offices and generate huge amounts of hazardous and non-hazardous wastes every year. Clinical laboratories can limit their environmental impact and provide sustainable laboratory services making reductions in four key areas—energy consumption, water consumption, waste production, and use of hazardous chemicals. Establishing sustainable development goals and applying multiple means for reductions in these key areas, clinical laboratories can reduce their environmental impact. By being mindful of the environmental impact of everyday actions in a lab, and by taking steps to minimize energy, water, and hazardous chemical use, as well as waste generation, a clinical lab can be transformed into a safe, sustainable space. Sustainability measures should be a key feature in the rapidly changing healthcare environment to reduce their negative impacts on the environment and economy. EFLM Task Force-Green and Sustainable Laboratories (TF-GSL) aims to implement sustainable practices in medical laboratories in the EFLM Member Societies leading the laboratory medicine community for the shift to carbon neutrality in line with the European Green Deal (EGD) Investment Plan, which is aimed at making Europe the world's first climate-neutral continent by decreasing their deleterious environmental impact and implementing efficient approaches to address the effects of climate change and pollution without compromising the quality of healthcare. In order to provide high-quality, effective, and safe healthcare services, sustainable healthcare systems need to overcome major economic and social challenges. Though there will be initial capital costs, there is a long-term cost-saving potential of ordering only necessary tests, adopting a green purchasing policy and a more efficient use of energy and other resources in healthcare systems. Despite this, there is a long way to go for environment-friendly hospitals, healthcare structures, and clinical laboratories to become the norm. Good collaboration among the healthcare systems and a common vision for future actions would help to achieve such goals.

PLENARY LECTURES

Thrombophilia Screening – Not So Straightforward

Gary W. Moore[1]

[1]*Addenbrooke's Hospital, Cambridge University Hospitals, NHS Trust, Cambridge, United Kingdom*

Even before any laboratory testing is done, the decision of whether or not to initiate thrombophilia screening is not straightforward as debate persists on which patients should receive the testing in terms of clinical benefit. Where testing does go ahead, it commonly comprises single phenotypic assays to detect each of antithrombin (AT), protein C (PC), and protein S (PS) deficiencies, and assessment for activated protein C resistance (APC-R) by a functional assay, and/or genetic analysis for FV Leiden (FVL).

Antigenic assays alone are insufficient to detect the deficient states as they will identify quantitative but not qualitative defects, so first line screening with functional assays is recommended as they will detect both. AT activity assays are based on inhibition of FIIa or FXa, and some antithrombin variants preferentially manifest in one assay type but not the other. PC activity assays employ either clotting or chromogenic end-points, the latter being considered more reliable due to reduced susceptibility to interfering factors. However, whilst the analytical principle of clotting-based assays permits expression of all functional anticoagulant-related activities of activated PC, chromogenic assays are limited to detection of defects in PC activation or it's active site. PS activity assays are notoriously variable and prone to interference. Consequently, most laboratories screen for the deficiency with an antigenic assay, with the inevitable loss of ability to detect functional deficiencies. A given assay repertoire will detect the majority of deficiencies, yet use of single assays for each parameter leaves patients needing a diagnosis that have rarer defects at the mercy of the particular assays in use in the laboratory to which their blood samples are sent. Interpretation of abnormal results must consider the possibility of acquired deficiencies.

A common misconception is to consider FVL and APC-R as interchangeable terms. Whilst FVL is responsible for >90% of hereditary APC-R, other clinically significant F5 variants conferring APC-R are known, and there are numerous causes of acquired APC-R. A variety of phenotypic APC-R assays are available, with varying sensitivities to different forms of acquired APC-R, but with the advantage of detecting hereditary APC-R irrespective of the causative F5 variant. Direct genetic analysis for FVL will detect most but not all hereditary APC-R, but not acquired APC-R.

Approximately 50% of thrombophilia screens will detect no abnormalities, partly because of inappropriate patient selection or insensitivity of the local repertoire to the defect that is present. Rare thrombophilias, such as dysfibrinogenemias, are probably under investigated, and other areas of haemostasis, such as fibrinolysis, currently lack sufficient evidence for inclusion in routine analysis but potentially contribute to the

thrombotic phenotype in certain individuals. Emerging thrombophilias, such as antithrombin resistance, may yet prove sufficiently 'common' to warrant routine testing.

Aging and Big Data

***Maurizio Ferrari*[1, 2]**

[1]*Synlab Italia*
[2] *Vita-Salute San Raffaele University, Milan, Italy*

The aging population is becoming predominant in many parts of the world, raising new challenges. Many industrialized countries are getting ready to deal with an impending 'silver tide', which could overload current healthcare systems and reduce overall economic productivity.

Aging is a multifactorial biological process of declining physiological functions increasing the susceptibility to aging-related chronic diseases, such as cancer, metabolic, cardiovascular, musculoskeletal, as well as neurodegenerative diseases. Hallmarks of aging include stem cell exhaustion, altered intercellular communication, senescence, genomic instability, and epigenetic deregulation.

The goals of aging biology research are broad and ambitious: to understand how a multitude of genes, pathways and mechanisms at multiple scales contribute to declines in function, health and lifespan in ways that can vary across populations, environments and species. Enormous progress has been made identifying individual genes, pathways, molecules and their connection in mechanisms that modulate aging.

Considering the biological complexity and heterogeneity of the aging process, it is now clear that full understanding of mechanisms underlying aging can only be achieved through the integration of different data types and sources, and with new computational methods capable to achieve such integration.

Big data" is the assessment of massive amounts of information from multiple electronic sources in unison, by sophisticated analytic tools to reveal otherwise unrecognized patterns. We also talk about "big data" in the context of analyzing large, complex data sets such as those derived from omics experiments.

Big data require collection and analysis of data at an unprecedented scale and represents a paradigm shift in health care, offering: the capacity to generate new knowledge more quickly than traditional scientific approaches; unbiased collection and analysis of data; and a holistic understanding of biology and pathophysiology.

Big data promises more personalized and precision medicine for patients with improved accuracy and earlier diagnosis, and therapy tailored to an individual's unique combination of genes, environmental risk, and precise disease phenotype.

Big Data are radically changing biomedical research. The unprecedented advances in automated collection of largescale molecular and clinical data pose major challenges to data analysis and interpretation, calling for the development of new computational

approaches. The creation of powerful systems for the effective use of biomedical Big Data in Personalized Medicine will require significant scientific and technical developments, including infrastructure, engineering, project and financial management

Mass Spectrometry-Based Omics

Julieta Hristova[1]

[1]*Medical University, Sofia, Bulgary*

Mass spectrometry (MS) is the most powerful research and analytical tool used in a variety of technological configurations for simultaneous detection and quantification of millions of atoms and biomolecules to ultra-trace levels at the highest resolution, thus granting a deep understanding of the biological system of focus. Unravelling the interactions of components even in a single living cell through the leading omics approaches – genomics, transcriptomics, proteomics, and metabolomics, provides valuable insights of their behavior in health and disease.

Genomics is the large-scale study of all of the DNA in a given system and matrix-assisted laser desorption/ionization (MALDI) MS is applicable for determining the nucleotide sequence of native molecules which is essential in studying their transformations. Given the fact RNA is more stable under MALDI-MS conditions, DNA are preliminary converted into RNA by in vitro transcription, allowing the technology to decode post-translational modifications that appear to be critical on the regulation of a variety of physiological processes and influencing the gene expression.

Proteomics is among the most intensively developing omics studying all the proteins in a biological system with their multiple post-translational modifications (glycosylation, phosphorylation, acetylation, methylation, etc.). MS has been the primary tool for peering into the depths of proteomic science. Currently, electrospray ionization (ESI) MS and MALDI MS in a variety of tandem and high-resolution modality configurations are most widespread in studying protein structure, function, and global dynamics. The role of these MS-based technologies in the field of interactomics – a branch of functional proteomics is uncontestable, allowing for high-accuracy determination of noncovalent protein interactions.

The metabolome is the last link in understanding the chain genome and refers to a variety of compound classes resulting from metabolic processes. The inhomogeneity of these components requires a complex approach for their investigation. Presently, MS-based metabolomics is the main fingerprinting tool exploring the changes of metabolite profiles in disease by target and off-target analysis of low-molecular-weight metabolites resulting from pathological processes, thus indicating presence or absence of a disease.

Mass spectrometry imaging (MSI) is a remarkably sophisticated molecular imaging technique providing a multi-omics approach for enhancing the current knowledge on the interaction mechanisms in health and disease. Initially developed for studying proteins and peptides, the current potentialities of MSI extend far beyond proteomics combining

biochemical and positional data with almost limitless application in omics profiling through imaging lipids, sugars, metabolites, oligonucleotides, and xenobiotics for a better overall individual patient care.

Keywords: mass spectrometry, genomics, transcriptomics, proteomics, metabolomics

SESSION 1: BONE AND MINERAL METABOLISM

Hyperparathyroidism - Diagnosis with Many Horizons

Emir Muzurovic[1,2]

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[2]*Faculty of Medicine, University of Montenegro, Podgorica, Montenegro.*

Hyperparathyroidism (HPT) is a condition in which one or more of parathyroid glands (PTGs) become overactive and release/secrete too much parathyroid hormone (PTH). There are three types of HPT: primary, secondary, and tertiary. Primary HPT (PHPT) is the third most common endocrine disorder after diabetes mellitus and thyroid disorders, and the diagnosis of PHPT is biochemical. The reported prevalence of PHPT has evolved over time, increasing as measurement of serum calcium (Ca) has become routine. From a clinical point of view, it is important to distinguish between a primary disorder (and tertiary) of the PTGs (PHPT or tertiary HPT [tHPT]), in which there is incompletely regulated, excessive secretion of PTH, and physiological situations in which the PTGs have responded to a stimulus that appropriately leads to increased PTH secretion (known as secondary HPT [sHPT]). More importantly, PHPT is now recognized to include three clinical phenotypes: overt target organ involvement (the symptomatic PHPT), mild asymptomatic PHPT, and high PTH levels with persistently normal albumin-corrected and ionized serum Ca levels (the normocalcemic PHPT), which makes the laboratory diagnosis and differential diagnosis of HPT very complex. The high frequency of HPT in the general population, very wide use of laboratory diagnostics in this field in recent years, and poor knowledge of the nature of HPT, leads to unnecessary laboratory diagnostics, which are usually clinically unnecessary and expensive. It is very important to clarify the causes and course of different forms of HPT, clinical presentation and possible complications, in order to carry out meaningful and justified laboratory diagnostics.

Updates in PTH

Etienne Cavalier[1]

[1]*University of Liege, Belgium*

Rosalyn Yallow, who was awarded the Nobel Prize in Medicine in 1977, was the first person to develop a competitive immunoassay for PTH determination. This initial assay utilized a single antibody and was later replaced by a sandwich immunoassay known as "intact PTH". While this assay was more specific, some resistance was observed in hemodialyzed (HD) patients. Clinicians were advised to maintain PTH concentrations between 1.5 and 2.5 times the upper limit of normal. It was later discovered that the

antibodies used in second-generation assays cross-reacted with a non-(1-84) PTH molecular form present in the blood of HD patients. Subsequently, a new generation of PTH assays was introduced, known as "bio-intact," "whole," or third-generation PTH assays, utilizing N-terminal antibodies targeting the first four amino acids of the peptide. These assays no longer cross-reacted with the 7-84 PTH fragment, which was commonly used as a surrogate for non-(1-84) PTH forms. Both generations of assays are still in use today, with third-generation PTH assays being better standardized to the WHO 95/646 International Standard compared to second-generation assays. However, the lack of standardization for second-generation assays has resulted in significant result disparities, leading to clinical issues in both CKD and non-CKD patients. Standardization process need higher order methods. The last two years, a high-resolution (HR) mass spectrometry and a triple quadripole LC-MS/MS were published, the latter being proposed as a candidate reference method. The HR-MS allowed the description of circulating fragments that accumulate in CKD patients. Yet, the only fragment presenting any cross-reactivity with the Roche cobas 2nd generation assay was the 7-84 one. But the authors showed that this fragment was not present in human blood, questioning the cross-reactivity of the fragments with 2nd generation assays. The LC-MS/MS method confirmed the absence of 7-84 PTH in blood and allowed a “in-silico” restandardization of 2nd and 3rd generation PTH assays, which resulted in an important reduction of the disparity of the results observed in patients. Finally, both methods failed to prove the presence of oxydized PTH in the blood of HD patients, challenging the findings of the authors proposing the “non-oxydized” PTH as the new target for immunoassays. Standardization of PTH assays is thus totally possible and will result in much better homogeneity of the results in CKD and non-CKD patients.

Sclerostin and Dickkopf-1 as a New Marker of Bone Disease

***Aylin Sepici Dincel*[1]**

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Bone metabolism is a dynamic process related to the alteration of specific circulating metabolites such as serum amino acids, lipids, and urine metabolites that can be associated with bone mineral density. Mainly the clinical uses of bone turnover markers are for risk assessment (bone loss, prediction of fracture and identification of secondary osteoporosis), selection of treatment and monitoring of response and offset of effect. Nowadays, the role of Wnt/beta-catenin pathway and its inhibitors in the osseointegration process is a challenging topic as a new approach that bases on our understanding of bone physiology. Extracellular Wnt antagonists (Sclerostin; Scl and dickkopf-1; DKK1) regulate bone formation by binding directly to Wnt ligands or by competing with Wnt ligands for binding to the co-receptors lipoprotein-related proteins 5 and 6 expressed on the surface of bone cells. In order to quantify the serum levels of Scl, there are ELISA, EIA and automated chemiluminescent Scl assay results can be observed from different studies. However, accurate measurement of Scl from serum and plasma sources remains a significant impediment and need more detailed structural definitions

for preanalytical phase. Besides, to determine the serum levels of DKK1 limited ELISA kits are available for scientific purposes. The talk will address the potential pathophysiological role of both Scl and DKK1 mainly in bone disease, preanalytical issues related to the measurements in serum/plasma together with their potential and future use as a biochemical marker of health and disease.

Bone Metabolism Markers in Physically Active Postmenopausal Women

Nela Raseta Simovic[1,2], Tatjana Bucma[2,1], Slobodan Simovic[3]

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[2]*Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotovic" Banja Luka, Bosnia and Herzegovina*
[3]*Faculty of Physical Education and Sport, University of Banja Luka, Bosnia and Herzegovina*

Introduction: Postmenopausal women are a risk group for the development of osteoporosis. Physical activity is important in bone remodeling. Bone markers indicate the state of bone metabolism. The aim of this research was to examine the influence of organized recreational exercise on bone metabolism in postmenopausal women.

Material and methods: The examined group consists of postmenopausal women (*n* = 51), average age 68, who were included in the recreational exercise program at the Faculty of Physical Education and Sports in Banja Luka for three months, one hour three times a week. The control group is postmenopausal women (*n* = 56), average age 63, who were not involved in organized physical activity. DXA and total serum concentration of 25(OH)D, Ca2+, Ca, N-MID Osteocalcin and β-CrossLaps were determined at the Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotovic" Banja Luka.

Results: According to the DXA findings, most of the subjects in the test group are in the zone of osteopenia (52.3%), and in the control group, 67.9% of the subjects have osteoporosis. The values of 25(OH)D total are significantly higher in the examined group (p = .003), while the values of CaS, osteocalcin and CrossLaps are lower compared to the control group. CaS is significantly lower in physically active subjects with osteoporosis than in the control group with osteoporosis (*p* = .005).

Conclusion: Regular and organized physical activity in postmenopausal women has a protective effect on bone metabolism, reduces the resorption and release of calcium from the bone. Vitamin D status is better in physically active postmenopausal women.

Key words: bone markers, physical activity, postmenopausal women.

SESSION 2: TOTAL TESTING PROCESS IN CLINICAL LABORATORY

Standardization and Harmonization in Laboratory Medicine: Data Comparability Is Needed

***Mario Plebani*[1]**

[1]*Honorary Professor of Clinical Biochemistry and Clinical Molecular Biology, University of Padova, Italy and Adjunct Professor, Department of Pathology, University of Texas, Medical Branch, Galveston, USA*

In the last decades increasing efforts have been made to promote standardization and harmonization in laboratory medicine, but during the COVID-19 pandemic the issue of comparability of laboratory information received an even increased concern as this may avoid confusion and wrong interpretation of laboratory results. In addition, standardization and harmonization are fundamental issues for improving patient safety and for the accreditation of medical laboratories according to ISO 19189. The terms standardization and harmonization are often used interchangeably, probably because the aim is the same: to provide the clinicians and patients with laboratory results that are comparable and equivalent among different laboratories and over the time. However, it should be taken into account that the two terms refer to different concepts. Standardization should be used when the results are uniform among routine measurement procedures and traceable to a recognized standard reference material defined by the International System of Units (SI) through a high-order primary reference material and/or a reference measurement procedure. Harmonization is aimed to make the results comparable irrespective of the measurement procedure, mainly because neither a reference measurement procedure nor a primary reference material is available. Notably, this is the case of the vast majority of the measurands determined every day in clinical laboratories. The other important difference between the two terms is that the harmonization process includes all the aspects of the total testing process (TTP) besides the analytical quality, taking into consideration pre-analytical issues such as harmonization of patient preparation, sample collection and handling, and post-analytical aspects such as harmonization of measurement units, reference intervals and decision limits. In the last few years, many interesting contributions have been published to improve the knowledge on the harmonization in laboratory medicine but further efforts are needed.

Closing the Gaps in Adults and Pediatric Reference Intervals: Global Initiatives

Khosrow Adeli[1, 2]

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[2]*International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)*

Clinical laboratory reference ranges serve as health-associated benchmarks that enable clinicians to interpret laboratory test results and facilitate clinical decision-making. Unfortunately, critical gaps currently exist in accurate and up-to-date pediatric reference ranges for accurate interpretation of laboratory tests performed in children and adolescents, which may contribute to erroneous diagnosis or misdiagnosis of many diseases. Several initiatives have been established internationally to address these gaps, including the KiGGS initiative in Germany, the Aussie Normals in Australia, the AACC-National Children Study in the USA, the NORICHILD Initiative in Scandinavia, and the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) program in Canada (www.caliperproject.ca). Since 2009, CALIPER (www.caliperproject.ca) has recruited more than 12,500 healthy children and adolescents, establishing a comprehensive database of pediatric reference ranges for over 200 biomarkers of health and disease (www.caliperdatabase.org); currently used by thousands healthcare institutions in North America and around the world to improve clinical decision making in children and adolescents with medical concerns. In this presentation, I will review the evidence supporting the critical role of laboratory medicine in public health and patient care and describe the major progress made in closing the evidence gap in pediatric reference intervals through the CALIPER program.

In adults, several national surveys have reported wide variation in reference intervals across healthcare centres in certain regions, even those using the same analytical platform and reagents for the same assay. There is a high risk of inappropriate test result interpretation when reference intervals are not appropriately harmonized. The Canadian Society for Clinical Chemistry (CSCC) Working Group on Reference Interval Harmonization was established in 2015 to develop evidence-based harmonized/common reference intervals (hRIs) and support their implementation in laboratories across Canada. Harnessing the power of big data, laboratory results were collected across populations and testing platforms to derive common adult RIs for 16 biochemical markers. A novel comprehensive approach was established, including: (1) analysis of big data from community laboratories across Canada; (2) statistical evaluation of age, sex, and analytical differences; (3) derivation of hRIs using the refineR method; and (4) verification of proposed hRIs across nine laboratories with different instrumentation using serum and plasma samples collected from healthy Canadian adults. Harmonized RIs were calculated for all assays using the refineR method, except free thyroxine. Derived hRIs met proposed verification criterion across nine laboratories and five manufacturers for alkaline phosphatase, albumin (BCG), chloride, LDH, magnesium, phosphate, potassium (serum), total protein (serum). Further investigation is needed for select analytes due to lower verification in one or more laboratory (albumin

(BCP), calcium, total CO2, total bilirubin, sodium) or concern regarding too wide hRIs (alanine aminotransferase, creatinine, TSH). In this presentation, we will discuss the work completed by the Working Group on Reference Interval Harmonization in Canada, challenges encountered, and future plans to support implementation.

In this presentation, I will review the current evidence for the central role of the intestine and the gut-brain axis in mediating nutrient sensing to control energy balance and body weight. Molecular and physiological mechanisms will be discussed focusing on the key role of gut peptides, particularly GLP-1 and GLP-2.

How to Use Biological Variation Data to 1) Set Performance Specifications 2) Monitor Patients 3) Generate Personal Reference Intervals.

Sverre Sandberg[1]

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Biological variation (BV) data have many applications in laboratory medicine. However, these depend on the availability of relevant and robust BV data fit for purpose. BV data can be obtained through different study designs, both by experimental studies and studies utilizing previously analysed routine results derived from laboratory databases. The different BV applications include using BV data for setting analytical performance specifications, to calculate reference change values, to define the index of individuality and to establish personalized reference intervals.

A WG and TG in EFLM have developed a critical appraisal check list to evaluate literature on biological variation and extract essential information from the papers as well as to summarise the information. The groups have categorised papers as A, B, C and D depending on their methodological quality, with category A papers indicating high-quality and D poor quality using a checklist that contains 14 items. From each paper 22 items are extracted and presented in the database. In addition, the WG on biological variation has collected data from about 100 healthy persons in 6 different European countries and is now generating new data for a lot of measurands. The results from this study are compared with data after the literature search. One of the end-results from these initiatives has been to deliver a database on biological variation on the EFLM website. The biological variation database on the EFLM website (https://biologicalvariation.eu) gives essential information about biological variation and derived analytical performance specifications and reference change values for different measurands as well as the evidence behind.

In the lecture, the use of biological variation data to set analytical performance specifications, to monitor patients and to develop personalized reference intervals will be discussed as well as how these data can be used and implemented in practical laboratory life.

SESSION 3: LABORATORY HEMATOLOGY

An Overview on How to Diagnose and Monitor the Different Porphyrias

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The porphyrias are a group of rare inherited disorders caused by partial deficiencies of certain enzymes in the haem biosynthetic pathway. Among clinicians they are often referred to as "Obscure diseases with confusing names considered only when the need for a diagnosis is desperate". Each enzyme deficiency is associated with a specific pattern of accumulation, and excretion of pathway intermediates, which can be detected in erythrocytes, plasma, urine, or faeces. The clinical consequences depend on the nature of the metabolites that accumulates. When porphyrin "ring" structures accumulates, photosensitization of sun exposed areas of the body can occur. This is the case in the so called "cutaneous porphyrias". In the acute porphyrias, excess delta aminolevulinic acid, ALA, and porphobilinogen, PBG, accumulate. Acute porphyrias is associated with acute neurovisceral attacks e.g., with abdominal pain, paresis or psychoses. Diagnosis depends on laboratory investigations to demonstrate the pattern of heme precursor accumulation and excretion specific for each type of porphyria, and it requires examination of appropriate specimens for the key metabolites using adequately sensitive and specific methods. The present lecture will present the recommendations from the International Porphyria Network (Ipnet) on how to diagnose and monitor porphyrias both in a general hospital and in an expert porphyria centre.

What Makes You Stronger Can Kill You: Use of Poly-Basic Peptides as New Drug Candidates Against *Plasmodium Falciparum*

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Plasmodium falciparum, the causative parasite of malaria, is the primary source of infection-related deaths globally. The current treatment of choice is artemisinin-combination therapy. Still, due to the development of malaria parasite's resistance to

existing drugs, there is an increased need for new drugs to be introduced. Here we show that a common feature of *P. falciparum* proteome, runs of poly-lysine residues found in adhesion and pathogenicity-related proteins, can be used as a successful peptide treatment of erythrocyte-infected parasites. Our data indicate that a single dose of poly-basic peptides can successfully reduce parasitemia in human erythrocytes *in vitro* with a favorable low or no toxicity. The treatment efficiency is correlated with the length of poly-lysine peptide, with 30 lysine peptides being sufficient for complete clearance of the parasites in erythrocytes at nanomolar concentrations. The treatment with poly-basic peptide shows equal results in artemisinin-resistant and non-resistant strains. Additional modifications of the poly-lysine peptides through PEG-ylation or the use of poly-lysine dendrimers increase the efficacy of parasite clearance and provide further stability to a new potential drug. Finally, our affinity pull-downs followed by mass-spectrometry and use of biotinylated poly-lysine peptides indicate *P. falciparum*'s membrane proteins as targets of polybasic peptide drugs. We hypothesize that polybasic peptides prevent infection by blocking merozoites interaction and invasion of human erythrocytes, and given the current use of poly-lysine dendrimers as an FDA approved drug-delivery agents, their repurposing as antimalarial drugs would be a useful therapeutic option.

Iron Deficiency in Nonanaemic COPD Patients – Could Low Haemoglobin Density and Microcytic Anaemia Factor Be Useful?

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Introduction: Erythrocyte indices LHD and Maf are complementary parameters to complete blood count and have been shown as reliable iron deficiency markers in different clinical settings. The aim of the study was to assess diagnostic performances of LHD and Maf in detecting iron deficiency in nonanaemic stable COPD patients.

Methods: A total of 93 nonanaemic stable COPD patients were classified as either iron deficient (ID, N = 15) or non-iron deficient (non-ID, N = 78). Iron deficiency was defined as a ferritin level < 100 μg/L with a transferrin saturation (TSAT) <20%. A complete blood count, including LHD and Maf as well as other relevant inflammation and iron status parameters were obtained for all participants.

Results: Both LHD and Maf have shown significant differences between the ID and non-ID group with p = .003 and p = .007 respectively. The AUC for LHD was .744 (95% CI: .626–.863, p = .003) with the best cut-off of 5.85 and sensitivity of 80% (95% CI: 76.0–84.0) and specificity of 61.5% (95% CI: 58.4–64.6). The AUC for Maf was .707 with optimal cut-off value 12.65 and sensitivity of 83.3% (95% CI: 79.1–87.5) and specificity of 60.0% (95% CI: 57.0–63.0). Furthermore, LHD performance was not affected by vitamin B_{12} status.

Conclusion: LHD and Maf are useful for iron deficiency diagnosis in stable COPD patients. LHD was shown to be resistant to vitamin B_{12} deficiency, which is of substantial importance in specific patient subpopulations. Both parameters are not technology-dependent and do not require additional sample and/or reagent volume, which makes them cost-effective and convenient for everyday use.

The Importance of Determining Hepcidin-25 for Treating Anemia in Patients with End-Stage Renal Disease

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Background: This study aimed to investigate the correlation of hepcidin-25 with hematological parameters, markers of iron status and inflammation, to determine its relationship with the treatment being applied to manage anemia in End–Stage Renal Disease (ESRD) patients, and to explore its diagnostic properties for discriminating anemia of chronic disease from sideropenic anemia (IDA). Also, our goal was to establish the influence of polymorphisms C282Y and H63D in the *HFE* gene and A736V in the *TMPRSS6* gene on the hepcidin-25 level and iron status parameters and to evaluate the diagnostic properties of hepcidin-25 in the assessment of positive iron balance in ESRD patients.

Methods: The study included three groups of participants: 126 patients with ESRD, 31 patients with IDA and a control group (CG) comprised of 30 subjects Hepcidin-25 was determined by a Hepcidin-25 Chemiluminescent Direct ELISA assay.

Results: Hepcidin-25 was significantly higher in ESRD patients compared to IDA (53.98 μg/L vs 3.00 μg/L) and CG (8.69 μg/L), and significantly higher in CG compared to the IDA group ($P < 0.001$, for all). ESRD patients on maintenance hemodialysis (ESRD-D) receiving erythropoiesis-stimulating agents therapy (ESAs-th) had significantly higher hepcidin-25 (59.34 μg/L vs 16.36 μg/L, $P < 0.001$) and ferritin levels (421.4 μg/L vs 90.0 μg/L, $P < 0.001$) in comparison to patients without ESAs therapy (ESAs-n). After correction for ferritin levels, hepcidin-25 levels were not significantly different between ESAs-n and ESAs-th groups ($P = 0.250$). The results of our multivariable model in ESRD-D group indicate that hepcidin-25 levels are independently and positively associated with iron stores and inflammation, and inversely with active erythropoiesis, regardless of ESAs administration. Maintenance ESAs and *i.v.* iron dose, as well the ESAs resistance index (ERI) were not related to hepcidin-25 levels. The cut-off values with the best diagnostic properties in distinguishing ACD from IDA was found for hepcidin-25 ≥ 9.32 μg/L. Interaction between gender and *HFE* haplotypes (H63D/C282Y) on hepcidin-25 ($P = 0.005$) and ferritin ($P = 0.027$) in ESRD patients was found. Transferrin concentration was influenced by the joint effect of gender and the *TMPRSS6* A736V polymorphism in

the ESRD group (P = 0.002). In multivariable models, hepcidin-25 was independently negatively associated with the most recent iron availability for hemoglobin synthesis reflected by CHr (β = - 0.493, P = 0.004) and RSf (β = - 0.334, P = 0.036) levels, only in the group of anemic ESRD patients with positive iron balance (PB). The best hepcidin-25 value to exclude PB was 66.13 μg/L.

Conclusions: Our results suggest that hepcidin-25 may prove to be a valuable additional tool in the evaluation of iron status and highlight the important gender-related involvement of the *TMPRSS6* and *HFE* polymorphism on anemia in ESRD patients.

Keywords: Hepcidin-25, *HFE* and *TMPRSS6* gene polymorphism, anemia, inflammation, end-stage renal disease, hemodialysis, percentage of hypochromic erythrocytes, reticulocyte hemoglobin content, erythropoiesis-stimulating agents, intravenous iron therapy

SESSION 4: THROMBOSIS AND HEMOSTASIS

Laboratory Diagnosis of Thrombotic Microangiopathies: Measurement of ADAMTS-13

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Thrombotic microangiopathies (TMA) are pathological conditions characterized by microangiopathic (non-hemolytic) anemia, thrombocytopenia and microvascular thrombosis with ischemic injury. TMA is divided into the primary and secondary. Secondary TMA is associated with many syndromes, conditions and diseases (e.g., preeclampsia, HELLP syndrome, malignancy, infections, autoimmune diseases (SLE), DIC). Primary TMA, which occurs spontaneously without underlying disorders, are thrombotic thrombocytopenic purpura (known also as Moschcowitz syndrome) (TTP) and hemolytic uremic syndrome (HUS). The later one may be a consequence of Shiga toxin–producing E. coli (typical HUS, primarily occurring in children) or complement abnormalities (atypical HUS).

Metalloproteinase ADAMTS-13 (a disintegrin with a thrombospondin type 1 motif, member 13) which cleaves large multimers of von Willebrand factor (VWF) is normally decreased in both HUS and TTP. Differential diagnosis is based on presence/absence of renal impairment (typical for HUS) but the most important is the level of ADAMTS-13 (moderately decreased in HUS and severely decreased in TTP). Although additional laboratory findings (LDH increase and schistocytes) are almost always present in TTP, definitive diagnosis is usually based on severely decreased ADAMTS-13 (level < 10% is diagnostic criteria for TTP), finding highly sensitive and specific for the diagnosis. TTP, in rare cases, is congenital due to the ADAMTS-13 gene mutations but more common it is a consequence of development of antibodies to ADAMTS-13. Irrespectively of the mechanism TTP is a life-threatening condition and proper and rapid diagnosis is inevitable for prompt and adequate treatment (usually plasma exchange and immunosuppressive treatment, while recombinant ADAMTS-13 has recently been approved for congenital deficiency).

As mentioned, diagnosis and treatment follow-up are based on laboratory determination of ADAMTS-13 level. It became available after findings which enabled development of monoclonal antibody to VWF 73 peptide. The first available assay (still considered as a "gold standard"), FRET (fluorescence resonance energy) requires specific instrumentation (fluorometric scan). Introduction of ELISA ADAMTS-13 assay about 10 years ago made testing easier for the coagulation laboratories. However, both methods are time consuming (more than half-of working day of a biomedical scientist) and are performed only during working hours. A typical Friday afternoon referral for ADAMTS-13 therefore uses to wait until Monday before ADAMTS-13 is analyzed. As a consequence,

treatment which is not possible to be delayed is just empirical and post-festum analysis is just confirmational or even worse indicates that expensive (and more important not always complications-free) plasma-exchange was unnecessary performed.

A semiquantitative rapid ADAMTS-13 Screen assay has been commercially available several years ago. Sensitivity was perfect and we considered low specificity still satisfactory for a rapid test with less than 30 minutes turnround time. Too many samples in "grey" zone which had to be retested with ELISA, in our hands, made this assay inappropriate and after certain-time period we replaced it with fully automatized ADAMTS-13 chemiluminescence assay. For almost 3 years, in our hands, sensitivity/specificity profile of this assay has been fully comparable with ELISA. We performed assay in our routine coagulation laboratory all weekdays (08-20 h) with a turnround time of about 30 minutes, helping our clinicians to establish or dismissed TTP diagnosis. However, in our laboratory evaluation of ADAMTS-13 antibody titer is still based on ELISA-Bethesda testing during office-hours only.

In conclusion, TTP is rare but potentially life-threatening TMA and rapid ADAMTS-13 assay saves time (and money?) necessary for diagnosis and treatment decision.

One Disease, Numerous Challenges: How to Successfully Diagnose von Willebrand Disease in the Laboratory?

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Von Willebrand disease (VWD) is an inherited bleeding disorder caused by quantitative deficiency and/or qualitative defects of von Willebrand factor (VWF), a large multimeric glycoprotein that has two crucial roles in haemostasis. In primary haemostasis VWF mediates platelet adhesion and aggregation at the site of vascular injury by binding collagen within the endothelium of an injured blood vessel and glycoprotein Ib (GPIb) on platelet surface, while in secondary haemostasis it serves as a carrier and stabilizer of coagulation factor VIII (FVIII) in circulation, thus extending its half-life and allowing adequate function. Multiple VWF functions in haemostasis are regulated by different regions within the encoding gene. To date, more than 700 unique mutations have been discovered throughout the whole VWF gene, affecting either VWF assembly, secretion, proteolysis, clearance, and/or binding affinity to GPIb, collagen, and FVIII. Any impairment of VWF function results in bleeding phenotypes whose severity depends on the causative pathophysiological mechanism and the remaining levels of functional VWF.

VWD is classified into three main categories: type 1 characterized by partial quantitative deficiency, type 2 that encompasses qualitative defects and is subdivided into four subgroups (2A, 2B, 2M, 2N) relative to the underlying structural disorder and

consequent impaired function of VWF, and the most severe type 3 where virtually complete absence of VWF is observed.

Although VWD is considered to be the most common bleeding disorder, due to the heterogeneity of underlying genetic variants that result in variable, usually mild bleeding tendency, it remains largely unrecognized and is one of the most underdiagnosed haematological disorders indeed.

Diagnosis of VWD relies on clinical features that include personal and family bleeding history, and is confirmed by laboratory testing. Initial laboratory evaluation includes determination of both VWF antigen (VWF:Ag) and activity, as well as FVIII activity. However, due to the multifunctional nature and structural complexity of VWF, accurate differential diagnosis of VWD is often challenging and the exact nature of causative VWF defect might remain incompletely revealed when commonly available laboratory assays are used. Therefore, for differential diagnosis of VWD subtypes or clarification of ambiguous cases, a selection of specialized coagulation assays that assess functional and structural characteristics of VWF are used. These include VWF collagen-binding assay, multimeric analysis, FVIII-binding assay and ristocetin induced platelet aggregation. Genetic analysis provides identification of the underlying genetic cause of the bleeding phenotype, however, due to the size of the VWF gene and the occurrence of mutations throughout the whole gene, it is still not part of routine diagnostic algorithms for VWD. With the introduction of next-generation sequencing (NGS) that enables the investigation of the entire VWF gene coding region, as well as analysis of multiple genes simultanoeusly, molecular diagnosis of VWD could become more accessible in the near future.

In this lecture, an overview of the challenges in the diagnostic pathway of VWD will be presented with focus on real-life clinical cases from the first study performed among Croatian patients with VWD in whom a comprehensive diagnostic approach comprising coagulation testing and genetic analysis by means of NGS was applied. This study revealed that due to large heterogeneity of the underlying defects of VWF but also effects of other physiological characteristics (age, AB0 blood group) on VWF activity, patients' clinical presentation and laboratory testing results can largely differ not only among patients with the same VWD subtype, but also among those carrying the same mutation or even patients who are blood-related. In addition, in the differential diagnosis of VWD, patients presenting with ambiguous mild bleeding phenotypes coupled with borderline levels of VWF (30–50 %) are especially hard to be properly characterized. Difficulties may also arise due to similarities in clinical presentation and laboratory results with patients suffering from the mild form of haemophilia A or women that are carriers of haemophilia A.

In the diagnostic management of patients with VWD it is of upmost importance not only to take into consideration anamnestic, clinical and laboratory data, but also to understand the characteristics of the used coagulation assays in order to properly interpret the obtained results. Identification of causative mutations, together with detailed analysis of structural and functional characteristics of VWF using specific coagulation assays, enhances the understanding of the pathophysiological mechanism

underlying the patient's bleeding disorder and can improve the differential diagnosis of VWD.

Mainstream in Hemostasis in Montenegro, Can We Keep Up?

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To who it may concern, about coagulopathies in Montenegro. The population is about 650.000, but with wast medical issues, due to low medical education, rural regions. This is going to change and has been changed through media, excellent collaboration between colleagues, education and off course our laboratory that we are proud of. Our goal is to expend our field of laboratory and clinical practice, to achieve the perfect relationship with our patients thus treating them. Hemophilia association is present in our country where we are freely giving advisees to our patients, making the plans for future life, their children and offering them comfort and communication with other colleges for other treatments. Numbers are small but obvious, hemophilia A 20 patients, hemophilia B 2 patients, vWD 2 patients. We are small but we are strong, 5 of are patients are on treatment with Emicisumab and we are continuing to encourage others to start with this treatment. We offer them non-stop ambulance treatment, regular check-ups. Through our and their education they are aware of the risk for trauma and of course future interventions (dental/orthopedic/injuries). Because of this small population we are in relationships with other renowned centers for genetic evaluation for us to gain the main knowledge in diagnosis of other hematological diseases (leukemias). We are dealing with acquired coagulopathies daily and we are in reach of results within 1-2 hours, treatments are available. So, to who it may concern, I think that with our resources we can keep up with you Europe, through education, collaboration, technical improvements. The mainstream in hemostasis in Montenegro is finding new friends, making new contacts and expanding our future.

Optimal Utilization of Thrombophilia Testing in Asymptomatic Individuals

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Thrombophilia is a complex, multi-factorial disease involving both genetic and circumstantial risk factors that affect a delicate balance between procoagulant and anticoagulant forces and may result in VTE. The increased risk of thrombosis is lifelong, and thrombotic events tend to occur when there are one or more of environmental risk

factors. The incidence of thrombosis in individuals having genetic defects is highly variable and some individuals never develop thrombosis, whereas others develop recurrent severe thrombotic events at an early age. This depends on the particular genotype, the coexistence of other genetic defects, and the influence of environmental triggers such as oral contraceptives, trauma, surgery, and pregnancy. Currently, routine testing of coagulation and genetic factors in asymptomatic individuals to assess the risk of thrombosis is not recommended. Screening asymptomatic individuals of any age may result in overtreatment and risk for bleeding rather than clot prevention. If they are asymptomatic family members of patients with VTE with Factor V Leiden or prothrombin 20210G>A mutation, especially a first-degree relative with VTE at an early age, they are proposed to be evaluated in consideration of oral contraceptives/estrogen therapy, big surgery and pregnancy. Additional data about the costs and potential benefits of screening asymptomatic relatives for thrombophilia are needed to optimize our personalized approach to these patients.

SESSION 5: CARDIOVASCULAR DISEASES AND CARDIOVASCULAR DISEASE RISK PREDICTION

Clinical Implications of High Sensitivity Cardiac Troponin Assays in Acute Coronary Syndrome

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Acute Coronary Syndrome (ACS) is a term used to describe a range of cardiovascular conditions that result from a sudden reduction or blockage of blood flow to the heart muscle. It typically includes unstable angina and myocardial infarction (NSTEMI and STEMI). ACS is an emergency condition that requires immediate attention and intervention. High sensitivity cardiac troponin assays have significantly revolutionized the diagnosis and management of acute coronary syndrome (ACS). These assays detect cardiac troponin, a protein released into the bloodstream following damage of the heart muscle, with greater sensitivity and precision than previous generations of assays. This increased sensitivity has important clinical implications in the context of ACS.

High sensitivity cardiac troponin assays allow earlier and more accurate diagnosis of ACS. These assays can detect even minor elevations in troponin levels, enabling clinicians to identify myocardial injury at an earlier stage. This early detection facilitates prompt initiation of appropriate treatment strategies.

These assays have improved risk stratification and prognostication in ACS. The precise measurement of troponin levels allows for a more accurate assessment of the extent of myocardial damage and subsequent risk of adverse cardiac events. Higher troponin levels correlate with increased mortality and the risk of future cardiovascular events.

Hs-cTn assays play a crucial role in the rule-out strategy for ACS. With their enhanced sensitivity, these assays can accurately identify patients at very low risk of adverse cardiac events, enabling safe discharge from the emergency department. Incorporating hs-cTn assays into algorithms, such as the high-sensitivity troponin rule-out protocol, allows for efficient and resource-effective patient management.

High-sensitivity cardiac troponin assays facilitate individualized treatment strategies in ACS.

Serial measurements of hs-cTn levels provide insight into the effectiveness of therapeutic interventions in ACS. A declining trend in troponin levels indicates a favorable response to treatment, while persistently elevated or rising troponin levels may suggest ongoing myocardial injury.

In conclusion, the introduction of high sensitivity cardiac troponin assays has had significant clinical implications in the management of ACS. The use of high sensitivity troponin assays has undoubtedly contributed to better patient outcomes by enabling more timely and targeted interventions in acute coronary syndrome.

Clinical and Laboratory Applications of Kynurenine and Its Pathway Products

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The kynurenine pathway, a key metabolic route responsible for tryptophan degradation, has garnered significant attention due to its implications in various physiological and pathological processes. This abstract aims to provide insights into the clinical and laboratory applications of kynurenine and its pathway products, shedding light on their significance in medical research.

Neurological disorders, including Alzheimer's disease, Parkinson's disease, and Huntington's disease, have been associated with dysregulation in the kynurenine pathway. The altered levels of kynurenine metabolites have been linked to disease progression, suggesting their potential utility as diagnostic markers and therapeutic targets.

Psychiatric disorders, such as major depressive disorder, schizophrenia, and bipolar disorder, have also exhibited perturbations in the kynurenine pathway. The dysregulation of kynurenine metabolites in these conditions implies their involvement in the underlying pathophysiology, thereby providing opportunities for targeted interventions.

In the realm of immune system regulation, the kynurenine pathway has emerged as a crucial modulator of immune responses. Kynurenine metabolites have been implicated in autoimmune diseases and chronic inflammation, underscoring their potential as targets for immunomodulatory therapies.

Laboratory applications of the kynurenine pathway have contributed significantly to medical research. The identification and quantification of kynurenine metabolites serve as valuable biomarkers for various diseases, aiding in early detection and monitoring of disease progression. Moreover, efforts to develop drugs targeting specific enzymes within the pathway offer new avenues for therapeutic interventions.

Experimental models, including animal and cell models, have played a pivotal role in elucidating the mechanistic aspects of the kynurenine pathway and its potential therapeutic implications. These models have yielded crucial insights into disease mechanisms and have facilitated the development of novel treatment approaches.

This abstract underscore the importance of exploring the clinical and laboratory applications of the kynurenine pathway and its metabolites. By unraveling the intricate

connections between kynurenine and disease pathogenesis, researchers can contribute to the advancement of personalized diagnostics, targeted therapeutics, and improved patient care.

Keywords: kynurenine pathway, tryptophan degradation, neurological disorders, psychiatric disorders, immune system regulation, biomarkers, drug development, experimental models.

Early Vascular Ageing, Arterial Stiffness and Target Organ Damages in Treated Hypertensive Patients

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Age related functional and structural alteration in hypertensive patients are accelerated thought life and early vascular aging (EVA syndrome) is the new condition described, with target organ damages (hearth, brain and kidney). This leads to increased CV morbidity and mortality. EVA syndrome refers that the vascular alterations occurring in apparently healthy old people may be present in young hypertensive patients. In last two decades scientific interests put lights on molecular pathways involved in EVA and reliable techniques how to access vascular ageing in order to get more reliable estimation of global CV risk.

In the last few years, a special attention has been given to a new method of measuring PVW, i.e., dynamic, 24-hour measurement of the pulse wave velocity, which provides data on circadian rhythm, night values, and variability.

In this study we included 160 patients with essential arterial hypertension, older than 18 years with signed informed consent.

The main goal of this study was to determine the association of 24h PVW measurements with target organ damages. Additional goals include analysis of 24h PVW characteristics in our group of subjects: variability, circadian pattern, night stiffness, increased morning stiffness, masked stiffness, and "white coat stiffness", and comparison of PVW values measured during 24 hours with values measured in the office.

The obtained results confirmed the clinical value of 24-hour pulse wave velocity (PVW) measurements in treated hypertensives and confirmed the correlation with target organ damages (kidney, hearth and brain)

Keywords: 24h pulse wave velocity, arterial stiffness, cardiovascular risk, target organ damage, arterial hypertension

Protein Bound Uremic Toxins (PBUTs); Potential Impact of Implementing Test Panels for PBUTs on Patient Outcomes

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Protein-bound uremic toxins (PBUTs) play a significant role in kidney disease, inflammation, and the complex interplay with microbiota, in patients undergoing hemodialysis. These toxins accumulate in the bloodstream due to reduced renal clearance provoking inflammation and oxidative stress, contributing to the progression of kidney disease. PBUTs encompass a diverse range of compounds, including indoxyl sulfate, p-cresyl sulfate, phenylacetic acid, phenyl sulfate, and hippuric acid, among others. These toxins are typically derived from the metabolism of dietary proteins by gut bacteria in the colon. Once absorbed into the bloodstream, they bind to proteins, predominantly albumin, forming complexes that are resistant to filtration by the kidneys.

The dysbiosis observed in patients with kidney disease alters the gut microbial composition and their metabolic activities, leading to the generation of uremic toxin and consequently, establishing a detrimental feedback loop.

Hemodialysis, the primary treatment modality for end-stage kidney disease, aims to remove toxins from the bloodstream. However, traditional hemodialysis techniques are limited in their ability to efficiently eliminate these toxins, as they are bound to large plasma proteins. Innovative strategies, such as high-flux membranes and adsorbent technologies, are being explored to enhance toxin removal during hemodialysis. Developing effective interventions to reduce their burden represents a recently active area of research. Furthermore, approaches targeting the gut microbiota such as probiotics – prebiotics and special diets are also being investigated as a potential approach to mitigate their accumulation and associated complications.

Taking all these into consideration measuring the concentrations of primary uremic protein bound toxins like p-cresyl sulfate (pCS), indoxyl sulfate (IS), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), and indole 3-acetic acid (IAA) metabolites in the serum of patients with chronic kidney damage would help understanding the role of PBUTs in kidney disease. Also monitoring these analytes' free forms in the serum is crucial for assessing the effectiveness of the treatments used and determining the prognosis of the patients.

Developing reliable liquid chromatography-tandem mass spectrometry (LC-MS MS) based methods for these toxins to monitor the challenges and solutions in managing PBUTs in chronic kidney patients, including inflammation, hemodialysis, and microbiota-targeted interventions, would be beneficial.

Implementing these test panels will have a positive impact on patient care, serving as a tool for future research and therapeutic advancements, aiming to improve patient outcomes and reduce the burden of kidney disease and associated complications, shaping personalized treatment plans for patients with chronic kidney disease.

Key words: Protein bound uremic toxins, chronic kidney disease, hemodialysis, microbiota, LC-MSMS

Cathepsin D in Cardiovascular Diseases and Its Relationship with Cardiovascular Mortality

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Cathepsin D, an enzyme involved in the breakdown of proteins within cells, plays a significant role in cardiovascular diseases and is associated with cardiovascular mortality. When Cathepsin D activity is disrupted, it contributes to the development and progression of various cardiovascular conditions, including atherosclerosis, myocardial infarction, and heart failure.

In atherosclerosis, Cathepsin D is involved in the formation and progression of plaques in the arteries. It breaks down proteins in the plaque's protective cap, making it more vulnerable to rupture and potentially leading to a heart attack. Cathepsin D also promotes the accumulation of fatty macrophages, contributing to inflammation and the development of atherosclerotic lesions.

Cathepsin D is also implicated in the progression of myocardial infarction. After a heart attack, Cathepsin D levels increase in the damaged heart tissue, leading to the breakdown of proteins and tissue remodeling. This remodeling affects heart function and can have negative outcomes.

In heart failure, Cathepsin D contributes to cardiac remodeling. It breaks down contractile proteins and components of the extracellular matrix, causing the heart to enlarge and impairing its ability to pump effectively. Elevated levels of Cathepsin D are found in failing hearts, and its activity correlates with the severity of heart failure, suggesting a role in disease progression and mortality.

Cathepsin D is involved in the development and progression of cardiovascular diseases, such as atherosclerosis, myocardial infarction, and heart failure. Disrupted Cathepsin D activity contributes to plaque instability, tissue remodeling, and impaired heart function. Additionally, elevated levels or activity of Cathepsin D are associated with a higher risk of cardiovascular mortality. In this presentation, Cathepsin D and its relationship between cardiovascular diseases and its mortality are being presented.

SESSION 6: IMMUNOLOGY / ALLERGOLOGY

Exploring the Latest Innovations in Laboratory Diagnostics for Drug-Induced Anaphylaxis: Towards Personalized Approaches and Biomarker Development

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Anaphylaxis is a systemic allergic reaction that, in the case of respiratory or cardiovascular involvement, can result in death. Symptoms appear within minutes of exposure to an allergen (most commonly insect stings, food, or medications).

Measurement of **acute serum tryptase,** released by mast cells during activation/anaphylactic reaction, is currently one of the main diagnostic tests to confirm an anaphylactic reaction. However, in up to 30% of cases, tryptase levels may not increase during the reaction, especially for food-related systemic reactions and certain medications. Therefore, extensive studies are being conducted to identify a reliable biomarker associated with basophils and mast cells for predicting anaphylactic reactions. The reference value for measuring acute serum tryptase is 11.4 µg/L. The measured value of acute serum tryptase may be slightly lower or slightly elevated compared to the reference range, known as the gray zone of measured values. In such cases, it is recommended to collect a paired sample after at least 24 hours, which provides both the acute and **basal values of tryptase**. For easier evaluation of both tryptase values, the following formula can be used: "basal tryptase + 20% + 2". However, the formula should not mislead us. In the case of low acute serum tryptase (e.g., <4 µg/L), this calculation method is not appropriate.

Therefore, the value of acute serum tryptase predicts the level of tryptase during an anaphylactic reaction, while the value of basal tryptase predicts the severity of the anaphylactic reaction.

In the search for a better biomarker for predicting anaphylaxis, the chemokine **CCL2** has shown great promise (Figure 1), as studies suggest that measuring serum CCL2 has the highest specificity and sensitivity for confirming anaphylaxis compared to previous methods (tryptase, BAT, etc.) (Figure 2). Importantly, it has been found that therapy administered during anaphylaxis does not affect CCL2 levels. The results of a large international multicenter study are aiming to demonstrate the clinical and laboratory value of routine CCL2 measurement for diagnosing anaphylaxis. Preliminary results also indicate a correlation between the severity of the reaction and the level of CCL2 elevation.

In our ongoing research, we also developed a **mast cell activation test (MAT)**, which shows great potential for predicting the severity of the reaction.

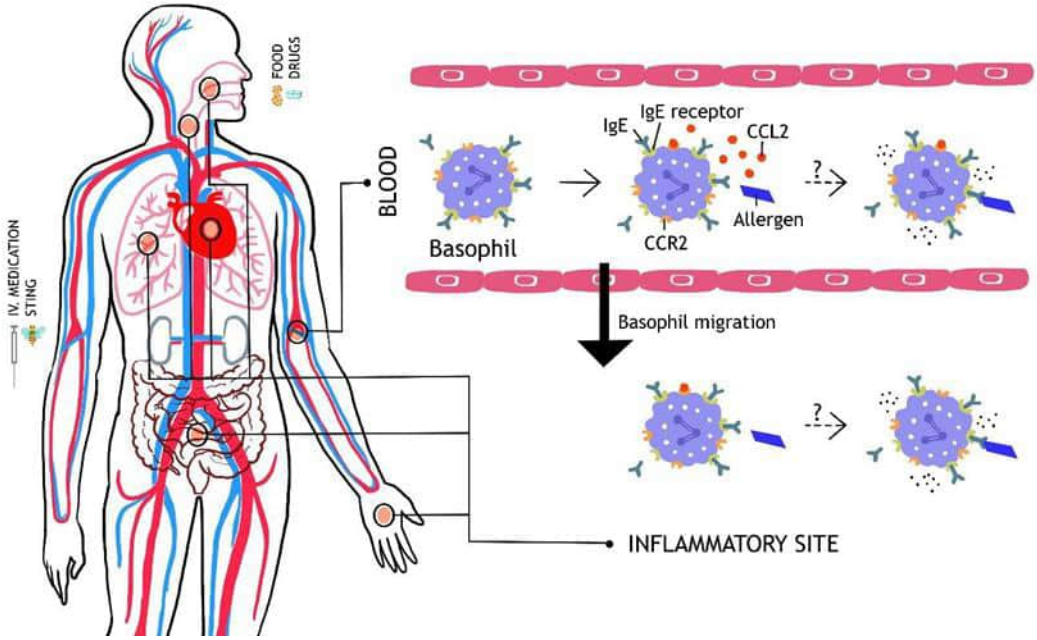

Figure 1: Hypothetical model of the role of basophils in anaphylaxis. The migration of basophils from peripheral blood to target tissues is key. This migration is associated with the chemotactic factor for basophils, CCL2. Activation and degranulation of basophils likely occur in target tissues.

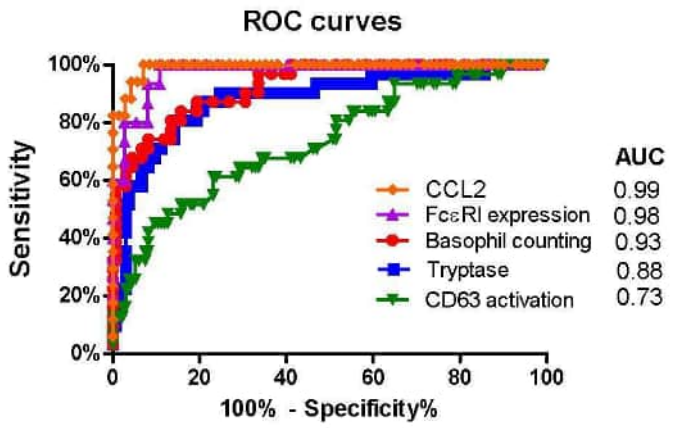

Figure 2: Comparison of different novel laboratory tests for confirming the clinical diagnosis of anaphylaxis. The serum basophil biomarker chemokine CCL2 measurement demonstrates the highest specificity and sensitivity (ROC=0.99).

Basophils and mast cells are key effector cells in immediate-type allergic reactions. The clinical impact of **basophil activation test (BAT)** is due to the unique ability of these cells to degranulate upon cross-linking of the specific IgE bound on membrane-bound high-affinity IgE receptor (FceRI) by allergen exposure. Compared with the determination of IgE in serum, the basophil activation test (BAT) offers a major advantage of being able to demonstrate the allergenic activity of IgE as positive test

results will only occur after successful cross-linking of the FcεRI and not by monovalent binding as seen in IgE assays. Currently, BAT with CD63 is the best clinically validated test. Some commercial BAT protocols are standardized; however, different in-house protocols are still wildly used, and in that case, the lab has to validate its own method. Researchers in Slovenia pioneered BAT research (with its first publication in 2005), and University Clinic Golnik is currently one of the most recognized groups in the global field of BAT, publishing more than 20 per-reviewed articles and more than 1000 citations.

Selection of allergen preparations

Perioperative hypersensitivity reactions pose a significant health problem with severe consequences. Various sources report different incidences of anaphylaxis during general anesthesia. Jamma Li et al. estimated the incidence of anaphylaxis to be between 1/1250 and 1/20000 procedures, while Spoerl et al. reported it between 1/4000 and 1/25000. Anesthesiologists correctly identified the cause of anaphylaxis in one-third of the cases. 60% of perioperative anaphylaxis is IgE-mediated, while the remaining 40% is non-IgE mediated. Muscle relaxants are the most common cause of perioperative hypersensitivity reactions (perioperative hypersensitivity - POH) among the drugs used in general anesthesia. A patient allergic to one muscle relaxant, neuromuscular blocking agent (NMBA), may also be allergic to another NMBA due to possible cross-reactivity. Other common causes of allergic reactions in the perioperative period include antibiotics, chlorhexidine, latex, opioids, hypnotics, and others.

Protein allergens are used in concentrations starting from the ng to μg/ml and are usually diluted for 4 log concentrations before maximal reactivity is achieved. Drug allergens are typically active in the mg/ml range and can be diluted 2 to 3-fold. It is important to compare data only when the allergen preparation and concentrations are comparable; failing this, the test can only give a limited result.

Evaluation of BAT (Basophil Activation Test) results for non-protein allergens, particularly medicinal substances and certain chemicals, poses challenges due to the involvement of mechanisms beyond IgE mediation. The existing literature reflects a lack of consensus regarding the optimal approach for establishing reference values for BAT-NMB (Neuro Muscular Blockers). Although the discrepancies between the different criteria are not substantial based on the available literature, the utilization of the Stimulation Index (SI) has demonstrated favourable outcomes. Our findings indicate that the implementation of criterion a (SI > 2) yielded the most robust diagnostic parameters, which we consider when providing test reports. The gold standard for confirming hypersensitivity reactions, the provocation test is rarely mentioned due to its high risk and limited justifiability.

Applying these findings in clinical practice necessitates considering that the diagnostic utility of BAT-NMB is limited due to its lower sensitivity, although the test maintains high specificity. A positive result suggests a high likelihood of a true positive outcome, warranting caution against the reuse of the same NMB during subsequent procedures. Exploring alternative options is preferable. Conversely, a negative test result cannot definitively exclude the possibility of a recurrence of Postoperative Hypersensitivity

(POH), as the low sensitivity of the test primarily stems from false-negative results. Therefore, a negative test result does not preclude the potential for a POH recurrence. It is crucial to interpret BAT results within the context of the comprehensive clinical picture.

Diagnostics

Tests and procedures used in allergology to diagnose the cause of perioperative anaphylaxis are; history and anesthesia records, skin tests (prick and intradermal), specific IgE antibodies, tryptase, basophil activation test (BAT) and provocation tests.

Skin tests for muscle relaxants are relatively easy to perform. When conducting in vivo diagnostic tests, caution must be exercised, particularly for patients who have experienced severe allergic reactions. Skin prick tests are performed first, and if they are negative, intradermal tests are conducted. If skin prick tests are positive, intradermal tests are not performed. Intradermal skin tests, in particular, can trigger a systemic reaction. Skin tests provide immediate results within 15 minutes. All muscle relaxants used in anesthesia can be tested. The majority of studies, as well as our own experience, indicate a good predictive value of skin tests for testing muscle relaxants. Therefore, skin tests are the most relied-upon diagnostic tool for muscle relaxant hypersensitivity. The actual predictive value of skin tests for muscle relaxants has not been definitively determined because provocative tests are usually not performed in cases of positive skin tests with drugs.

Specific IgE tests for muscle relaxants are straightforward to administer. Results are received quickly, usually the next day. However, they rarely identify perioperative anaphylaxis. In our clinic, specific IgE tests are only performed for succinylcholine. Some centres report good diagnostic value of sIgE for morphine and fentanyl in diagnosing POH due to NMBA. Morphine and fentanyl contain a similar ammonium group as NMBA.

BAT for muscle relaxants is an equally safe test as determining sIgE because it only requires a blood sample, and the test is performed in vitro. When determining BAT for muscle relaxants, the test is considered more relevant than determining sIgE. This has been proven by the majority of studies. BAT and skin tests for NMBA (neuromuscular blocking agents) are mostly coherent and provide more meaningful results for the IgE mechanism. Various studies have calculated different specificities and sensitivities for individual NMBA agents. BAT is determined for a mixture of muscle relaxants and separately for specific muscle relaxants.

Provocation tests for drugs used in anesthesia are not always feasible, most commonly due to the properties of anesthetic drugs. Performing drug provocation tests requires highly skilled personnel and equipment. They are carried out in rare centres where anesthesia allergologists lead the diagnosis of POH (perioperative hypersensitivity). At Clinic Golnik, we perform provocation tests with antibiotics, hypnotics, and opioids, but not with muscle relaxants. Patients undergo other possible/accessible tests for the planned drug for provocation (skin tests, BAT, sIgE) beforehand. In a study by Marie-Line et al., it is described that in the vast majority of cases, provocation testing would not contribute significantly to the diagnosis or determination of a clear trigger for patients

who have negative skin tests and BAT for a specific drug. Specifically, they mention that if provocation testing is not performed, they would miss 1 out of 150 patients tested for anesthetics, 1 out of 129 patients tested for opioids, and 1 out of 34 patients tested for NMBA.

Proposed Guidelines for the Management of perioperative anaphylaxis in Slovenia

During anesthesia-related complications suspected to be anaphylaxis, a blood sample for tryptase determination should be obtained immediately after initial patient stabilization. The optimal time for blood sampling is 15 to 120 minutes after the reaction. Normal tryptase concentration does not exclude anaphylaxis.

Blood samples are collected according to professional recommendations in additive-free tubes (red stopper) or K3 EDTA tubes for haematological tests (lavender stopper). Along with the blood sample, a referral form for laboratory testing at the Golnik Clinic is completed and sent. Referral forms can be obtained or ordered from the Laboratory of Clinical Immunology and Molecular Genetics, Golnik. The serum or plasma is pipetted off and stored in a refrigerator at 4°C for up to five days. If the testing is not performed within this time, the sample should be frozen at -20°C.

The time elapsed between the reaction and sample collection should be recorded. To confirm the basal level of tryptase and monitor its dynamics, a repeat blood sampling is recommended within 48 hours after the reaction. Baseline sampling can also be performed during the patient's visit to the allergist.

In the case of suspected mastocytosis, an additional blood sample should be collected 1-2 weeks after the reaction. In the event of a fatal outcome, it is advisable to obtain a blood sample for tryptase from the femoral artery or vein before resuscitation ends. Postmortem samples can be collected up to 48 hours after confirming death.

Tryptase determinations are performed daily, and the results are issued immediately after the test. They are sent to the requester. In urgent cases, the result can also be communicated to the treating physician over the phone.

Every patient with suspicion of perioperative anaphylaxis requires diagnostic evaluation to identify the causative factor and to search for possible cross-reactivity.

In addition to completing the anesthesia-specific card, the anesthesiologist prepares a detailed written description of the reaction. The documentation should also include a copy of the anesthesia record, which must be accurately and legibly completed, as well as the recovery room report and the therapeutic record before and after the procedure.

The patient needs to be informed about the reaction during anesthesia, explain the nature of the reaction, and emphasize the importance of additional diagnostics.

The reaction should be reported to the pharmacovigilance centre.

The patient should be referred to an allergology centre with experience in the management of perioperative anaphylaxis. Good documentation is important for allergological evaluation. Searching for the cause of anaphylaxis without information

about the medications received, and the timing of symptoms during anesthesia does not make sense. The temporal relationship between the reaction and perioperative events is crucial in identifying the potential causative factor. Sometimes it is necessary to contact the referring anesthesiologist, so it is advisable for the anesthesiologist who treated the patient during the reaction to provide their contact information. It is important to consider the possibility of a reaction associated with the surgical procedure or anesthesia.

Skin allergy tests are usually performed 4-6 weeks after the reaction. If the patient urgently requires surgery before that, tests can be performed earlier, but in this case, the sensitivity of allergy tests may be lower. If the tests are negative, the diagnostic evaluation should be repeated after 4-6 weeks for a definitive determination.

The diagnostic value of tests decreases over time, so it is advisable to start them as soon as possible after the reaction.

In cases where skin tests are negative or not feasible (e.g., for nonsteroidal anti-inflammatory drugs - NSAIDs), provocation tests are performed. The same medication is administered in the same way it was applied during anesthesia (oral, intravenous, subcutaneous, or intramuscular). We do not perform provocation tests with muscle relaxants (MR).

Results of allergological diagnostics:

The allergological diagnosis of perioperative anaphylaxis is based on positive skin tests, results of laboratory examinations, and the consistency of findings with the clinical picture and the course of anesthesia.

The allergological report should be sent to the anesthesiologist who treated the patient. The report should list the tests that were performed and interpret the results. If a specific allergen is identified, possible cross-reactivities should be mentioned, and advice regarding the use of related medications in case of a need for re-anesthesia should be provided. A copy of the report should be sent to the regional pharmacovigilance centre and the selected physician. The patient should receive an "allergy card for medications."

In case of difficulties in interpreting the tests, a team consultation of allergologists and anesthesiologists experienced in the management of perioperative anaphylaxis is advisable.

Thyrotropin Receptor Antibodies: Clinical Relevance and Methodological Aspects

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Thyrotropin receptor antibodies (TSH-R-Ab) represent highly heterogeneous class of autoantibodies expressing variable biological function. They are considered the major

culprits in the pathogenesis of autoimmune thyroid diseases (AITD), primarily Graves' disease (GD). Their direct action on TSH receptor target cells appears to be the main provoker of the underlying autoimmunity.

Hyperthyroidism in GD originates from persistent, uncontrolled stimulation of thyroid cells by TSH-R-Ab. Apart from their direct action on thyrocytes, these antibodies initiate disruptions in other tissues harboring TSH-R expressing cells, orbital fibroblasts and adipocytes among others. This leads to the development of Graves' orbitopathy (GO), a specific inflammatory disorder of the orbit characterized by a highly variable clinical phenotype.

TSH-R-Ab are classified as stimulating (TSAb), blocking (TBAb), and neutral antibodies, based on their functionality and the ability to mimic the natural ligand of TSH-R.

Although first described as the long-acting thyroid stimulators, TSH-R-Ab have been widely researched for their role in both GO and GD. However, to this day, their exact pathogenetic role has not yet been established. Apart from being a valuable research tool, these antibodies demonstrated a significant clinical potential as highly sensitive and specific biomarkers of both thyroid and orbital autoimmunity. Given the unpredictable course of both conditions and lack of optimal treatment choices, a specific, early biomarkers of both GD and GO are well needed.

Total concentration of TSH-R-Ab has traditionally been measured by competitive binding immunoassays; however, they do not distinguish between their functionality (stimulating, blocking, or neutral). Next generation bioassays have been developed to detect both stimulating and blocking TSH-R-Ab. This has been a major breakthrough in their analytical assessment since the clinical course of both thyroid and orbital autoimmunity is defined by their functional activity.

This lecture addresses the current issues in the field of GO, its perplexing relationship with thyroid autoimmunity, and actual challenges in the laboratory assessment and diagnostics. It offers several novel findings pertaining to the clinical relevance and utility of functional TSH-R-Ab in a carefully evaluated Serbian collective with GO of various degrees of clinical activity and severity.

Analytical And Clinical Characteristics of Serological Immunoassays of Anti-SARS-Cov-2 Antibodies

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Literature data indicate that serological immunoassays can be an adequate complement to molecular diagnostics of SARS-CoV-2 infection. The aim of this study is to evaluate the agreement of SARS-CoV-2 serological tests from five different manufacturers and to examine the immune response to different types of vaccines.

Methods used: Sars-CoV-2 IgG (Beckman Coulter) (I), SARS-CoV-2 Total Antibodies (BioRad) (II), SARS-CoV-2 IgG II Quant (Abbott) (III), SARS-CoV -2 IgG (Abbott) (IV) and Elecsys Anti-SARS-CoV-2 (Roche) (V). 44 samples, I and III 84 samples, I and IV 53 samples, I and V 80 samples were tested in parallel using method I and II. 153 samples were analyzed to evaluate the immune response to infection and different types of vaccines. Subjects were checked for the presence of antibodies due to suspicion of asymptomatic SARS-CoV-2 infection or due to the presence of symptoms.

A greater number of reactive results were obtained with methods II and III compared to method I (65.9% and 91.7% vs 52.3%). Methods I and IV showed agreement in reactivity (60.4% vs 69.8%), while method I and V did not yield agreement in reactivity results (58.8% vs 93.8%). The most intense immune response was achieved after vaccination with the Pfizer vaccine. The immune response after Pfizer and Sinopharm vaccines showed a correlation with the time elapsed since immunization (P=0.032 and P=0.012). Women had a higher antibody titer after vaccination compared to men (P=0.006). No difference was obtained in relation to the type of vaccine and the age of the subjects (P=0.197).

Serological immunoassays show significant analytical and clinical characteristics for the detection of SARS-CoV-2, but there is still the problem of complete test evaluation and uncertainty regarding the accuracy of the results and their comparability. Although it can be said that the COVID 19 pandemic is behind us, the knowledge and experience gained will be helpful for some future situations so that humanity does not stop again at some point.

The Bright Side of COVID-19 Pandemic for Patients with Allergic Conditions

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Background: The novel SARS CoV-2 virus is the cause of acute viral respiratory disease. The symptoms it causes are different, from a common cold and headache, to massive pneumonia and death. In the course of dealing with its spread, measures were implemented to protect the population from the spread of the virus, and one of those measures was the defining the list of chronic diseases that are a risk for patients if they become infected with SARS CoV-2. Working in the largest Covid-19 Center in Macedonia during the Covid-19 pandemic, I noted groups of patients, with chronic allergic diseases who were expected to be patients with higher risk and comorbidities. But practice has shown that these patients are somehow more protected than other patients.

Methods: 65 allergy patients with acute covid-19 and 65 convalescent patients with allergic disease and also 65 patients in the control group without covid-19 were inspected in this study with analysis of their peripheral blood samples. In this study was used automatic analyzer Immulite 2000 xpi to present the results for concentration on serum levels of inflammatory factors of first response and concentration of Immunoglobulin E antibodies. Allergy patients had asthma, allergic rhinitis and atopic dermatitis. Internal Hospital system was used to detect reported deaths and hospital time for treatment.

Results: Mortality is one of the main problems of the new disease, and the existence of risk factors and chronic diseases are considered the main reason for the high mortality rate of covid. But in the research, although all subjects had a history of chronic allergic diseases, there was not a single fatal outcome. Although convalescent patients were significantly older patients and age is one of the risk factors for mortality in patients infected with covid, there was not a single patient with a fatal outcome. Treatment time in the hospital for convalescent patients was significantly longer than for acute covid subjects and thus a higher risk of death would be expected, however, there were no deaths in all groups of patients with allergic diseases.

Conclusions: A higher level of IgE has a protective role in patients with allergic diseases during the course of covid and the existence of a chronic disease such as allergy in any form is not a risk factor for mortality from viral infection with SARS CoV-2.

SESSION 7: BIOMARKERS IN NEUROBEHAVIORAL AND PSYCHIATRIC DISORDERS

Multimodal Prediction of Age of Onset of Alzheimer's Disease with Potential for Treatment Stratification

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There is a need for cost-effective tools for early detection of Alzheimer's disease (AD), and for early-stage stratification to identify who can benefit from new pharmacological agents.

We have developed a new multimodal hazard score (MHS) combining age, a polygenic hazard score (PHS), brain MRI abnormalities, and cognitive testing to predict conversion from mild cognitive impairment (MCI) to AD in a Memory Clinic Setting. This was tested in the Alzheimer's Disease Neuroimaging Initiative (ADNI). We also did power calculations to estimate required clinical trial sample sizes using the MHS. We also applied the PHS to determine the predicted age of onset for AD pathology.

The analysis of the MHS performance showed that it predicted conversion from MCI to AD with a clinically relevant hazard ratio (HR) =27.03 (80th vs 20th percentile). The power calculations from the MHS results estimated that application of the MHS based on MRI, cognition and genotypes can reduce the clinical trial sample sizes by 67%. The analysis of age of onset of pathology showed that the PHS predicted age of onset of amyloid and tau markers.

The new multimodal prediction tool MHS improves early detection of AD with clinically relevant effects sizes which makes it useful in memory clinics for early detection of AD. Further, it seems to be a valuable tool for reducing the variation in disease stage in a clinical trial, and thus reduce the number of participants to reduce costs of clinical trials.

Using Antisense Oligoes for Treatment of Diseases with Genetic Haploinsufficiency

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Many disorders are caused by inherited gene mutations that result in lower protein expression. Genetic haploinsufficiency, in which partial or complete loss of one normal allele is sufficient to cause disease, is the basis of numerous human neurodegenerative as well as other diseases. In such disorders, increasing protein levels might potentially slow or cure the disease. Current strategies use deliverables of protein products, mRNA

transcripts or adenoviruses with gene replacement strategy to replace our increase reduced amounts of haploinsufficent genes. However, these strategies cause often immune responses, reduced efficiency during the long periods of treatment and are often not deliverable to all organs including Central Nervous System (CNS).

We have identified a novel strategy using a safe and well-validated DNA-based technology called antisense oligonucleotides (ASOs) that might be able to benefit numerous brain and other disorders associated with genetic haploinsufficiencies. The untranslated regions (UTRs) that flank the coding sequence of mRNA are critical regulatory hubs that influence RNA metabolism, including translation and stability. In particular, the 3'UTR encodes numerous regulatory elements that engage trans-acting elements such as RNA binding proteins (RBPs) and microRNAs, many of which repress translation and reduce mRNA stability. ASOs bind complementarily to target RNA with high affinity and block the binding of trans-factors, thereby altering aspects of RNA metabolism. Importantly, ASOs targeting non-coding regions like UTRs can modify stability and translation without interfering directly with translation machinery. We are now applying these ASOs to treat two inherited neurodegenerative conditions, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We identified ASO molecules that may be effective at treating these conditions in patient cells and are now working to refine these drugs to treat humans with disease.

Modeling Psychiatric Disorders with Patient-Derived Brain Organoid Cultures

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Bipolar disorder (BD) and Schizophrenia (SCZ) are two psychiatric disorders amongst the leading causes of disability worldwide. There is still limited understanding of which specific biological features are associated with diagnosis, response status and drug treatment and their clinical management is limited by trial-and-error approach to pharmacological treatment and descriptive diagnostic criteria.

A major limitation in BD and SCZ research is the poor accessibility to post-mortem diseased brain tissue and the lack of proper animal models, as most psychiatric phenotypes are unique to humans and they cannot fully recreate the complexity of polygenic diseases, which resulted in slow advances in our understanding of the disease.

Induced pluripotent stem cells (iPSCs) technology emerged as a powerful technique for in vitro disease modeling, as they preserve the genetic background of patients, as we have reviewed. This is a major breakthrough, as it directly links underlying disease pathology to mechanisms of action of psychopharmacological agents. Recently, the discovery of iPSC-derived 3D brain organoids has raised great promise for brain development and disease modeling in vitro, as they add an extra layer of complexity compared with 2D iPSC-derived models. They reproduce key in-vivo features like cell-to-

cell interactions or tissue-specific brain organization and architecture. Thus, the methods and tools now available can form the basis for a transformative change in clinical infrastructure.

Our recent results illustrate how patient iPSC-derived 2D cells and 3D cortical spheroids can be used as a biological model to provide new insight into BD pathophysiology and to advance our understanding of the cellular mechanisms underlying the therapeutic effects of Lithium.

Moreover, high-throughput screening of new classes of compounds capable of pharmacological amelioration of the diseased phenotypes is now feasible. Promisingly, if the diseased phenotype in iPSC-derived cells can be lessened, the same result may be achieved directly in the patient. In addition, if adverse drug responses are identified in specific subpopulations, it may be possible to predict these before clinical complications appear in patients. Taken together, the findings revealed by iPSC technology-based BD and SCZ brain cortical organoids models will represent an important step in further improving and perhaps developing novel therapeutic treatments.

The influence of the COMT Polymorphisms on the Severity of presentation of Negative Symptomatology in Schizophrenia Spectrum

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Introduction: Schizophrenia spectrum disorders represent a group of severe psychiatric illnesses that lead to disability. During treatment, 20 to 45% of patients show signs of therapeutic resistance and only partial symptom reduction. Negative symptomatology poses the greatest challenge for clinicians due to poor to moderate therapeutic response, pervasiveness of negative symptoms, and significant impact on patients' quality of life. The aim of this research is to investigate the association between the rs4680 and rs4818 polymorphisms in the COMT gene and the severity of negative symptomatology in patients with schizophrenia spectrum disorders.

Materials and Methods: The sample consisted of 122 participants. The participants were divided into groups based on the presence of therapeutic resistance using Suzuki's criteria. Phenotypic characteristics of patients were assessed using standardized scales: M.I.N.I., CAINS, CGI, and GAF. Clinical status and sociodemographic data were obtained using a Clinical Assessment Questionnaire. Molecular methods were used to determine the rs4680 and rs4818 polymorphisms of the COMT gene. The influence of polymorphisms and haplotypes on the degree of severity of negative symptomatology was analyzed.

Results: Patients with therapeutic resistance had significantly higher average scores on the CAINS compared to patients without therapeutic resistance. Statistical significance

was obtained in the analysis of the association between the rs4680 polymorphism in the COMT gene and negative symptomatology, where carriers of the AG genotype had significantly more severe negative symptoms compared to carriers of the homozygous AA and GG genotypes. Analysis of haplotypes showed that the A-G/C-G haplotype was a prognostic marker of more pronounced negative symptomatology and more severe clinical presentation of the disorder.

Conclusion: The research results suggest that genetic investigations of the COMT gene can help predict the occurrence of negative symptomatology. The importance of these results lies in the potential for practical application in terms of early interventions and personalized therapy for patients with psychotic disorders within the schizophrenia spectrum.

Keywords: schizophrenia spectrum psychotic disorders, negative symptomatology of schizophrenia, COMT gene.

SESSION 8: PRECISION MEDICINE TECHNOLOGIES AND MOLECULAR DIAGNOSTICS

Pharmacogenetics: prevention is key!

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The discovery that the metabolism of drugs is highly variable between patients, and can be predicted by performing DNA analysis of genes encoding drug metabolizing enzyme, paved the way for translating pharmacogenetics into clinical care. A major role for the cytochrome P450 enzymes, with CYP2D6 (involved in the metabolism antidepressants, antipsychotics, AHDH medication, beta-blockers, opioids and tamoxifen) and CYP2C19 (antidepressants, clopidogrel) as major targets. However, for CYP2D6, 5-10% of the population is deficient and for CYP2C19 2-4%, making that standard doses prescribed to these patients increase the risk of adverse drug reactions (CYP2D6/antidepressants, CYP2D6/metoprolol, or predispose patients for suboptimal therapy (CYP2C19/clopidogrel, CYP2D6/tamoxifen). Specific use of pharmacogenetics exists also in oncology (DPYD/capecitabine, TPMT/6-MP), internal medicine (e.g. HLA-B*5701/abacavir) and hematology (CYP2C9/warfarin), making that an approach for preemptive testing, and introducing a general DNA-passport for medication may be helpful to increase patient safety with respect to drug therapy. A recent publication on this approach showed that a 30% in adverse drug reactions could be established.

Currently, 15-30 genes for drug metabolizing enzymes and drug transporters that can be used clinically for optimizing personalization of drug therapy. Our international (IFCC) expertcenter for pharmacogenetics at the Dept. of Clinical Chemistry has been providing this clinical service since 2005. In the Netherlands, pharmacogenetic testing can be done in 16 ISO15189 certified laboratories, and advices regarding dosing information based on pharmacogenetics can be obtained at every community pharmacist.

In this presentations, successes and challenges in implementing pharmacogenetics into routine health care will be highlighted.

RNA Editing as a Diagnostic Test to Diagnosis Bipolar Disorders

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New technologies have led to the development of several tests and epigenetics in psychiatry is now a reality. Epigenetics is a branch of genetics that is now attracting

attention concerning human disease conditions. The environment, lifestyle, nutrition, and psychological factors impact the epigenetic machinery. One of the epigenetics mechanisms is RNA editing, which is a post-transcriptional modification of an RNA.

It consists of the modification of adenosine to inosine (A to I) that could be studied by sequencing of specific regions in the target biomarkers. A to I is a common type of RNA editing, catalyzed by ADAR (Adenosine Deaminase Acting on RNA), which leads to post-transcriptional modification regulating protein function.

The accurate differentiation between Bipolar Disorder (BD) and unipolar depression poses a significant challenge due to the overlapping depressive symptoms that form the core presentations of both disorders. Misdiagnosis during depressive episodes (in particular first-ever depressive episodes) not only leads to a delay in appropriate treatment, but also contributes to the inadequate management of the condition. To address this critical issue, a novel diagnostic test myEDIT-B™ has been developed that combines RNA editing variants modifications with depression subtypes and the utilization of artificial intelligence (AI) algorithms.

By integrating 8 epigenetic markers with clinical information, the test can effectively discriminate BD patients from those with unipolar depression. The implementation of the novel diagnostic test myEDIT-B™ promises to significantly reduce the misdiagnosis delay of bipolar patients, enabling a timelier initiation of appropriate treatment strategies. By providing clinicians with an objective and reliable tool, early identification of BD among depressed patients becomes achievable, leading to improved outcomes and enhanced management of the condition. Moreover, the utilization of RNA editing variants and AI techniques paves the way for personalized medicine approaches, facilitating the tailoring of treatments.

Clinical Mass Spectrometry Becomes a Major Tool for Precision Medicine

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Introduction: There is an extraordinary flood of new technologies in medicine nowadays - sophisticated diagnostics based on genome assays, mass spectrometry, magnetic resonance spectroscopy and cell sorting platforms combined with machine learning and artificial intelligence are driving significant technological transfer in laboratory medicine, enormously enhance the informative value of assays, and promote the entrance of individualized patient management in clinical practice.

Aim: This work overviews the role of Clinical mass spectrometry (MS) in the medical laboratory and its place as a major tool for the introduction of precision medicine in clinical practice.

Methods: Literature review and personal experience. While genetic testing allows the physician to check personal genetic program and choose appropriate medicine, the performance of MS assays provides the patient's actual phenotype, with all of the environmental, pharmacological and pathological variables. Therefore, MS is essential technology for ultimate personalization of patient management. LC-MS/MS (QQQ) is the today's most utilized analytical platform, but high-resolution MS systems are also employed to resolve challenging analytical demands.

Results: The great technological advance of MS resulted in the introduction of methods with unprecedented identification power, extreme sensitivity, specificity and extended linearity range, which are based on the current reference analytical principles. Further, the ability to perform panel profiling with simultaneous measurement of bioactive compounds, their precursors and metabolites in a single sample, enormously amplifies the informative value of results, with ultimate improvement of patient care. Typical examples include new born screening, TDM, toxicology, endocrinology, microbiology, clinical omics assays and others. Experience with clinical mass spectrometry of over 18 years in the field of immunosuppressive drug monitoring, 13 years for assessment of vitamin D status, 5 years analysis of individual steroids, 3 years of steroidomic diagnostics encompassing 456 patient profiles, 3 years of dihydropyrimidine dehydrogenase (DPD) phenotyping via measurement of endogenous uracil (U) assessing over 400 patient samples will be presented and discussed. Steroid profiling assured improved research and diagnostics for primary and secondary hyper-aldosteronism, incidentalomas/ adenomas with autonomous cortisol secretion and non-secrering ones. DPD activity was categorized as sufficient if U<16.0 μg/L, as fully deficient if U>150 μg/L, and as partially deficient: over 17% of our patients were with U>16 μg/L, highest 66.9 μg/L, and another 10% with U between 14.0÷15.9 μg/L (proposed grey zone).

Conclusion: Clinical MS paves the introduction of precision medicine and integrates chemical and anatomical pathology via MS imaging and I-knife-MS guidance in surgery, thus opening new horizons for personalized treatment and individualized patient care.

SESSION 9: ADVANCING HEALTHCARE FOR TRANSGENDER PATIENTS

Genital Surgery and Bioethical Aspects in Transgender Persons

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Surgical treatment, i.e., the conversion of genitals from birth to the desired gender represents the final stage of treatment in the transgender population. Reconstruction includes the creation of new, desired genitalia from the existing ones, with which the transgender person was born. In the direction from male to female, new female external genital organs, clitoris, vulva and vagina are formed. In the opposite direction, the male sex organ is formed either by the reconstruction of the enlarged clitoris or by the formation of a new phallus using extragenital tissues (forearm, abdominal skin, latissimus dorsi muscle).

Through the spectrum of possible surgical techniques, presentation of results and complications, we will present modern methods of surgical treatment of this entity. Also, a special review will be given to all bioethical issues, such as treatment in puberty and adolescence, the possibilities of parenthood for transgender persons, transplantation of genital organs as an ideal way in this transition, and the treatment of persons who have shown remorse for the previous transition.

Hormone Treatment in Transgender Persons, and Interpreting Laboratory Results

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Context: Healthcare professionals rely on accurate reference values to interpret clinical biochemical test results. Reference values can vary significantly between different population subgroups. In transgender individuals receiving gender-affirming hormone therapy (GAHT) these reference values are poorly defined. The uncertainty as to which reference values to use, may lead to clinical decision making conundrums.

Synthesis of Sources: A series of cases will be presented, illustrating the influence of reference values on clinical decision making in transgender persons. The cases are gathered from recent publications on gender-affirming care, as well as from the clinical practice of the Department of Endocrinology of the Ghent University Hospital, Ghent, Belgium. The Ghent Endocrinology Department is a national centre of expertise on gender-affirming care. Each case will be framed against a background of the current

scientific knowledge on GAHT effects. To this end, results from the European Network for the Investigation of Gender Incongruence (ENIGI) study will be discussed. Results from this prospective, multicentric study already helped to define global guidelines for GAHT.

Results and Conclusion: Considerable changes in body composition, sex steroid levels and common laboratory values can occur quickly after the initiation of GAHT. It is advisable that the reference values of the gender the patient identifies with are used after three months of GAHT. This recommendation does not apply to some organ-specific or organ volume-dependent tests, such as cardiac troponins, prostate-specific antigen or ß-human chorionic gonadotropin. Due to the large heterogeneity of the transgender population, an individualized approach to reference values and biochemical test results interpretation may be required.

Psychiatric Aspects in Transgender Population

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The terms "gender identity disorder", "gender dysphoria", "transgenderism", "gender incongruence" are synonyms for the various intensities of disharmony between biological sex by which a person is assigned at birth and the individual perception of gender which can be experienced by some people. Mild cases of this dysharmony less frequently seek help from professionals from our team but visit sensitized psychologists We mostly deal with transsexualism as the most severe form of this dysharmony. Transsexual people cannot imagine continuing their life in the body they were born in and want other people to perceive them as such. They insist on harmonizing the body with their inner, psychological experience of the other sex and gender by hormone therapy and surgical interventions. Diagnostics and psycho-social preparation are the first steps in the process of gender affirmation (sex change). Transsexual individuals are supported by psychiatrists, psychologists and social workers through this part of the transition process. The experience of the Department of Psychiatry Counseling Service for Gender Dysphoria team in "Dragisa Misovic" Clinical and Hospital Center and my one in private practice in Belgrade, Serbia will be presented in this paper.

Key words: gender dysphoria, transsexualismus, psycho-social preparation

SESSION 10: DYSLIPIDEMIA, DIABETES, ADIPOSITY

Neuroendocrine Regulation of Food Intake and Metabolism via Gut Hormones and Enteric Neurons: Implications in Obesity & Type 2 Diabetes

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Over the past few decades, there has been an alarming rise in incidence of both pediatric and adult obesity and their metabolic complications worldwide leading to much higher rates of type 2 diabetes in children and adults. Despite decades of research, the molecular and physiological mechanisms underlying metabolic abnormalities that lead to obesity and type 2 diabetes remain elusive. Emerging evidence indicates that obesity and type 2 diabetes may be an intestinal disease. The intestine plays a key sensing and signaling role in the physiology of energy homeostasis by controlling satiety, energy intake, and energy expenditure. Upon nutrient ingestion, an intestine-brain axis is triggered involving signals from the upper intestine by vagal afferents that communicate with the nucleus of the solitary tract (NTS) in the brain. Satiety signals from the gastrointestinal tract such as small lipids in the upper intestine control food intake by afferent sensory neurons that signal to the NTS to regulate nutrient consumption. Besides this neuronal pathway, many hormones (leptin, insulin, ghrelin, and peptides like cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) contribute to the regulation of food intake by modulating the responses of the brain to the intestinal signals. Lipid ingestion can also regulate glucose homeostasis involving a gut-brain-liver axis. As part of the brain-liver axis, the hypothalamus plays a major role in this process as experimental lipid infusion in this area blocks hepatic glucose production, an effect that is reverted after hepatic vagotomy. Moreover, blocking the sensitivity of the hypothalamus to lipids promotes overfeeding resulting in obesity and insulin resistance. During the transition from fasting-to-fed states (immediately following meal ingestion), enteroendocrine cells (L- and K-cells) in the proximal and distal small intestine are stimulated, possibly through a combination of neuro-hormonal pathways and direct nutrient stimulation, to secrete incretins, GLP-1 and gastric inhibitory polypeptide (GIP). GLP-1 together with postprandial hyperglycemia stimulates the pancreatic beta-cells to secrete insulin, thereby increasing circulating insulin levels. Postprandial hyperinsulinemia acutely suppresses lipoprotein assembly and secretion in the liver, and increased GLP-1 secretion may also have a similar function, reducing hepatic lipid export postprandially. There is further evidence in animals that a gut-brain-liver axis is operative and thus the accumulation of certain lipids (such as long-chain fatty acids) in the upper intestine suppresses liver glucose production through action of the gut-peptide hormone CCK.

In this presentation, I will review the current evidence for the central role of the intestine and the gut-brain axis in mediating nutrient sensing to control energy balance and body weight. Molecular and physiological mechanisms will be discussed focusing on the key role of gut peptides, particularly GLP-1 and GLP-2.

Diabetes and Lipids

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Lipid abnormalities, most notably mixed dyslipidemia is intertwined with the pathophysiology, in particular to that of type 2 diabetes. Optimizing lipid control is one of the cornerstones of treating both type 1 and 2 diabetes. The evidence from clinical trials is the strongest for type 2 diabetes following notable trials such as the Heart Protection Study which also included the largest cohort of patients with type 1 diabetes. I will discuss this evidence base and the challenges when it comes to treating these patients in the clinic whilst looking at both the well-established treatments and the more recently employed novel treatment options whilst debating the value and interpretation of recent studies.

Familial Hypercholesterolemia – Diagnosis and Management, UK Experience

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Familial hypercholesterolemia (FH) is a common, autosomal dominant, genetic disease caused by mutation in one or more of the genes critical for low-density lipoprotein cholesterol (LDL-C) catabolism. FH is characterised by elevated levels of LDL-C and a predisposition to early onset atherosclerotic cardiovascular disease (ASCVD), which is associated with significant morbidity and mortality.

Genes, most implicated in FH are genes coding for LDL-receptor (LDLR), PCSK9 and ApoB. Those patients who have two pathogenic mutations (compound heterozygotes and homozygotes, HoFH) are more adversely affected than those with one pathogenic mutation (heterozygotes, He). According to the Dutch Lipid Network criteria, it is estimated that prevalence of HoFH is 1:160,000-1:300,000, whilst prevalence of HeFH is 1:250.

Most used criteria which aid diagnosis of FH in primary practice are the Simon Broome criteria and the Dutch Lipid Clinic Network (DLCN) criteria. They consider **(i)** personal and family history of ASVCD in men before age of 55 and in women before age 60,

(ii) LDL-C levels (>4.9mmol/l), **(iii)** presence of tendon xanthomas or arcus cornealis, and **(iv)** presence of genetic mutation in LDLR, PCSK9 or APOB.

In lipid clinic across UK, at initial consultation, depending on the personal and family history, cascade testing of first-degree relatives (children, siblings, parents) is often suggested. It involves lipid profile testing (it's offered to children at the age of 9), or genetic testing in cases where FH has been confirmed in such a way. This allows early identification of potentially affected individuals, and starting treatment before cardiovascular disease may have developed.

Approach to management is non-pharmacological and pharmacological. Patients are advised on specific lifestyle modifications (Mediterranean style diet, moderate physical exercise, and smoking cessation), and signposted to relevant resources to achieve these. Pharmacological management involves use of medications such as statins, ezetimibe, PCSK9inhibitors, bempedoic acid and inclisiran. Rare patients may be offered nicotinic acid or cholesteryl ester transfer protein inhibitors. As PCSK9 inhibitors, bempedoic acid and inclisiran are relatively novel medications, their prescription is regulated by specific The National Institute for Health and Care Excellence (NICE) guidelines, and they are initiated and continued under the guidance of specialists in lipid clinics.

With the current National Health Service (NHS) strategy in place which aims for detection of 25% individuals with FH by 2025, the aim is to achieve earlier diagnosis and improved treatment for affected individuals, and prevention of premature stroke and myocardial infarction, and morbidity and mortality associated with it.

Association Between Vitamin D Status and Hypertension in Overweight and Obese Children in Montenegro

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Introduction: Obesity represents one of the most important risk factors for development of childhood hypertension. Correspondingly, low vitamin D (VD) status is considered associated with increased blood pressure in children.

Objective: To investigate the association between vitamin D levels and arterial hypertension in overweight and obese children in Podgorica, capital of Montenegro.

Materials and methods: The survey included 202 schoolchildren aged 7-15 (63.9% boys, 36.1% girls) from Podgorica. Participants were divided into 3 groups according to nutritional status (International Obesity Task Force criteria): normal weight (42.1%), overweight (40.6%) and obese (17.3%). Anthropometric measurements were performed: body weight (kg) and body height (cm), though body mass index (kg/m2) was calculated. Blood pressure was measured using an oscillometric monitor (Omron HEM 907 XL). Hypertension was defined as an average systolic blood pressure (SBP) and/or

diastolic blood pressure (DBP) greater than or equal to the 95th percentile for gender, age, and height. The value of 25 (OH) VD (nmol/L) was determined from the serum of 176 children (immunochemistry, Cobas 6000, Roche). Vitamin D values ≤ 50 nmol/L were considered deficient.

Results: The median value of VD for normal weight children was 77.2 (interquartile range (IR) 67.70-95.10), overweight 70.1 (IR 56.00-86.60) and obese 69.6 (59.30-85.87), this difference was borderline statistically significant ($p < 0.046$). Vitamin D deficiency was found in 4.3 % of normal weight, 16.0 % of overweight and 12.5 % of obese children. There was no statistically significant difference in the frequency of VD deficiency in relation to nutritional status of the examined children ($\chi2 = 5.185$, $p = 0.075$). Similarly, there was no statistically significant difference in the frequency of VD deficiency in relation to hypertension ($\chi2=0.240$; $p=0.624$), even though VD status was inversely related to SBP in obese children.

Conclusions: Low vitamin D status was not associated with childhood hypertension in overweight/obese children in Podgorica.

Dyslipidemia and Relevant Disorders to Female Reproductive Health

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Dyslipidemia is a multifactorial disorder, which arises from complex interactions among genetic and environmental risk factors. During their lifetime, from puberty, reproductive age, and perimenopause to menopause stages women experience hormonal changes. These changes induced variations in the amounts of blood lipids. Starting from birth, LDL concentrations are progressively increasing in the first two years in both men and women. During teenage period, ranging from 10 to 17 years old, the LDL concentrations are lower in men than in women. After 20 years of age, both men and women show an increase in their LDL concentrations, greater in men than in women. During the adulthood the HLD-c level is lower in men compared to women, resulting in a cardio-protective factor for women at this age. It is relevant to consider the modifications that the lipid profiles show during pregnancy, in which an increase of the hormones gonadotropin, β-estradiol, insulin, and progesterone occurs. These hormones are associated with an increment of total cholesterol (TC), TG, LDL-c, HDL-c, and Apo protein A1 concentrations, having their highest peak at the week 36 of pregnancy. Other stages that nowadays have become a challenge for lipidologists are pre-menopause and menopauses, as well as conditions such as polycystic ovary syndrome (PCOS) where the expression of risk factors joined the hormonal suppression, require multidisciplinary vision of the practice. PCOS is the most common endocrine abnormality in women of childbearing age and may be accompanied by dyslipidemia, hyperandrogenism, hyperinsulinemia, oxidative stress and infertility. Dyslipidemia is now known to play an

important role in the development of PCOS. Lipid abnormalities, including elevated low-density lipoprotein and triglyceride levels and reduced high-density lipoprotein levels, are often found in women with PCOS and play an important role in PCOS; therefore, we summarize the effect of lipid abnormalities on hyperandrogenism, insulin resistance on patients with PCOS.

SESSION 11: MASTERCLASS FROM BALKAN REGION I

The Science of Women Leadership

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Why are there so few women leaders?

Traditional gender stereotypes operate in virtually all organizations — including health institutions and medical laboratories — to slow, obstruct and block women's progress up the career ladder. They do this by fostering five distinct sorts of biases against women in the workplace:

1. Negative biases
2. Benevolent biases
3. Agentic biases
4. Self-limiting biases
5. Motherhood biases

Leadership Double Bind

You can be a "good" woman but a "bad" leader.

You can be a "good" leader but a "bad" woman.

The data suggest that men were more likely overall to be chosen or rated as leaders, in part because they had more assertive personalities and thus spoke up more. Women are already significantly underrepresented in leadership. For years, fewer women have risen through the ranks because of the "broken rung" at the first step up to management. Now, companies are struggling to hold onto the relatively few women leaders they have. Women leaders are switching jobs at the highest rates we've ever seen—and at higher rates than men in leadership. That could have serious implications for organizations.

Male and female leaders perform equally well. Considering the gender gap at the top of organizational charts, women should not be asked to change their behavior as a way of addressing this situation; rather organizations should train employees to change their perspectives. To make meaningful and sustainable progress toward gender equality, organizations need to go beyond table stakes.

Organizations as Leaders:

1. Pipeline women from early career to executive level;
2. Provide external support for women's advancement;
3. Ensure inclusion from supply chain through decision making;
4. Build into performance measures;
5. Monitor progress and outcomes from equality initiatives and activity.

Men as Leaders:

1. Ask and listen to women;
2. Mentor, coach, and sponsor women;
3. Give women meaningful feedback;
4. Place women in stretch assignments/roles;
5. Equality starts at home.

Women as Leaders:

1. Question everything;
2. Leverage your difference;
3. Create a support network;
4. Amplify other women;
5. Equality starts at home.

An advice for men: "Be less dominant. Let other people have some time to talk."

An advice for women: „Focus on your values. Be BRAVE."

Key words: science, women, leadership

Sepsis Biomarkers: From Bench to Bedside

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Sepsis is characterized by organ dysfunction due to severe host immune response to pathogen or tissue damage signals. Early diagnosis of sepsis and, more importantly, monitoring of sepsis progression, are important for patient management. Several biomarkers have been identified to date, some being indirect indices of severe inflammation and others direct markers of cytokine storm and immune system dysfunction. These markers include expression of HLA-DR, suPAR, procalcitonin, or different immunomodulatory cytokines such as IL-10, Monocyte Distribution Width (MDW) and other. Even though several biomarkers to support sepsis diagnosis have been established, timely diagnosis of sepsis immunosuppression remains unclear. Results utilizing functional assays such as responsiveness to TLR ligands or effective autophagy, that determine the capacity of immune cells to mount immune responses to secondary infections, will be presented and their potential use as tools to stratify patients and potentially serve as biomarkers will be discussed. Following the discovery of miRNAs and elucidation of their role in regulating immune responses their potential as functional serum biomarkers has emerged. Association of functional changes with expression of miRNAs will also be discussed. A distinct population readily exhibiting sepsis immunosuppression are neonates, which are inherently immune compromised. Functional analyses in mouse models of sepsis, coupled with studies in patient samples

from our group has shown that another secreted protein Del-1 is a potent regulator of immune responses during sepsis and can serve as biomarker of sepsis both in adult and neonatal septic patients. An overview of the current knowledge and experimental data on sepsis biomarkers will be presented.

Medical Waste Management at The Biochemistry Laboratory

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Background: Clinical laboratories generate large amounts of biomedical waste. The generation of biomedical waste has been increasing in quantity and variety due to the wide acceptance of single-use disposable materials, such as gloves, plastic syringes, reagent bottles and laboratory tubes. The management of biomedical waste has been of major concern due to potentially high risks to public health and the environmental degradation.

Methods: Biomedical waste programs in Greece, with reference to overall hospital waste management, are based on a strict legal framework since many years. The present study focuses on biomedical waste generated at a biochemistry laboratory and the practices implemented in their management. The laboratory is part of the Naval and Veterans Hospital of Athens, a tertiary care hospital in Athens, Greece. The laboratory receives approximately 500 samples per day (serum, whole blood, urine and biological fluids). An audit was performed during one year, in order to detect the type and quantity of waste generated and to formulate the plan for segregation, waste handling and management.

Results: The audit revealed solid non-infectious waste, waste sharps, infectious waste and liquid waste generated from analyzers, labware washing and laboratory cleaning. Waste minimization and recycling practices where applied, wherever possible. Moreover, ordering of reagents and consumables is centralized, in order to promote bulk ordering with fewer shipments and reduced levels of packaging. A major problem still remains with the plastic bottles of reagents. If they are autoclaved to be rendered safe, they become unsuitable for recycling.

Conclusions: Since public concern surrounding the levels of single use plastic has increased, we are seeking for a more sustainable approach, without compromising work quality.

Lipid Profile in the Context of Cardiovascular Risk: A New Approach of ASoLaM in the Role of Laboratory Specialists in Patient Counseling

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Background: Cardiovascular disease is a major cause of mortality and hospitalization all over the world. Our understanding of this disease nowadays is quite comprehensive and the main focus is on preventive actions and patient counseling to avoid and delay atherosclerotic changes and cardiovascular events associated with them. Our aim was to provide clear recommendations for Albanian laboratory specialists to unify lipid profile reporting in the context of cardiovascular disease.

Methodology: The Albanian Association of Clinical Biochemistry and Laboratory Medicine (ASoLaM) set up a working group of laboratory experts to review all documents of consensus published by the EFLM, EAS/ECS and other European Laboratory Associations, and come up with a series of unified recommendations for Albanian medical laboratories and specialists regarding pre-analytical, analytical and post-analytical phase of lipid testing and dyslipidemias in the context of cardiovascular risk.

Results: The working group's recommendations encompassed the entire lipid testing process. As a result, we now have preanalytical recommendations on fasting requirements before lipid testing, as well as analytical recommendations on what should a standard lipid profile include and what methods should be used to test lipids. We have post-analytical recommendations on the reference/target values of lipid tests taking in consideration the cardiovascular risk. The working group also assessed what should be considered alarming values and how should they be reported and communicated to patients and physicians. The recommendations also cover Lp(a) and ApoB testing, as well as target LDL-C values associated with cardiovascular risk. ASoLaM recommendations include instructions on when to provide counseling and/or refer to a specialist, based on a patient's lipid profile.

Conclusions: The collaboration between laboratory specialists and cardiologists, as well as the collaboration between International Societies of Cardiology and Laboratory Medicine is of outmost importance for defining effective strategies for the early prevention and treatment of atherosclerotic disease.

Immunomodulatory Effect of Nanomembrane-Based Low-Volume Plasma Exchange on Intestinal Permeability in Metabolic Syndrome

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Background: Intestinal permeability, or "leaky gut," refers to an increased passage of molecules across the intestinal lining, which can allow the entry of bacteria, toxins, and other substances into the bloodstream. This increased permeability can lead to chronic low-grade inflammation and metabolic disturbances, potentially contributing to the development of metabolic syndrome (MetS). Among the non-invasive markers of intestinal permeability, lipopolysaccharide binding protein (LBP) and Zonulin (Zon) stand out due to their sensitivity.

Low-volume plasma exchange (LVPE), using a novel nanomembrane-based technology, is an innovative approach to blood purification that removes toxic and inflammatory blood components. It basically consists of a device that pumps and filters the patient's blood through nanopores in a multi-membrane layout. Another benefit of LVPE is its immunomodulatory effect, which aims to achieve the desired immunological balance and response. Interleukin-6 (IL-6) plays a crucial role in immunomodulation, as it has both pro-inflammatory and anti-inflammatory properties.

The aim of this study is to present the immunomodulatory effect of nanomembrane-based LVPE on markers of intestinal permeability and low-grade inflammation.

Methods: This prospective study included 48 outpatient participants, 31.3% female and 68.7% male, with an average age of 50 years. They underwent four cycles of nanomembrane-based LVPE, performed every other day, as a safe and minimally invasive procedure using the Hemofenix device and the nanotech membrane PFM 500. Risks of allergic reactions and viral disease transmission were excluded. During each cycle, 30% of circulating plasma was removed, which was then replaced only with a saline solution. LBP, Zon, high-sensitive C-reactive protein (hsCRP), and IL-6 were measured in serum samples immediately before the first and after the fourth LVPE cycle.

Results: Although four cycles of LVPE led to a decrease in serum concentrations of LBP, Zon, IL-6, and hsCRP, the obtained differences were not statistically significant. Serum concentrations of LBP and Zon significantly correlated before the first ($p<0.05$) and after the fourth cycle ($p<0.001$) of LVPE. However, serum concentrations of IL-6 before the first LVPE cycle significantly correlated with LBP concentrations ($p<0.007$) as well as with hsCRP concentrations ($p<0.001$), but not after the fourth cycle.

Conclusion: Four cycles of nanomembrane-based LVPE, as a safe and minimally invasive procedure, can have a significant immunomodulatory effect on low-grade

inflammation and intestinal permeability through the modulation of anti-inflammatory IL-6 activities.

Early Prediction of Bloodstream Infection with Complete Blood Count Parameters: An Ex-Vivo Human Whole Blood Model

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Background: Despite the advanced laboratory technologies available today, blood culture is the gold standard method in the diagnosis of bloodstream infections. The causative agent in circulatory infections is mostly bacterial. Although there is availability of automated blood culture devices for antimicrobial susceptibility tests, in vitro proliferation of microorganism is required. It takes at least 2-3 days up to 6 days for laboratories to give blood culture results. Technically early detection of causative agent in bloodstream infections is limited. Especially in serious conditions such as sepsis, early diagnosis and treatment are crucial. We aimed to investigate complete blood count (CBC) parameters as potential early markers in Gram-positive and Gram-negative bloodstream infections using an ex vivo whole blood model.

Methods: Standard strains of Escherichia coli (ATCC 25922) and S. aureus (ATCC 29213) were incubated on 5% sheep blood agar in air with 5% CO_2 at 37 °C for 1 day. Bacterial colonies were suspended in phosphate buffered sterile PBS (pH 7.4) and a concentration of stock bacterial suspension was adjusted to 108 CFU/mL using a spectrophotometer at 600 nm wavelength. Whole blood was collected from 10 healthy participants (between 20-40 years of age) in Na-heparin tubes. Each patient's blood sample was transferred to 9 tubes (1, 2, 3, and 4 hours incubation for each bacteria and one baseline) containing 450 µL of blood sample. Each whole blood sample (450 µL) was mixed with 50 µL of bacterial stock suspension for bacteria-induced and 50 µL PBS for baseline samples. Thus, the final bacterial concentration in the samples was set to 10^7 CFU/mL. At the end of each incubation period, the results of the complete blood count parameters were analyzed with Mindray BC-6800 hematology analyzer. Statistical analysis was performed by one way ANOVA Tukey-Kramer post-hoc multiple comparison test, statistical significance is accepted as $p<0.05$.

Results: In both E. coli and S. aureus induced whole blood model, white blood cell (WBC), platelet, immature platelet fraction (IPF), P-LCC (platelet large cell count) baseline values were compared with the 1st, 2nd, 3rd and 4th hour values. The baseline values were significantly different when compared to all other values of incubation. There was no difference between baseline immature granulocyte (IMG) and 1st, 2nd, 3rd hour IMG results, whereas 4th hour IMG results were statistically different compared to baseline IMG. In the E. coli model neutrophil baseline values were statistically significant

when compared to all incubation values whereas only 3rd and 4th hour incubation values were found to be significant.

Conclusions: Platelet derived parameters including P-LCC and IPF were detected as very early biomarkers in the ex vivo model induced by E. coli and S. aureus. Considering the limitations of the ex vivo model compared to real in vivo conditions, it can be used in future biomarker studies.

SESSION 12: MASTERCLASS FROM BALKAN REGION II

Old Biomarkers and Their New Use

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There is a constant need to investigate certain substances and relate them to the disease's stage, type, and severity, as we are defining the goals of drug or medical device development for the prevention, diagnosis, mitigation, or treatment of diseases. This need is the basis for the development of the biomarker concept.

The definition of biomarkers is based on their role in describing or measuring the state within certain biological systems. Biomarkers can be classified according to their application, such as diagnostic biomarkers, prognostic biomarkers, pharmacological biomarkers, and surrogate biomarkers.

According to their biophysical properties, molecular biomarkers are measured in different biological samples. Among key tools, they provide valuable information related to disease molecular mechanisms that are characteristics of various pathophysiologies.

The roles of many biomarkers have been well-known for decades (complete blood count, metabolic control parameters, enzymes, etc.), but novel researches bring insight into some of their possible new ones. Recently, alternative biofluids (e.g., saliva), containing a variety of biomarkers, have also shown great potential for many diagnostic purposes.

Various Types of Urinary Casts and Their Diagnostic Significance – Manual Microscopic Vs Automatic Method (IRICELL) – An Integrated View

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Introduction: Through optical microscopy Automatic Method (**IRICELL**), the casts present in the urinary sediment and the morphological differences between them can be highlighted. For safety and control, in addition to the automatic method, we will use, where appropriate, several types of manual microscopy (bright field, phase contrast and where appropriate polarized light).

Material and method: More than 150 patients were studied, all these patients were diagnosed with certainty as having kidney disease, hospitalized and treated in the Nephrology Department of Clinical County Emergency Hospital Timisoara. Fresh "morning" urine specimen was used for the analysis, as well as a second urine for which the urinary sediment was performed (both manually and automatically) and following the examination, several types of casts were highlighted.

Results and discussions: It were possible to highlight the following types of casts: hyaline casts, granular casts, waxy casts, cellular (red blood cell casts, white blood cells casts, macrophage and renal tubular cells casts), fatty casts, microbial casts, cylindroid and pseudo casts. The photos were taken in our laboratory – Urinalysis Department.

Conclusions: The lack of urinary casts does not exclude a kidney disease, only highlighting as precisely as possible the type of cast present in the urinary sediment can confirm or deny the diagnosis. For example, the presence of red blood cells casts can differentiate a glomerular bleeding from a non-glomerular one. Finally, we can also say that the final morphology of the casts depends on the diameter of the renal tubes in which they were formed.

Key words: urinary casts, microscopic techniques, automated method

Endocan as a Novel Marker of Cardiometabolic Disorders

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Although a wide array of cardiometabolic biomarkers has emerged in the last several decades, the quest for those that would be reliable to predict cardiometabolic risk, disease and its progression still continues. Endocan represents an intriguing biomarker that is not thoroughly investigated in cardiometabolic diseases, given that many discrepant results exist in scientific literature. Secreted by endothelial cells, this soluble dermatan sulphate proteoglycan was found to be increased in many inflammatory diseases with enhanced expression in highly proliferative tissues, such as lungs, hepatocytes, kidneys, etc. It was shown that pro-inflammatory mediators stimulate the secretion of endocan. In line with this, increased serum endocan levels were found in disorders tightly connected with insulin resistance and inflammation, such as metabolic syndrome, polycystic ovary syndrome, obesity, type 2 diabetes mellitus, non-alcoholic fatty liver disease, hypertension, coronary artery disease. Thus, the question arises whether endocan could be a novel diagnostic tool and treatment target in the mentioned disorders which could postpone and/or prevent the onset and progression of complications, especially cardiovascular ones.

Chemical Composition, Antioxidant and Antibacterial Activity of Essential Oil of *Satureja montana L.*

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Background: Differences in the chemical composition of the *Satureja* genus occur under the influence of climatic and environmental factors. Also, depends on the region where the plant material was cultivated. In numerous studies it was shown that thymol, carvacrol, p-cymene and γ-terpinene are the predominant chemical compounds of the essential oil of the species *Satureja montana* (SM).

Methods: The plant material used in this study was an aromatic plant species belonging to the family *Lamiaceae* - *Satureja montana L*. The plant was cultivated near Podgorica, under typical Mediterranean climate conditions. All of the bacterial strains used for evaluation of antimicrobial activity were obtained from The General Hospital "Blazo Orlandic" - Bar. Among the cultures tested were used Gram-positive (*Staphylococcus aureus, Enterococcus faecalis & Bacillus subtilis*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae & Pseudomonas aeruginosa*) bacterial strains. All of the microbial strains used were pathogens isolated from human samples. The volatile oil extracted from SM was analysed using gas chromatography–mass spectrometry (GC-MS) method. The antibacterial activity was performed using the microdilution method. The results were expressed as minimal inhibitory concentration MIC µL/mL. The *Satureja montana* essential oil (SMEO) was tested for antioxidant activity via a DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) spectrophotometric assay (the values were used to calculate EC50 via linear regression) and the FRAP (Ferric Reducing Antioxidant Power) assay - antioxidant activity expressed as µmol FRAP/L).

Results: In total, 40 chemical compounds were identified in the analysed essential oil of SM, the most abundant constituents were monoterpene hydrocarbons (86,64%), followed by sesquiterpene hydrocarbons (8,09%) followed by unclassified (others) compounds (1,68%). The major constituents of the EO were p-cymene (28,65%), thymol (22,1%), linalool (4,86%), trans-caryophyllene (4,52%) and carvacrol (3,28%). The antibacterial activity of the essential oil of SM was examined against a panel of 6 cultures. The results reveal that the oil of SM inhibited the growth of all of the tested strains. The most sensitive strain was *E. faecalis* (0,78125 µL/mL). Four tested strains (*E. coli, B. subtilis, S. aureus and K. pneumoniae*) showed the same level of sensitivity (3,125 µL/mL). The most resistant culture was *P. aeruginosa* (12,5 µL/mL). SMEO analyzed in our work showed a considerable amount of antioxidant activity in the DPPH assay (EC50 = 3.44 µL/ml) and reductive potential in the FRAP assay (1168.845 µmol FRAP/L).

Conclusions: The results we obtained in the antimicrobial assay showed that the essential oil of SM is a strong antibacterial agent. The SMEO inhibited growth of all tested

pathogenic bacteria. Also, strong antioxidant activity of an EO correlates with its strong antibacterial activity, which could be a good perspective and approach to the SMEO analysis in the future.

LAB CLINICAL CASE REPORTS

Blood Morphology. A Microscope: Between Reality and Mind's Eye

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The phrase mind's eye refers to the human ability for visualization.

The relation between reality and mind is usually perceived as an event that takes place in reality while we are simultaneously producing an internal image in our mind.

That is exactly what we do in medical practice: We examine a patient, then we get inspired in predicting what a patient's condition is all about and we choose appropriate diagnostic tools to get to the final diagnosis.

But what happens when there is a discrepancy between reality and mind's eye?

Three following case reports surprised us but gave us a new insight into the beauty of laboratory work.

Case #1

A 90-year-old patient from retirement home was brought to University Medical center Maribor to the Emergency department due to acute respiratory failure. At the admission to the hospital, she was afebrile and with arterial oxygen saturation 70% and evident dyspnea (30/min).

Auto anamnesis is not possible due to somnolence. Many associated diseases where already known (Alzheimer's disease, diabetes type 2, ICV, condition after heart attack...)

There was a suspicion of Covid pneumonia after lungs X-ray imaging even though nasopharyngeal swab tested negative.

Case #2

A 65-year-old woman was brought to University Medical center Maribor to the Emergency department due to worsening Chronic obstructive pulmonary disease with dull chest pain. Saturation measured in the ambulance car was below 93%. She suffered from dyspnea and chills but there was no fever or signs of febrile condition. She has lost 5 kg in 14 days.

Case #3

12-hour-old full-term singleton was born as a first child to a 32-year-old mother. A child had no particularities in terms of gestational age just like the delivery itself did not have any irregularity. But shortly after delivery respiratory distress of a newborn

occurred. Artificial ventilation with an oxygen mask was performed. Neonatologist noticed unusual baby's tongue and fists movements.

Antiphospholipid Syndrome – Diagnostic and Therapeutic Challenges

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The range of pathological features of the antiphospholipid syndrome (APS) includes thrombosis in the arterial and venous blood vessels as well as capillaries, together with obstetric morbidity in the presence of antiphospholipid antibodies (aPL). According to the classifications criteria, persistent presence of medium to high titers of aPL comprises antibodies against cardiolipin (aCL), β2glycoproteinI (anti-β2GPI) or positivity to lupus anticoagulant (LA).

The catastrophic antiphospholipid syndrome (CAPS) constitutes < 1% of all APS cases, though with 30-50% mortality. CAPS is a dramatic severe 'thrombotic storm', with thrombotic lesions in multiple locations occurring within a short period of time. A pathognomonic sign of CAPS is s.c. triple positivity for aPL. International consensus criteria from 2003 state that for classification of a definite CAPS diagnosis, four criteria have to be fulfilled: (i) involvement of three or more organ systems, (ii) presentation within a week, (iii) biopsy confirming small vessel occlusion in at least one organ and (iv) aPL positivity. Patients may however present with acute APS-related symptoms, but do not fulfil all four criteria. In these cases, they should, depending on symptoms, be considered as probable CAPS and be treated accordingly.

Small vessel occlusions dominate the clinical CAPS picture, but thromboses in large vessels may also occur. A major source of knowledge about CAPS comes from the CAPS registry, an online registry which has collected more than 500 international case reports. Information on initiating and descriptive factors, treatments and outcomes is collected in this registry (https://ontocrf.grupocostaisa.com/ web/caps), and all who care for these patients are asked to report their cases. The last updated cumulative data state that the average age at diagnosis is 38 years and 69% are female. The most common organs to be affected are kidneys (73%), lungs (60%), brain (56%), heart (50%) and skin (47%). Mortality rates have declined over the years but are still very high, 37%. A key observation is that precipitating factors, 'a second hit', especially infections (49%), but also surgery (17%), malignancies (16%), contraceptives (10%) and pregnancy-related complications (8%), precede CAPS in a majority of cases. Patients with autoimmune diseases, especially SLE, were overrepresented (40%) and these patients had a more severe prognosis.

Since APS is a treatable condition, which according to present guidelines in many cases comprises life-long anticoagulant treatment, it is important that these patients are recognized by the general health care so that they can be properly diagnosed and treated.

In the workshop on APS, two patients will be presented and discussed. One patient is a young women diagnosed with APS during pregnancy and the other is a patient with CAPS as a first clinical manifestation.

Lipoprotein (a)

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Lipoprotein (a) is a complex molecule which function and metabolism is incompletely understood. Epidemiological data clearly demonstrate that lipoprotein (a) levels correlate with an increased risk of atherosclerotic cardiovascular disease. Currently no specific treatment is available to reduce lipoprotein (a) concentrations, though several phase 2 and 3 trials are underway investigating therapies which target the lipoprotein A gene by way of small interfering RNA and antisense oligonucleotide technology. Whether lipoprotein (a) should be measured in routine clinical practice is debatable. Accurate measurement of lipoprotein (a) is challenged by the lack of method standardization, the numerous isoforms present which impacts on the choice of calibrant and reference material. I will discuss these challenges, providing a brief look at the past interventions and gazing to the future as we aim to reduce cardiovascular disease.

Curious Cases of Dyslipidaemia – A Series of Case Reports

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Dyslipidemia is an established risk factor for atherosclerotic cardiovascular disease (ASCVD). Identifying it and correcting it early, results in reducing the risk of atherosclerosis and premature cardiovascular disease. The following cases present patients with various underlying causes of dyslipidaemia, their management and lessons learned.

Case 1 – A 12-year-old girl presented with a 5-day history of extreme fatigue and tiredness, polyuria and polydipsia. She was previously fit and well. On examination, she was cachectic, mildly dehydrated, with no xanthomas. There was no organomegaly, or clinical signs of lipodystrophy. Eye examination showed lipemia retinalis. Initial blood tests showed total cholesterol 41mmol/L, triglycerides 251.2 mmol/L, HbA1c -174, random glucose 28.1 mmol/L and diabetic keto acidosis (DKA). She was admitted to hospital, put nil by mouth, and treated with fixed rate insulin infusion. Her insulin requirements were appropriate for her weight (0.05 units/kg/h). No further intervention

was required, and 10 days post admission, her TG were below 10 mmol/L. Diagnosis of Type 1 diabetes mellitus was confirmed, and patient discharged home on basal bolus insulin treatment. One month after initial clinical presentation, her lipid profile was normal.

Case 2 – A 50-year-old man was referred by GP to Cardiovascular Risk/Lipid clinic for urgent assessment due to increased triglycerides, at 41mmol/l. His past medical history included mixed dyslipidaemia, hypertension, obesity and type 2 diabetes mellitus. Repeat blood test revealed TG of 59 mmol/L, HbA1c was 76. Telephone appointment was scheduled. He complained of polydipsia and polyuria suggesting worsening of diabetes control. Initially, patient denied any alcohol intake. However later, he disclosed heavy alcohol intake in the past few months. Risk of pancreatitis was discussed, and patient signposted to Accident and Emergency department in case of abdominal pain. He was managed with lifestyle advice, insulin detemir, semaglutide (oral), empaglifozine, bezafibrate and atorvastatin. His diabetes and lipid control are improved, however suboptimal.

Case 3 – A 58-year-old woman with possible Familial Hypercholesterolaemia (FH) was referred to Cardiovascular Risk/Lipid clinic for investigation of possible statin intolerance manifested as liver function test (LFT) derangement. Patient has been on Atorvastatin 80 mg for 30 years before developing deranged LFTs (at peak, ALT 6.6 x upper limit normal (ULN), AST 4xULN, GGT 3.3xULN). Patient has undergone investigations which included repeated LFTs on and off statins (showing decrease in LFTs when off statin treatment), liver screen (ANA positive (deemed insignificant), remaining screen normal, including hepatitis B and C, CMV and EBV, ferritin, alpha-1-antitrypsin (A1AT) and Tissue Transglutaminase Antibody (TTG). Ultrasound liver showed mild fatty disease. FibroScan was consistent with minimal or no liver fibrosis. Liver biopsy demonstrated fatty liver with no evidence of other inflammation and no damage to the liver. Genetic studies confirmed heterozygous missense mutation in the LDL receptor gene (c.681C>G p. (Asp227Glu)). As her cholesterol rose to baseline levels (TC 11.6 mmol/L, LDL-C 9.1 mmol/L) with no statin treatment, patient was put on a PCSK9 inhibitor, initially evolocumab, later alirocumab. However, she showed poor response to both, with only around 25% LDL reduction. PCSK9i non-responders are very rare (<1% of all patients), and there are multiple factors involved. Treatment with alirocumab was continued, and additionally, patient was started on bempedoic acid in combination with ezetimibe. She continues to be monitored in lipid clinic.

Poster Presentations

ALLERGOLOGY

Cross-Reactive Anti Carbohydrate Determinants Antibodies Impact on Inhalant Allergens Sensitization Testing

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Background: Glycoproteins found in plants and insects, more precisely N-glycans with specific epitopes, are known as cross-reactive carbohydrate determinants (CCDs). These can be viewed by the human immune system as foreign and, in some individuals, may elicit the production of IgE antibodies. CCDs IgE antibodies have limited or no clinical significance, however, can impact the diagnostic accuracy of the quantitative measurement of IgE antibodies to inhalant allergens in a patient's serum. The aim of this study was to assess the impact of anti CCDs IgE interference on inhalant sensitization serum testing.

Material and methods: Serum samples from 344 subjects with respiratory symptoms were tested for presence of 29 inhalant allergens and CCD specific IgE antibodies with Inhalation 30-I assay (Polycheck, Germany). CCD positive samples were treated with Anti-CCDs absorbent (Euroimmun AG, Germany).

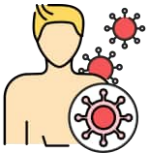
Results: We found that 12.4% of patients who were sensitised to inhalant allergens, had significantly high values of anti CCDs antibodies (≥0.35 kU/L). Anti CCDs antibodies did not interfere with mesurement of following inhalant allergens specific IgE: Rye pollen, Cultivated Oat pollen, Kentucky Blue Grass pollen, Timothy Grass pollen, Cultivated Wheat pollen, Cocksfoot pollen, Cat epithelia and Dog epithelia.

False positive results (≥0.35 kU/L) were detected for: Cypress pollen, Hazel pollen, White Ash pollen, White Oak pollen, Olive pollen, Birch pollen, Bermuda Grass pollen, Plantain pollen, Goosefoot pollen, Wall Pellitory pollen, Ragweed pollen, Mugwort pollen and Latex.

Equivocal results were observed for anti-Blattella germanica IgE, with 50% results staying positive after performance of anti CCDs inhibition test.

Conclusion: Falsely elevated allergens IgE results can commonly occur due to the presence of CCDs IgE. To exclude potential inadequate diagnosis, results should be evaluated carefully, and CCDs absorbent used in samples with significant presence of anti CCDs and specific IgE antibodies to some, but not all inhaled allergens.

AUTOIMMUNE DISEASE



Diagnostic Value of Indirect Immunofluorescence for the Autoimmune Bullous Dermatoses. Our Experience in Albania.

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Background: The autoimmune bullous diseases are characterized by pathogenic autoantibodies directed at target antigens whose function is either cell-cell adhesion within the epidermis or adhesion of stratified squamous epithelium to dermis or mesenchyme.

Aim of the study: To assess the correlation between clinical diagnoses and Indirect Immunofluorescence patterns in autoimmune bullous diseases and to assess the diagnostic value of Indirect Immunofluorescence (IIF) in various autoimmune bullous diseases.

Materials and Methods: The study was conducted for a period of 10 years from February 2011 to January 2020. Total of 208 patients aged 21-89 years with vesiculobullous lesions of both sexes (58.2% of them females), attending the Department of Dermatology were sent for the determination of the autoantibodies in the Laboratory of Immunology. These samples were tested and analyzed under Fluorescence microscope and IIF testing.

Results: Among the patients of vesiculobullous disorders studied, the most common disorder was found to be pemphigus vulgaris, constituting 40.4% cases. About 25% of patients resulted with bullous pemphigoid. Mucosal pemphigoid was found in 3.8% of cases. One case resulted positive for pemphigoid gestationis and four cases resulted positive for Epidermolisis bullosa acquisita. Out of 208 cases of autoimmune vesiculobullous disorders, only 149 (71.6%) cases correlated clinically with IIF patterns.

Conclusion: Our study proves Indirect Immunofluorescence is not only diagnostic but also confirmatory for the autoimmune bullous disorders.

Keywords: Indirect Immunofluorescence

Significance of Paraprotein Determination in Systemic Connective Tissue Diseases

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Background: Monoclonal gammopathy of undetermined significance (MGUS) is a non-cancerous condition in which the body produces paraprotein. The existence of MGUS is one of the most common premalignant disorders, and considering its occurrence in association with specific systemic connective tissue diseases, it is considered that the possible mechanisms of MGUS are various rheumatic and autoimmune diseases. People with MUGS have a slightly higher risk of developing myeloma and lymphoma. The aim of this study was to prove the presence and significance of paraproteins in a selected sample of patients with systemic connective tissue diseases.

Methods: The cross-sectional study included 48 patients with some of the systemic connective tissue diseases (38 women and 10 men). According the diagnosis, patients were divided into six groups: patients with systemic lupus erythematosus (14), patients with primary Sjögren's syndrome (15), patients with rheumatoid arthritis (2), patients with vasculitis (6), patients with mixed diseases connective tissue (10), patients with dermatopolymyositis (1). The available diagnostic data, clinical pictures and demographic data were taken for each patient. Laboratory diagnostics of these patients included determination of serum total protein (TP), complement component 3 (C3), complement component 4 (C4), rheumatoid factor (RF), immunoglobulins IgA, IgG and IgM concentrations. Serum protein electrophoresis (albumin, alpha-1, alpha-2, beta 1, beta 2 and gamma globulins) and immunofixation of all samples were performed.

Results: The presence of paraproteins was proven in 13 patients (27%). The most common type of paraprotein was IgG kappa (18.8%). There is a significant association between the diagnosis and the presence of paraproteins ($p = 0.039$), but the association between diagnosis and presence of IgG kappa, IgG lambda, IgM kappa, and IgM lambda were not significantly different ($p=0.157$, $p=0.837$, $p=0.087$, $p=0.837$, respectively). There was significant correlation between C4 and IgG concentrations and RF and IgG concentrations ($p <0.05$). The significant correlations between C3 concentration and the gamma globulin ($p = 0.001$) and between C4 concentration and the gamma globulin ($p = 0.001$) were also found. There was no significant difference ($p>0.05$) in TP, C3, C4, EF, IgA, IgG, IgM, albumin and globulin concentrations between six patients' groups.

Conclusion: It is recommended to monitor patients with present paraproteins regularly in order to detect the possible progression of MGUS to multiple myeloma or other lymphoproliferative diseases and start appropriate treatment on time.

Rods and Rings Antinuclear Antibodies (ANA) Pattern Unrelated with Hepatitis C Treatment, A Case Report

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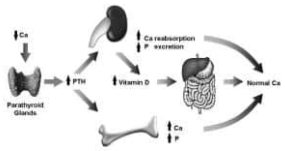
Background: The pattern of ANA-Hep2 called Rods and Rings (RR) consists of circular and stick fibrillar structures located in the cytoplasm. These structures have at least two enzymes as targets: Inosine monophosphate dehydrogenase type 2 (IMPDH2) and CTPS1 - Cytidine Triphosphate Synthase type 1. There are some hypotheses that try to explain the appearance of these antibodies: Apoptotic bodies, epigenetic modifications or cross-reactivity between self and foreign proteins, playing a role in the generation of autoantibodies. The presence of anti-RR antibodies has commonly been associated with ribavirin/interferon-α treatment of Hepatitis C virus (HCV) but there are also reports in healthy population as well as in patients receiving multiple medications or with underlying autoimmune conditions.

Methods: We report a case of RR fluorescence unrelated with antiviral therapy in a patient with antibodies anti-HCV and a complex autoimmune condition. Semiquantitative determination of IgG ANA was performed with indirect immunofluorescence using Euroimmun Mosaic HEp-20-10/Liver kit. Titers equal or higher than 1:160 are considered positive. AntiHCV was tested with chemiluminescent microparticle immunoassay (CMIA). HCV RNA viral load was analyzed with Abbot Real Time HCV assay. The clinical parameters of biochemistry, immunology, and hematology were performed on Alinity c, i, hq series, Abbot.

Results: A 57 years old woman was hospitalized in the Hepatology Department with ascites as the main objective finding. Biochemical testing showed elevated total and direct bilirubin, AST and ALP, low albumin, prolonged Prothrombin Time and low complement C3 and C4. The patient had macrocytic anemia. Anti HCV antibodies tested positive while HCV RNA viral load was negative. Autoimmunity testing showed positive ANA on 1:640 titer and speckled fluorescence pattern. There was also observed high Rods and Rings cytoplasmic fluorescence. The patient tested negative for anti-ds-DNA and anti-mitochondrial antibodies. Rheumatoid Factor and anti-transglutaminase IgA antibodies were found positive. There is no evidence of prior usage of any antiviral therapy.

Conclusion: Rods and Rings ANA pattern is a rare finding that is not exclusively related to HCV antiviral treatment, as it has been suggested before. Exact inciting role, pathogenesis and their prognostic significance is still a mystery that requires further investigations.

BONE AND MINERAL METABOLISM



Laboratory Assessment of Bone Metabolism in Patients with Hemoglobinopathy by Dosing the Bone Resorption Marker, β-Crosslaps

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Introduction: Osteoporosis is an important cause of morbidity in hemoglobinopathy patients. It is characterized by low bone mass and disruption of bone architecture, resulting in reduced bone strength and increased risk of fractures.

Hemoglobinopathies represent a group of quantitative/qualitative genetic disorders. The pathogenesis of bone involvement in hemoglobinopathy is multi-factorial. β-CTX (β-CrossLaps) is a marker of bone resorption.

The aim of this study is to evaluate the role of serum β-CrossLaps in patients suffering from hemoglobinopathy complicated with osteoporosis.

Materials and Methods: We measured in 102 patients with Thalassemia (TM) and Sickle cell disease (SCD) and in 67 healthy control subjects' serum β-CrossLaps levels and determined correlations with vitamin D, calcium and ferritin.

Results: 31.1% of our patients with TM & SCD have osteoporosis and 21.6 % have osteopenia.

β-CrossLaps in thalassemia major is 0.7±0.55ng/ml, in sickle cell disease is 1.1±1,1 ng/ml and in the control group 0.2±0.17 ng/ml.

We find correlation between β-CrossLaps, ferritin, vitamine D and calcium (β-CrossLaps-ferritina (r=0.472; p<0); β-CrossLaps-Vitamin D (r0.43; p<0); β-CrossLaps-calcium (r-0.23; p<0.002).

Conclusion: β-CrossLaps should be considered a useful marker of bone resorption.

Keywords: Thalassemia, Sickle cell disease, β-CrossLaps, osteoporosis

Prevalence and Impact of Vitamin D Insufficiency/Deficiency in Multiple Myeloma Patients

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Background: Multiple myeloma (MM) is one of the most frequent hematological malignancies, characterized by malignant proliferation of monoclonal plasma cells in the bone marrow. The major feature of MM is myeloma bone disease (MBD) and at diagnosis up to 80% of patients present with osteolytic bone lesions and are of increased risk of pathological fractures leading to serious consequences on morbidity and mortality of patients. Vitamin D is an important hormone for bone health and proper functioning of the immune system. The purpose of this study was to assess the vitamin D status of newly diagnosed MM patients (NDMM).

Patients and methods: In the present study 45 patients (41 NDMM and 4 with MGUS – monoclonal gammopathy of undetermined significance) and 33 healthy controls, age and sex matched, were enrolled. Vitamin D status was determined as follow: deficiency (25OHD≤25.0 nmol/L), significant insufficiency (25OHD: >25.0 to ≤50.0 nmol/L), insufficiency (25OHD: >50.01 to 80.0 nmol/L), sufficiency (25OHD>80.01 nmol/L). According to the International Scoring System NDMM patients were divided into three groups: ISS-1 (n=16), ISS-2 (n=7), ISS-3 (n=18) and according to whole body low dose CT evaluation of MBD – into two groups: G1 (n=9) – with no to 3 osteolytic lesions and G2 (n=32) – with more than 3 osteolytic lesions and/or pathologic fractures. 25-hydroxyvitamin D (25OHD) was measured by LC-MS. Standard statistical methods were performed with GraphPadPrism software, v8.01.

Results: The 25OHD serum levels of NDMM patients were lower than those of controls (respectively 43.55 nmol/L vs 57.08 nmol/L, p<0.01) and to MGUS (53.03 nmol/L, p=0.4266). The season of sampling influenced the patients 25OHD levels with higher values in warm months (June-October): 48.70 nmol/L and lower values in cold months (November-May): 40.78 nmol/L, without reaching statistical significance (p=0.2033). Frequency distribution of NDMM patients according to their vitamin D status was worse than those of controls (χ^2=10.28, p=0.0163). Patients in ISS-3 had significantly lower values in comparison with patients in ISS-2 (24.03 nmol/L vs 53.13 nmol/L, p=0.0003) and to ISS-1 (63.21 nmol/L, p<0.0001). G2 patients had lower levels compared with G1 (33.14 nmol/L vs 60.05 nmol/L, p=0.0288) and with controls (57.08 nmol/L, p=0.0010).

Conclusions: Although vitamin D insufficiency/deficiency is prevalent in the general population, there is a higher prevalence of vitamin D insufficiency/deficiency in NDMM patients. Moreover, patients with worse clinical characteristics had significantly lower 25OHD serum levels. Vitamin D insufficiency/deficiency could induce secondary hyperparathyroidism, thus increasing osteoclasts number. This additionally could impact the disturbed bone remodeling process, observed in MM pathogenesis. Perhaps

supplementation with vitamin D could have beneficial effects at least on symptoms such as fatigue, muscle weakness, and bone pain.

Comparison of Lipid Status in Premenopausal and Menopausal Women

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Background: During menopause there is a change in lipid metabolism, due to hormonal changes, such as decrease in estrogen levels, leading to increased risk of cardiovascular diseases. The aim of this study was to analyze the difference in the lipid profile in premenopausal and menopausal women, thus assessing the risk of cardiovascular diseases.

Methods: The study involved 36 women, not receiving antilipemic therapy, divided into two groups. Group of premenopausal women, N=18 (with an average age of 39.3±7.9 years, median=40) and group of menopausal women, N=18 (with an average age of 58.2±7.4 years, median=59). The measure of every parameter was made after overnight fast.

Total Cholesterol was measured with CHOD-PAP enzymatic colorimetric method. HDL was measured with a direct enzymatic method without precipitating. LDL was calculated using the Friedewald equation. Triglycerides were measured with the GPO-PAP enzymatic colorimetric method. All parameters were measured on an automated analyzer (MINDRAY BS-240).

Student's t-test was used to analyze the data, p values <0.05 were taken as statistically significant.

Results: In the group of premenopausal women the average values of Total Cholesterol were 5.4±0.7 mmol/L (median=5.4 mmol/L), HDL 1.6±0.4 mmol/L (median=1.55 mmol/L), LDL 3.5±0.7 mmol/L (median=3.5 mmol/L) and Triglycerides 0.8±0.6 mmol/L (median=0.7 mmol/L). In the group of menopausal women, the average values of Total Cholesterol were 6.5±1.2 mmol/L (median=6.5 mmol/L), HDL 1.5±0.4 mmol/L (median=1.45 mmol/L), LDL 4.3±0.9 mmol/L (median=4.3 mmol/L) and Triglycerides 1.6±0.7 mmol/L (median=1.5 mmol/L).

The results showed statistically significant higher levels of Total Cholesterol ($p<0.05$) and LDL ($p<0.05$) in menopausal women compared to premenopausal women. The levels of HDL in menopausal women were slightly lower, but the difference was not statistically significant ($p>0.5$). We found significantly higher levels of Triglycerides ($p<0.05$) in menopausal women compared to premenopausal women.

Conclusion: The levels of Total Cholesterol, LDL and Triglycerides in women in menopause were higher, as evidenced by the results. This is due to the decrease of estrogen in menopausal period. Lower levels of estrogen result in emphasizing the

anabolic processes in lipids and increasing the depo-lipides. Thus, the risks of cardiovascular diseases are higher in this group of women. These findings suggest the importance of prevention (healthy lifestyle, exercise, supplements, etc.) in menopausal women, to reduce the risk of cardiovascular diseases.

Soluble ST2 Is Associated with Inflammatory and Calcification Markers in Patients with Atrial Fibrillation or Heart Failure

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Background: There is no single diagnostic test for heart failure (HF) and atrial fibrillation (AF). As AF and HF often co-exist, it would be of great importance to find a biomarker with diagnostic utility in predicting HF in AF patients. Soluble Suppression of Tumorigenesis-2 (sST2) is a part of the cardioprotective IL-33/ST2 signaling pathway and may serve as a candidate biomarker for HF and AF. Therefore, the aim of the current study was to evaluate the serum levels of sST2 in patients with AF and heart failure with preserved ejection fraction (HFpEF) and to explore potential relationships with traditional risk factors for cardiovascular diseases (CVD) and with novel biomarkers for vascular calcification like undercarboxylated matrix Gla-protein (ucMGP).

Methods: In the study were included 99 patients stratified into 3 groups: HFpEF (n=19), paroxysmal or persistent AF in sinus rhythm (n=33), and control group without CVD but at moderate-to-high CVD risk (n=47). Hemodynamic and anthropometric measures, coronary artery calcification (CAC), routine laboratory parameters, circulating undercarboxylated matrix-Gla-protein (ucMGP) and sST2 were measured.

Results: sST2 levels were highly elevated in HFpEF patients. Significant positive correlation was found between sST2 and CAC-score (r=0.237, p=0.039), negative relations with serum lipids in AF patients, and positive association with serum C-reactive protein (r=0.609, p=0.018) in HFpEF patients. Soluble ST2 positively correlates with ucMGP in the entire studied population (r=0.252, p=0.006) and in the combined CVD group (AF+HFpEF) (r=0.254, p=0.036).

Conclusions: sST2 levels emerge as a novel biomarker in CVD and may have prognostic importance for HF prediction in AF patients.

Keywords: cardiovascular diseases; matrix Gla protein; ST2 protein; vascular calcification.

Presentation of the Results of BNP Testing for a 3 Years Period From 2020 To 2023 in the Bitola Clinical Hospital, North Macedonia

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Brain natriuretic peptide (BNP), also known as B-type natriuretic peptide, is a hormone secreted by the cardiomyocytes in the heart ventricles. Increasing ventricular wall stress causing myocyte stretch is the primary stimulus for secretion and activates synthesis of the BNP, which is cleaved into two major molecules—an inactive marker molecule (NT-proBNP) and the bioactive BNP molecule. Both peptides are released into the circulation and are readily measured using commercially available assays. BNP is currently used as a biochemical marker for heart failure in clinical settings because it reflects the state of heart failure extremely well.

During 2020-2023 in the department of medical biochemistry in PHO Clinical Hospital dr. Trifun Panovski in Bitola, North Macedonia, we analyzed blood samples from a total of 1544 patients using ABBOTT ALINITY CI ANALYZER. Males were 918 patients with median age 69 and 626 patients were females with median age 68. We detected 354 males with BNP above reference value and 245 females.

We separated the patients into 5 groups according to age. Age group 0-45 years old males included 52 patients, 33 of them had BNP above reference value (< 85 pg/ml). In this age group, the values of BNP ranged between 107,4 to 4531 pg/ml with median value of 284 pg/ml. In the same age group for females, we had 33 patients, 16 had BNP value above reference value. BNP ranged between 95,7 to 1218 pg/ml with median value of 407,8 pg/ml. Age group 46-54 years old males included 87 patients, 48 of them had BNP values above reference value (< 87 pg/ml) BNP ranged between 100 to 3617 pg/ml with median value of 278 pg/ml. In the same age group for females, we had 42 patients, 25 had BNP above reference value. BNP here ranged between 112,2 to 3579 pg/ml with median value of 320,6 pg/ml. In the age group 55-64 years old males, 192 patients were included, 79 of them had BNP above reference value (< 119 pg/ml). BNP ranged between 119,9 to 3304 pg/ml with median value of 386.9 pg/ml. In the same age group for females, we had 148 patients, 73 had BNP above reference value. BNP here ranged between 124,6 to 5129 pg/ml with median value of 305,4 pg/ml. Age group 65-74 years old males included 335 patients, 122 of them had BNP above reference value (< 160 pg/ml) BNP ranged between 160,4 to 5000 pg/ml with median value of 494,4 pg/ml. In the same age group for females, 233 patients were included, 84 had BNP above reference value. BNP here ranged between 164,7 to 12933 with median value of 439 pg/ml. Age group 75-100 years old males included 252 patients, 72 had BNP above reference value (< 254 pg/ml). BNP here ranged between 273,4 to 2594 pg/ml with median value of 752,6 pg/ml. In the same age group for females, we included 170 patients, 47 had BNP above reference value. BNP ranged between 266,1 to 4224 pg/ml with median value of 480 pg/ml.

We can conclude that with age increasing in males, BNP also increases while in females it is not expressed with a significant difference. The most affected age group for both genders is 65-74 years old.

A wide range of cardiovascular disorders that result in the impairment of the heart's ability to fill or to pump out blood may eventually lead to the clinical syndrome of heart failure (HF). The most common causes are coronary artery disease and hypertension. In other cases, heart valve disease is to blame. Less often, HF can also be caused by heart muscle diseases, cardiomyopathies from a viral infection, over use of alcohol, or certain genetic disorders. Early diagnosis is very important for better therapy optimization and outcome improving. Plasma BNP is used clinically to guide the management of patients with HF and cardiac dysfunction, and it is also used as a prognostic indicator which can help clinicians adjust their therapy strategy and determine therapy effectiveness to improve a patient's survival. Therefore, BNP is one of the best prognostic indicators in all stages of heart failure predicting outcome in both hospitalized and outpatients.

Could Determination of Bcl2 and Caspase 3 Activity Indicate Plaque Evolution in Ischemic Heart Disease Patients?

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Background: Apoptotic cell death may play a critical role in a variety of cardiovascular diseases, especially in those developing on the basis of atherosclerosis. The goal of this study was to compare the activity of caspase-3 and values of Bcl-2 protein in sera in patients with various forms of ischemic heart disease, and to correlate these markers with inflammatory and lipid parameters.

Methods: We studied 30 patients with chronic stable angina pectoris (SAP), 27 with unstable angina pectoris (USAP), 39 with acute ST-elevation myocardial infarction (STEMI) and 27 age-matched healthy volunteers (Control group). Caspase-3 activity was determined by a colorimetric commercially available method while serum Bcl-2 concentrations were determined using commercially available immunoassays (ELISA).

Results: Caspase-3 was significantly higher only in the USAP group (0.122±0.062 μmol/mg protein, $p<0.05$) in comparison with the control group (0.092±0.022 μmol/mg protein). Concentrations of Bcl-2 were significantly higher in patients with SAP (0.310±0.075 ng/mL) and USAP (0.329±0.102 ng/mL) compared to healthy (0.250±0.069 ng/mL, $p<0.01$) and the STEMI (0.266±0.041 ng/mL, $p<0.01$) groups. ROC curve analysis showed that Bcl-2 had the best characteristics in patients with SAP and USAP and represents the best indicator of atherosclerotic plaque activity. However, Bcl-2 could not be a marker of patients' stratification because there was no

significant difference between areas of Bcl-2 curves of these two patient groups. These results suggest that simultaneous determination of caspase-3 activity and Bcl-2 can indicate plaque evolution from stable to unstable one.

Conclusions: The studied markers of apoptosis present valuable parameters in evaluation of atherosclerotic plaque activity and a new target for drug therapy.

Tumor Necrosis Factor-Alpha, Selectin-E and Matrix Metalloproteinase-7 as Potential Biomarkers for Carotid Artery Disease

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Background: Carotid artery disease (CAD) presents an important healthcare burden as one of the leading causes of stroke. The need for the improvement of CAD stratification based on individual risk prediction is widely recognized, as well as the great potential of serum biomarkers for this use. Still, the complexity of CAD symptom development that is influenced by numerous factors makes it hard to come to agreement on the significance of specific parameters. For this reason, we investigated tumor necrosis factor-alpha (TNF-alpha), selectin-E and matrix metalloproteinase-7 (MMP-7) to try to define the way they relate to different aspects of CAD.

Methods: The study comprised 136 CAD patients and 45 healthy controls. Serum levels of TNF-alpha, soluble selectin-E and MMP-7 were measured by commercial enzyme-linked immunoassay. SPSS PASW 18 version was used for the statistical data analysis. Kolmogorov-Smirnov test was used to decide the normality of distribution. The non-parametric correlation and Mann-Whitney U test were used to test the correlation of parameters with the stenosis degree and to compare the difference between groups.

Results: CAD patients were found to have increased MMP-7 compared to controls ($p<0.001$), while the TNF-alpha and selectin-E were decreased (both $p<0.001$). There was no difference in any parameter relative to symptomatic status of patients. MMP-7 correlated positively to stenosis degree with $r=0.155$ ($p=0.007$), and selectin-E correlated negatively with $r=-0.248$ ($p<0.001$). Selectin-E was shown to correlate to TNF-alpha with $r=0.232$ ($p<0.001$) and MMP-7 showed no correlation to TNF-alpha.

Conclusions: TNF-alpha is possibly decreased by some of the therapeutics that CAD patients use which makes it an unreliable biomarker for risk prediction in this disease. Due to this fact, other parameters that highly depend on TNF-alpha, such as selectin-E, don't relate to the disease state directly, and further research should be more focused on biomarkers that are independent of TNF-alpha level, such as MMP-7.

Heart Failure During Hospitalization for Acute Myocardial Infarction: The Prognostic Role of Monocytes

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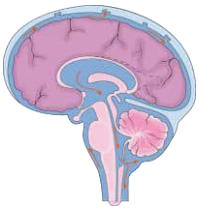
Background: Inflammation has a pivotal role in myocardial infarct healing and ventricular remodeling. After the onset of Acute Myocardial Infarction (AMI) inflammation is initiated with the infiltration of monocytes and lymphocytes to the infarct region. Acute monocyte recruitment and activation of macrophages stimulate fibroblast proliferation, angiogenesis and collagen deposition. Different studies emphasize the antagonizing effects of monocyte-mediated myocardial inflammation: regeneration and remodeling opposed to dilatation and fibrosis. It is not determined yet the role of high monocyte counts in the early progression of AMI to Heart Failure. The aim of this study is to explore the significance of the monocyte count in the development of Heart Failure during AMI hospitalization.

Methods: In this study were enrolled 230 patients diagnosed with AMI, confirmed with coronary catheterization. Venous blood was collected at admission. Complete blood count was performed in an automated blood cell-counter. Monocytes-Lymphocytes Ratio (MLR) was calculated. Patients were categorized in two groups according to the occurrence or not of Heart Failure during their hospitalization. Statistical analysis was performed using SPSS/IBM. P-value<0.05 was considered statistically significant.

Results: 73.5% of the patients were males and 26.5% were females. The mean age was 68+/-12 years. They had an average monocyte count of 4.56+/-1.39 /mm^3 despite their in-hospital outcomes. Heart Failure was diagnosed in 43% (99 patients) during the hospitalization. Patients with Heart Failure following AMI had a mean monocyte count of $4.1*10^3$, while the patients that didn't develop Heart Failure had a mean monocyte count of $4.9*10^3$. The monocyte count was significantly higher in the group of patients who didn't develop Heart Failure during hospitalization following AMI compared to those who did ($p<0.001$). MLR was also higher among patients that didn't develop Heart Failure but the difference was not statistically significant.

Conclusion: Monocytes serve as possible biomarkers of positive prognosis after AMI. In this study, patients with higher counts of monocytes were less likely to develop heart failure during the hospitalization for AMI.

CEREBROSPINAL FLUID ANALYSIS



Cell Analysis in Cerebrospinal Fluid (CSF) Using Sysmex XN 550 Hematology Analyzers

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Background: The analysis of the cell number and the cell types in the cerebrospinal fluid are important investigations in the routine workup when a neuroinflammatory disease is suspected clinically, as well as in subarachnoid hemorrhage and neoplastic meningitis. The analysis is also part of the diagnostic procedure in emergency medicine when a meningitis or encephalitis is suspected and thus, the technique has to be available 24/7. Cell differentiation by automated devices provides only a rough orientation for lymphocytes, granulocytes and monocytes.

Methods: Cell examination in cerebrospinal fluid represents one of the basic parameters of diagnosis to reveal hemorrhages and inflammations in human central nervous system. Platform Sysmex XN 550 assigned with body fluid mode (BFM) for counting white blood cells (WBCs) and red blood cells (RBCs) as well as differentiating WBCs into mononuclear cells (MNCs) and polymorphonuclear cells (PMCs) in CSF. The flow cytometry (FCM) techniques of both HAs were evaluated with the recognized gold standard techniques Fuchs–Rosenthal chamber, counting native cells, and standard FACS (fluorescence activated cell sorting)–FCM analysis of leukocyte subsets, using external CSF trials of DGKL, to check performance of both HAs for routine CSF diagnosis.

Results: During 2022 year in the Department of medical biochemistry in PHO Clinical Hospital dr. Trifun Panovski in Bitola, North Macedonia we made 10 tests for cell number and the cell types in the cerebrospinal fluid. We analyzed results and we conclude that 2 of them were positive, patients were male's age 25 and 40 years. Concentration of WBC in their samples were 9510 and 17588 cells, in both samples were dominated by polymorphonuclear cells. The remaining 8 samples were without significantly increased number of cells in cerebrospinal fluid.

Conclusions: The automated cytology and cell differentiation of CSF cells provides only a rough differentiation into granulocytes, lymphocytes and monocytes. Thus, it should only be used as a screening method for gross orientation. Other diagnostically important cell types such as tumor cells, siderophages, blasts and others are not reliably detected. Automated analysis of CSF cells may give a first orientation, but has its limitations, in particular in the low cell count range and in the differentiation of CSF cells. Therefore, conventional cytology continues to be an integral part of CSF analysis and should be carried out regularly at each diagnostic lumbar puncture, irrespective of the total number of cells.

Comprehensive Analysis of Cerebrospinal Fluid Samples Using Multiplex PCR - Biofire FilmArray Meningitis/Encephalitis Panel for 2022

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Background: Meningitis is a severe condition characterized by inflammation of the meninges, the protective membranes surrounding the brain and spinal cord. Rapid and accurate diagnosis is crucial for effective treatment and patient outcomes. In this study, we analyzed cerebrospinal fluid (CSF) samples from 77 patients using the multiplex PCR - Biofire FilmArray Meningitis/Encephalitis Panel, which simultaneously detects 14 pathogens associated with meningitis. We present the results of this comprehensive analysis for 2022 and discuss the implications.

Methods: In 2022 CSF samples from 77 patients presenting with suspected meningitis were collected and subjected to analysis. Cell count and differentiation, proteins levels, glucose levels, and lactate levels were determined using standard laboratory techniques. Additionally, samples were tested for infective pathogens using Biofire Meningitis/Encephalitis Panel. This multiplex polymerase chain reaction (PCR) assay detects and identifies nucleic acids from a panel of pathogens commonly associated with meningitis, including bacteria, viruses and yeasts.

Results/Discussion: All 77 CSF samples showed abnormalities in cell count, protein levels, glucose levels, and lactate levels. The cell count was consistently higher than the reference range in all samples, suggesting an inflammatory response. The levels of proteins, glucose, and lactate were also outside the reference range, indicating disruptions in the normal physiology of the central nervous system. These parameters can serve as valuable indicators of disease severity and progression.

Analysis of the CSF samples using the Biofire Meningitis Panel revealed positive results for 11 patients for Streptococcus pneumoniae, 3 patients for Listeria monocytogenes, and 1 patient for HSV-1. These findings indicate the presence of these pathogens in the CSF, providing valuable information for targeted treatment.

The detection of Streptococcus pneumoniae in 11 CSF samples suggests its significant involvement in meningitis cases. S. pneumoniae is a leading cause of bacterial meningitis, and timely identification can guide appropriate antibiotic therapy, as well as help in monitoring antibiotic resistance patterns.

The presence of Listeria monocytogenes in 3 CSF samples is noteworthy, as it is an uncommon but potentially serious cause of meningitis, particularly in immunocompromised individuals, the elderly, and pregnant women. Prompt recognition and treatment are crucial to mitigate the associated complications.

The identification of HSV-1 in one CSF sample underscores the importance of considering viral etiology in cases of meningitis. HSV-1 meningitis can be severe,

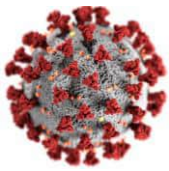
particularly in neonates and immunocompromised individuals. Antiviral therapy, initiated promptly, can improve outcomes in these cases.

Conclusions: The observed abnormalities in cell count, protein levels, glucose levels, and lactate levels emphasize the importance of comprehensive CSF analysis in evaluating meningitis cases.

Early detection and targeted treatment based on the identified pathogens can significantly improve patient outcomes.

Our study demonstrates the utility of the Biofire Meningitis Panel in diagnosing meningitis and identifying specific pathogens.

COVID-19



Changes in the Level of D-Dimers and Serum Concentration of CRP in Patients Who Had a More Severe Clinical Picture of COVID-19 - A Retrospective Study

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Objective: To evaluate changes in the level of D-dimers and serum concentration of CRP in patients who had a more severe clinical presentation of COVID-19 examined five days after the detection of Sars-Cov-2 infection.

Materials and methods: Retrospective research study was carried out in the biochemical laboratory in the hospital in Prilep, Republic of North Macedonia in the period 2020-2021. The study included 325 patients from 20 to 80 years old in the research and they were divided into three subgroups according to age (one group from 20 to 40 years old, the other from 41 to 60 and a third group from 61 to 80 years old). The level of D-dimers was determined by a fluorescent enzyme method in order to establish whether there was a blood clot. Most patients with a more severe clinical presentation had pulmonary thromboembolism, deep vein thrombosis and in younger patients, abnormal blood clotting in the brain that can lead to stroke. Serum CRP concentration was determined by the turbidimetric method. The obtained results were statistically processed with descriptive and analytical methods.

Results: In the first subgroup of 98 patients (20-40 years old), the level of D-dimers was increased by about 50% above upper normal limit in 43 patients, and a double increase above upper normal limit in 27 patients. CRP concentration was increased 3 to 10 times above upper normal limit in 8 patients.

In the second age subgroup of 140 patients (41-60 years old), the level of D-dimers was increased from about 50% above upper normal limit to two times above and five times above in 12 patients. CRP concentration was increased two to ten times above upper normal limit in 47 patients and twenty times increased in 13 patients.

In the third subgroup of 87 patients (61-80 years old), the level of D-dimers was increased in 48 patients from 50% above upper normal to four times above and twenty times above in 4 patients. The concentration of CRP was two to twenty-five times above upper normal limit in 42 patients.

Conclusion: The results showed that the highest values for the evaluated biochemical parameters were found in the age subgroup of 61-80 years, which confirmed the finding that old age is the most significant risk factor for an unfavorable course of the disease

together with the comorbidities that older patients have (e.g., diabetes, hypertension, increased body weight). The increased levels of analyzed biochemical parameters may facilitate the assessment of disease severity and may be useful in indicating the progression of COVID-19 from mild to more severe clinical presentation of COVID-19.

Key words: D-dimers, CRP, age, severe clinical presentation, COVID-19.

D-Dimer Levels as a Predictor of the Bad Progression of COVID-19 in Durres, Albania

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Introduction: Coronavirus disease was a global pandemic due to rapid human-to-human transmission. It caused mild to fatal respiratory, cardiovascular, and neurological diseases. We aimed to find out whether elevated D-dimer levels are a predictor of the bad progression of COVID-19 to help reducing the mortality.

Methods: the data of COVID-19 patients from March 21, 2020 to April 24, 2021 were retrieved from "Linda Lab" in Durres, Albania database. We used the receiver operating characteristic (ROC) curve to get the optimum cutoff value of D-dimer levels on admission and after 5 days. We used these cutoffs to divide patients into two groups and compare the in-hospital mortality between them to assess the prognosis value of D-dimer levels.

Results: In this study were included 189 patients, of whom 179 were discharged and 10 died in hospital. The optimum cutoff value to predict mortality in patients using D-dimer levels on admission was 768 ng/ml (sensitivity 90%, specificity 63.3%, Areas under the ROC curve 0,775). As for D-dimer levels on day 5, it was 1560 ng/ml (sensitivity 100%, specificity 88,6%, Areas under the ROC curve 0.946). The group with D-dimer levels on day 5 > 1560 ng/ml (26 patients) had a worst evolution and a higher incidence of mortality compared to the group with D-dimer < 1560 ng/ml (56 patients) (10/19 vs 0/69, P = 0,0002).

Conclusion: D-dimer greater than 1560 ng/ml on day 5 could help clinicians identify patients with poor prognosis at an early stage of COVID-19.

Keywords: Coronavirus disease; D-dimer; mortality; prognosis.

Comparison of Four Systems For SARS-Cov-2 Antibody at Five Time Points After SARS-Cov-2 Vaccination

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It is of great importance to evaluate the prevalence of both asymptomatic and symptomatic cases of SARS-CoV-2 infection and their differing antibody response profiles.

Methods: We compared SARS-CoV-2 antibody levels in serial samples from 7 vaccinated individuals at 20 time points with 4 assays: Elecsys Anti-SARS-CoV-2 S, BioMerieux, Vidas Sars-CoV-2 IgG II, Abbott SARS-CoV-2 IgG II Quant (CoV-2 IgG II), and Siemens SARS-CoV-2 IgG (sCOVG),

Antibody levels by time, the cumulative data and kinetics of antibody development after the combined adenovirus-based Gam-COVID-Vac vaccine were evaluated. Results: The median values at five selected time points were: 0.41; 0.19; 1.47;0.1 (WHO BAU/mL) at baseline, 75.3; 67.6; 57.08; 76.9 (WHO BAU/mL) for 21th day, 370.7 ;308.7; 260.3; 536.1 for 38th day, 175.1; 164.8; 67.0; 173.5 for 77th day, and 142.0; 59.98; 37.6; 45.9 for 170th day, respectively.

Conclusions: This study demonstrated the dynamic changes in antibody values at different time points using four test systems and is expected to provide useful baseline data for comparative studies and standardization efforts in the future.

Pediatric Vitamin D Levels from a Romanian Pediatric Tertiary Center during Pandemic Confinement

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Background: It is a truism that Vitamin D (VD) is essential during the growth period, especially for preventing rickets and with possible effects on non-calcemic functions, especially inflammation, infections or autoimmune diseases. Sunlight is the most important source of VD (>90%) through skin synthesis of Vitamin D3. Nowadays children experience a change in lifestyle, with an increasing degree of inactivity and unhealthy eating habits, more pronounced during COVID-19 confinement, which could negatively affect their development.

We aimed to evaluate the 25OH-Vitamin D (25OHVD) status in pediatric population referred to a pediatric tertiary center during the COVID-19 pandemic confinement.

Methods: A retrospective study (December 2019 – December 2020) on 25OHVD serum levels was carried out on a number of 3105 children, aged between 0-18 years, referred to our institute for different pathologies. 25OHVD levels were measured within National Program for VD Status Assessment, using an Enzyme Linked Fluorescent Assay (VIDAS, bioMérieux); 25OHVD status: deficiency <50 nmol/L, insufficiency <75 nmol/L, potential toxicity >175 nmol/L.

Results: Only 54.4% from children included in the study received VD supplementation; 20% from them revealed VD deficiency, with 25OHVD levels below 75 nmol/L and 5.3% exceeding the upper limit of the optimal range. 87.4% from children who did not receive prophylaxis therapy presented VD deficiency. We found the worst situation in children aged over 7 years (1219 children with a 25OHVD average of 65 nmol/L, and severe deficit in 43 cases (<20 nmol/L)). We noticed 25OHVD levels exceeding the safety limit of 175 nmol/L in a few cases of neonates and infants (5.3%), beneficiaries of prophylaxis, most probably because possible administration errors (too high doses/additional intake of vitamin D from milk formulas or multivitamin preparations).

Conclusions: The decrease of sun-exposure and physical activities during quarantine had a negative impact on 25OHVD levels in pediatric population, even in the conditions where they benefit from the correct prophylaxis of rickets. We highlight the severe 25OHD deficiency with increasing age, having deleterious consequences on pediatric population's general health conditions. The follow-up of infants, children and adolescents regarding the appropriate VD supplementation and an adequate sunlight exposure, physical activities and diet should be mandatory for pediatricians and general practitioners.

DIABETES, ADIPOSITY, METABOLIC SYNDROME

Melatonin, Growth Hormone and Cortisol Levels Depending on Body Mass Index in Women with Metabolic Syndrome

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Background: It is believed that hormonal factors are important for the appearance and development of the metabolic syndrome (MetS). Melatonin is the main hormone secreted by the pineal gland mainly at night. Scientific evidence shows involvement of the hypothalamic-pituitary-adrenal axis in the pathogenesis of MetS. According to International Diabetes Federation the main criteria for the diagnosis of MetS are the presence of central obesity with a waist circumference ≥94 cm for men, ≥80 cm for women and two or more of the following disorders: increased triglycerides, decreased high density lipoprotein cholesterol, increased fasting plasma glucose levels and elevated blood pressure. The study aimed to examine and compare serum melatonin, cortisol, and growth hormone (GH) levels in women with different body mass index (BMI).

Material and methods: Women with MetS were divided in two groups according to BMI: women with BMI≥26.6 kg/m^2 (n=26) and BMI<26.6 kg/m^2 (n=18). Anthropometric measurements included BMI, waist circumference, and homeostatic model assessment for insulin resistance. For melatonin and GH determinations, venous blood samples were taken at 03:00 AM and 08:00 AM, and for cortisol at 08:00 AM and 23:00 PM. Serum melatonin (Elabscience Biotechnology Inc, China) and GH levels (IBL-Hamburg, Germany) were determined using ELISA methods and concentrations were measured using Sirio S microplate reader (SEAC, Italy). Cortisol levels were analyzed with indirect chemiluminescence (Access, Beckman Coulter, USA). The statistical treatment of the data was carried out with independent sample t-test and Mann-Whitney test (P<0.05).

Results: There was no significant difference in the mean age of the women with BMI ≥ 26.6 kg/m^2 and BMI < 26.6 kg/m^2 (34.31±2.39 years vs 33.61±3.13 years, P=0.857). Patients with BMI ≥ 26.6 kg/m^2 had significantly higher waist circumference than those with BMI < 26.6 kg/m^2 (105.73±3.26 cm vs 79.94 ± 2.78 cm, P=0.0001). At 3:00 AM, the mean serum melatonin of women with BMI >26.6 kg/m^2 was 134.63±11.53 pg/ml, and of women with BMI < 26.6 kg/m^2 was 180.71±29.35 pg/ml, P=0.114. At 08:00 AM the mean serum melatonin of the women of both groups was 169.68±23.32 pg/ml and 184.62±125.70 pg/ml respectively, P=0.543). At 3:00 AM the mean serum GH of women with BMI ≥ 26.6 kg/m^2 was 2.56± 1.08 ng/ml and of women with BMI < 26.6 kg/m^2 was 4.68±1.99 ng/ml, U=164.00, P=0.213. At 08:00 AM mean serum GH of the women of both groups was 1.35±0.21 ng/ml and 1.56±0.27 ng/ml respectively, P=0.063. The mean

serum cortisol concentrations were not significantly different between the two groups at 23:00 AM (122.86±22.14 nmol/l vs 182.04 ± 44.74 nmol/l; P=0.575) and at 08:00 AM (515.72±44.74 nmol/l vs 606.19±73.62 nmol/l; P=0.274).

Conclusion: Data from the present study show that when dividing women with MetS by BMI, there is no statistically significant difference in day and night mean serum concentrations of melatonin, GH and cortisol.

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Evaluation of HOMA-IR and TG-HDL_C Ratio in Obese Pubertal Children in Bosnia and Herzegovina

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Background: Excessive body weight and obesity represent serious health concerns among the pediatric population. Childhood obesity is accompanied by impaired insulin signaling leading to impaired glucose transport, lipid metabolism disorders and diabetes later on in life. Evaluation of Insulin Resistance (IR) is a useful tool allowing early intervention. The Homeostasis Model Assessment for Insulin Resistance (HOMA_IR) index is a widely used, but not commonly available, measure of insulin resistance in adults and children. Triglyceride (TG) and HDL_C (HDL cholesterol), on the other hand, is a routine test and inexpensive compared to insulin. Conflicting findings exist on the usefulness of the triglyceride to HDL_C ratio (TG-HDL_C ratio) as predictor or marker of IR.

The aim of this study was to analyze the usefulness of triglyceride to HDL_C ratio and the HOMA-IR, as well as their association in obese pubertal children and compare it to their healthy peers.

Methods: The research was conducted at the Clinical Center of the University of Sarajevo on 70 obese children and 40 non-obese healthy peers, aged 12-18 years, with no other underlying conditions. The values of BMI, glucose, insulin, triglycerides, HDL cholesterol, HOMA-IR and TG-HDL_C ratio were determined for all study subjects according to standard laboratory procedures. Nonparametric tests were used in analysis and p=0.05 was considered statistically significant.

Results: The groups were age and gender matched. Median (IQR) BMI values in group of obese children was 31.4 (29.8-33.5) compared to 21.0 (18.6-22.8) kg/m^2 in non-obese controls. Median (IQR) values of insulin, triglycerides, HOMA-IR and TG-HDL were significantly higher in obese children compared to group of children with normal body weight, p<0.001 for all. Median (IQR) values of HDL cholesterol, were significantly lower in obese children compared to non-obese group, with p= p<0.001. Furthermore, we found significant positive correlations between BMI and: HOMA-IR (rho=0.448, p<0.001), TG-

HDL (rho=0.510, p<0.001). TG-HDL showed significant positive correlation with HOMA-IR, rho=0.398 with p<0.001.

Conclusion: HOMA-IR and TG-HDL_C ratio were higher in obese pediatric population compared to non-obese peers. TG-HDL_C shows significant positive correlation to HOMA-IR. Both of these tools are a significant early and sensitive predictor of insulin resistance in children. TG-HDL_C is a commonly available and inexpensive alternative to HOMA-IR.

An Association Between GST Polymorphisms and Diabetic Nephropathy Development

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Background: Diabetic nephropathy (DN) is one of the most significant microvascular chronic complications of type 2 diabetes mellitus (T2DM). Long-lasting hyperglycemia can induce increased reactive oxygen species (ROS) production which leads to oxidative stress. There is a body of evidence that oxidative stress plays an important role in DN development. The present study was designed to investigate the possible modifying effect of glutathione transferase polymorphisms (GSTM1, GSTT1, GSTP1 rs1138272/rs1695, GSTO1 rs4925 and GSTO2 rs156697) in the susceptibility to diabetic nephropathy. Because of the great significance of advanced glycation end products (AGEs) in oxidative stress-related diabetes complications, it was important to analyze the relationship of GST polymorphisms with the plasma AGEs in patients with or without DN.

Methods: GSTM1 and GSTT1 deletion polymorphisms were determined by multiplex PCR, whereas GSTO1, GSTO2, and GSTP1 polymorphisms were determined by the real-time PCR in 160 T2DM patients with or without DN. Plasma AGEs were measured by ELISA.

Results: A significant association between GST genotypes and susceptibility for development of diabetic nephropathy was found only for GSTO2 rs156697 polymorphisms. Individuals with the GSTO2*AG genotype were 2.6-fold more prone for symptomatic diabetic nephropathy development (OR = 2.59, 95%CI = 1.11–6.05, p = 0.028) in comparison to the carriers of the wild-type GSTO2*AA genotype. Similarly, individuals with the GSTO1*AA genotype rs4925 had higher odds of diabetic nephropathy development compared to the carriers of the wild-type GSTO1*CC genotype, however with borderline significance (OR = 3.81, 95%CI = 0.85–17.09, p = 0.081). The patients with DN who were carriers of GSTM1 null, GSTT1 null and variant GSTO1*AA genotypes had significantly increased levels of AGEs in comparison with carriers of the GSTM1 active, GSTT1 active and referent GSTO1*CC genotypes (p < 0.001,

p = 0.036, p = 0.053, respectively). The patients without DN who were carriers of the GSTM1 null and GSTT1 null genotypes had significantly increased levels of AGEs in comparison with carriers of the GSTM1 active and GSTT1 active genotypes (p < 0.001, p < 0.010, respectively). The carriers of the GSTP1*GG genotype had significantly decreased AGEs concentration compared to GSTP1*AG carriers (p = 0.025).

Conclusions: Despite the relatively small number of the participants, the present study supports the hypothesis that GST modulates the risk of diabetic nephropathy development and influences the AGEs concentration. By improving our knowledge of the diabetes and diabetic nephropathy genetics and the association between individual phenotype and genotype data, as well as the underlying mechanisms, we will be in the position to achieve individual medicine practices and develop target therapeutics.

Influence of Age on Hemoglobin Levels in Children with Type 1 Diabetes Mellitus

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Background: An association between diabetes and anemia in adults is well known. However, evidence for this association in children with Type 1 diabetes mellitus (T1DM) is lacking. A difference in hemoglobin concentration in adolescents with T1DM compared to the healthy population is noticed, which may be indicative of anemia development. It is necessary to examine whether these differences in children are evident in all age groups. The aim of this research is to determine hemoglobin (Hgb) status in children diagnosed with T1DM, and to assess hemoglobin levels in relation to the age of the children.

Methods: 150 children already diagnosed with T1DM and 122 healthy controls, aged 0 to 18, were included in the research. Children were divided into age groups: 0-6 years, 7-12 years and 13-18 years. Hemoglobin concentration was determined for all subjects, according to standard laboratory procedures. The possible presence of anemia is defined as a hemoglobin level two standard deviations below the mean value for a certain age group. The results were processed using nonparametric tests, with p<0.05 considered to be significant.

Results: A statistically significant difference was found in the blood hemoglobin concentration in children with T1DM and the healthy population. Patients with T1DM had lower hemoglobin values, a median of 140.0 g/L (IQR 132-146) vs 143.0 g/L (IQR 138-148) in healthy peers, p=0.001. No hemoglobin concentrations below the defined values for the diagnosis of anemia were observed in any group of subjects. Children with T1DM

had a significantly higher frequency of hemoglobin concentration below the reference mean value (22.0%) than their healthy peers (5.7%), p<0.001. However, when observed in age groups, the frequencies differed only in the adolescent group, i.e., p_{0-6}=0.957, p_{7-12}=0.178, p_{13-18}=0.001. In general, the occurrence of lower hemoglobin values was less common in prepubescent children, p=0.209, compared to those in puberty, p=0.001. The frequency of lower hemoglobin values in children with T1DM generally did not show gender dependence (p=0.101), except in the adolescent group (p=0.023). In that group, lower Hgb concentrations were unexpectedly more common in boys (40.9%) than in girls (17.1%), p=0.023. Note that different reference values were used for boys and girls in adolescence, although the age at which the physiological limits really start to differ is unclear.

Conclusion: Blood hemoglobin levels are significantly lower in children with T1DM than in healthy controls, although all are within reference values. The pubertal children contribute the most to this difference. Prepubescent children do not show a difference in hemoglobin status compared to their healthy peers. This indicates the possible development of anemia in children with diabetes, but probably only in puberty.

Polycystic Ovary Syndrome (PCOS) and Risk of Cardiovascular Disease

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Background: Polycystic ovary syndrome (PCOS) is a heterogeneous clinical syndrome characterized by hyperandrogenism and ovulatory dysfunction. It is associated with reproductive and metabolic abnormalities. Determination of hsCRP and lipid status as well as insulin resistance in women with PCOS enables timely and energetic prevention of the risk of cardiovascular diseases. Research objective: Evaluation of hsCRP and lipid status in women with polycystic ovaries.

Material and methods: In accordance with the set goals, the research was conducted by cross-sectional study type. The survey included 94 female respondents, aged 20 to 40. Body mass (kg), body height (cm), waist circumference (cm), hip circumference (cm) were measured for each subject, and the body mass index (BMI, kg/m^2) was calculated based on the obtained results. The laboratory examination included: measurement of the concentration of hsCRP (mg/ml), total cholesterol (mmol/L), cholesterol in HDL (mmol/L), LDL (mmol/L) and VLDL (mmol/L) lipoproteins, triacylglycerol (mmol/L), and also the concentration of LH (mIU/ml), FSH (mIU/ml), testosterone (ng/ml) and insulin (μIU/ml) in the serum. The subjects were divided into three groups: obese subjects with PCOS (N 32), normal weight subjects with PCOS (N 30) and a control group (N 30) of healthy women without PCOS.

Results: 72% of obese patients with PCOS have hsCRP >3 mg/ml (high risk for atherosclerosis), 28% hsCRP 1-3 mg/ml (medium risk for atherosclerosis). In the PCOS group of normal body weight, 20% of subjects have hsCRP >3 mg/ml (high risk), 70% have hsCRP between 1-3 mg/ml (intermediate risk) and 10% have hsCRP < 1 mg/ml (low risk). The values of hsCRP, total cholesterol, LDL cholesterol and triglycerides were the highest in the group of obese subjects with PCOS ($p<0.01$), while there was no significant difference between the group of PCOS subjects of normal body weight and the control group ($p<0.05$).

Conclusions: In women with polycystic ovaries, there is a statistically significant correlation of hsC-reactive protein and lipids with obesity and insulin resistance. Early recognition and treatment of obese women with PCOS and taking preventive measures are of key importance in reducing the risk of cardiovascular diseases.

Correlation Between Modified Neutrophil to Lymphocyte Ratio Indexes and Glycated Hemoglobin in Patients with Diabetes Mellitus Type 2

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Background and Aim: Recent studies suggested several indexes derived from the white blood cell (WBC) count as better determinants of many chronic diseases than each parameter alone. However, studies that explored the association between glucoregulation and modified neutrophil to lymphocyte ratio (NLR) indexes, such as derived neutrophil to lymphocyte ratio (dNLR) and monocyte/granulocyte to lymphocyte ratio (M/GLR) are scarce. Therefore, we aimed to investigate this potential relationship with glycated hemoglobin (HbA1c) in patients with diabetes mellitus type 2 (T2D).

Methods: A total of 301 patients with T2D were consecutively included in the study and were compared with 359 healthy controls. The indexes were calculated as follows: NLR=neutrophil to lymphocyte ratio, dNLR=neutrophils/(WBC-neutrophils), M/GLR=(WBC-lymphocytes)/lymphocytes. The results are presented as median (interquartile range). The associations were reported as Odds Ratio (OR) with 95% Confidence Intervals (CI).

Results: Patients with T2D had higher WBC, neutrophils and lymphocytes ($p<0.001$ for all). Also, T2D patients exhibited higher NLR [1.55 (1.18-2.01) vs 1.44 (1.11-1.81), $p<0.01$], dNLR [1.14 (0.91-1.44) vs 1.05 (0.84-1.29), $p<0.01$] and M/GLR than controls [1.91 (1.49-2.38) vs 1.78 (1.40-2.18), $p<0.01$]. Both, univariate [OR (95%CI) 1.799 (1.310-2.470), $p<0.001$; 1.350 (1.131-1.611), $p=0.001$, respectively] and multivariate

ordinal regression analysis revealed relationship between dNLR and M/GLR with HbA1c [OR (95%CI) 1.662 (1.189-2.326), p=0.003; 1.275 (1.057-1.540), p=0.012, respectively].

Conclusion: The independent relationship between novel indexes (such as dNLR and M/GLR) and HbA1c was found in the current study. These easy available and low-cost parameters could be reliable markers for glucose homeostasis disturbances in patients with T2D.

Serum Soluble Transferrin Receptor Levels in Relation with Obesity and Insulin Resistance in Adolescent Girls

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Background and Aim: Studies in children and adolescents show discrepant results related to biomarkers of iron homeostasis in obesity and insulin resistance (IR). Hence, we aimed to evaluate the potential relationship between serum transferrin, soluble transferrin receptor (sTfR) and ferritin levels, and IR.

Methods: A total of 60 girls were recruited from the third and fourth classes of the two secondary schools in Podgorica. Biochemical parameters were measured. Waist circumference (WC), body height and weight were provided. Body mass index (BMI) and HOMA-IR (as surrogate marker of IR) were calculated.

Results: Adolescent girls with HOMA-IR≥2.5 had higher BMI (p=0.001) and WC (p<0.001) than girls with HOMA-IR<2.5. Moreover, they had significantly higher transferrin (p=0.008), sTfR (p=0.003) and ferritin levels (p=0.047) than their counterparts. When divided into normal weight (NW) (BMI<25 kg/m^2) and overweight/obese group (OW/OB) group (BMI≥25 kg/m^2), significantly higher levels of sTfR and ferritin were observed in OW/OB group compared to NW counterparts (p=0.008 and p=0.002, respectively). Multivariate binary logistic regression analysis showed that only sTfR levels remained positively associated with higher HOMA-IR after an adjustment for WC (as a marker highly related to HOMA-IR) [OR (95% CI)=1.259 (1.006–1.577); p=0.044].

Conclusion: The association between sTfR and HOMA-IR is independent of obesity (as determined by WC).

Correlation Between Body Mass Index and Red Cell Distribution Width in Adolescents

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Aim: Studies that examined the relationship between red blood cell (RBC) indices in relation with obesity in adolescents are scarce and show contradictory results. The aim of the current study was to examine this potential relationship in late adolescents ages between 16-19 years.

Patients and Methods: A total of 156 participants voluntarily participated in the study. RBC indices were measured [i.e., RBC, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW)] on the automatic hematology analyzer, whereas their indexes were calculated. Adolescents were divided into subgroups according to their body mass index (BMI), i.e., normal weight (BMI <25 kg/m^2) and overweight/obese (BMI ≥25 kg/m^2).

Results: Overweight/obese adolescents had lower RDW (13.90±1.32% vs 14.76±2.27%, p=0.008) and lower RDW/MCV (0.16±0.02 vs 0.17±0.04, p=0.018) as compared to normal weight counterparts. There was no difference in other RBC indices. Spearman's correlation analysis showed negative association between BMI and MCHC (rho= -0.207, p=0.009) and RDW (rho=-0.254, p=0.001), respectively. However, after adjustment for confounding variables, RDW was the only independent predictor of BMI (p=0.038).

Conclusion: Lower RDW values were independently correlated with BMI. RDW as available and cost-effective parameter could be reliable diagnostic biomarker in overweight/obese adolescents.

Triglyceride to High Density Lipoprotein Cholesterol Ratio as Surrogate Marker for Insulin Resistance

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Background: Insulin resistance (IR) is closely associated with metabolic profiles, including obesity and dyslipidemia. The triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C) has been advocated as a simple clinical indicator of insulin resistance. However, results differ between populations, and presence of comorbidities.

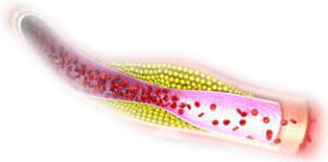
The aim of the present investigation was to evaluate TG/HDL-C ratio as an insulin resistance (IR) marker in 20-40 years old population suspected of insulin resistance.

Methods: The current research was conducted in a cross-sectional manner. Total of 116 serum samples from 51 males and 65 females, aged 20-40 years were analyzed for fasting glucose, lipids and insulin levels. Insulin resistance was defined by the homeostasis model assessment for insulin resistance (HOMA-IR) of at least 2.00.

Results: The average age of subjects was 29.5±7.6 years. In the IR group (63) the glucose level were higher, with borderline significance (5.54±0.45 vs 5.25±0.35, $p=0.05$), while insulin levels were more different (15.08±8.49 vs 6.16±1.52, $p<0.001$). TG/HDL-C ratio was significantly lower in non-IR group (1.18±0.66 vs 3.41±3.63, $p<0.05$), while triglyceride values were higher in IR group ($p<0.05$). There was no significant difference between total cholesterol, HDL-C and LDL-C values between the two groups. Values of TG/HDL-C ratio ≥2.0 had sensitivity of 70.4%, and specificity of 86.7%. All the subjects with TG/HDL-C ratio ≥3.0 had HOMA -IR ≥2.5.

Conclusion: TG/HDL-C ratio is associated with IR. It can be used as a sensitive surrogate marker for evaluation of impaired insulin sensitivity in 20-40 years old population, with the cut of value of ≥2.0.

DYSLIPIDEMIA AND ATHEROSCLEROSIS



Inflammatory Markers in Atherosclerosis-Related Diseases Patients and Healthy Subjects

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Background and aims: Atherosclerosis and its clinical manifestations still represent the leading cause of morbidity and mortality all over the world. Although atherosclerosis has long been considered a degenerative disease mainly determined by a passive accumulation of lipids, recent studies have shown that it is an inflammatory disease characterized by chronic low-grade inflammation, endothelial dysfunction and immune cell activation. This study aimed to evaluate the differences in concentrations of two inflammatory biomarkers: high-sensitive C-reactive protein (hsCRP) and pentraxin 3 (PTX3) between atherosclerosis-related diseases patients and healthy subjects.

Materials and Methods: The study included 21 patients with stable angina pectoris (SAP) aged 60 ±10, 69 patients with ST-segment elevation acute myocardial infarction (STEMI) aged 61±12 and 67 healthy individuals aged 56±10 respectively. hsCRP was determined using a latex-enhanced immunoturbidimetric method (Tina-quant CRP Roche, Indianapolis, USA) and PTX3 was measured by enzyme-linked immunosorbent assay (ELISA) (Human Pentraxin3 DuoSet ELISA R&D Systems, Minneapolis, USA).

Results: The inflammatory markers concentrations in the healthy control group were as follows: hsCRP 0.7 (0.3-1.9) mg/L; and PTX3 1.0 (0.7-1.2) ng/mL. SAP and STEMI patients had significantly higher values of both inflammatory markers: SAP- hsCRP 2.7 (1.9-4.9; P<0.001); and PTX3 1.8 (1.6-2.2; P<0.001); STEMI- hsCRP 3.7 (2.1-7.2; P<0.001); and PTX3 2.4 (1.5-5.4; P<0.001) compared to healthy controls.

Conclusions: The finding of elevated hsCRP and PTX3 values in both patients´ groups suggest that inflammatory markers are involved in the pathogenesis and development of atherosclerosis, but additional investigations on a large sample size are needed to more comprehensively assess their role.

Examination of Cholesterol Levels in Preschool Children in Dubrovnik

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Background: The national program for detection of familial hypercholesterolemia (FH) in Croatia was introduced in 2023. The program includes the measurement of cholesterol for all children during a systematic examination upon enrollment in the 1st grade of elementary school. The aim of this work was to present the results of cholesterol in preschool children tested in our laboratory.

Methods: The data were collected in the Medical Biochemistry Laboratory of Glavic Health Care Institution, Dubrovnik, Croatia in the period from January to May 2023. Venous serum was used as a sample for cholesterol determination. Cholesterol values were determined by the photometric method with cholesterol oxidase on the AU480 analyzer (Beckman Coulter, Brea, CA) according to the manufacturer's specifications and using proprietary reagent.

Results: The number of preschool children whose cholesterol value was determined was 324 (151 girls and 173 boys). In Croatia, the following recommended cholesterol values are used: for children <4.7 mmol/L, for adults <5 mmol/L. According to the Simon Broome Diagnostic Criteria for FH for children up to 16 years of age, the value of total cholesterol is >6.7 mmol/L. Cholesterol value over 4.7 mmol/L was observed in 79 children (24.4%), while cholesterol value over 6.7 mmol/L was observed in 2 children (0.62%).

Conclusions: Early detection of FH is important because this disease can be treated and thereby prevent the development of atherosclerosis and consequent morbidity and mortality from cardiovascular diseases. Cholesterol, as a simple routine laboratory test, can indicate an increased cardiovascular risk in preschool children who are referred for further diagnostics.

ENDOCRINOLOGY

Screening Test for Hashimoto's Thyroiditis and Hypothyroidism

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Background: Thyroid disorders are common and pose challenges for medical professionals. Early detection and diagnosis are crucial for effective treatment and prevention of hypothyroidism. This study explores the role of ultrasound screening and blood test in identifying Hashimoto's disease and its subsequent hypothyroidism. The aim is to determine the most cost-effective diagnostic approach for these cases, considering the range of available options including clinical evaluation, imaging, and laboratory tests.

Methods: Based on the WHO criteria, the study analyzed different methods for spontaneous or continuous screening of Hashimoto's thyroiditis. Ultrasound examination was selected as the primary screening method due to its acceptable cost, accuracy, and ability to detect hypoechoic areas such as pseudonodes and stretch marks. The clinical stages of Hashimoto's thyroiditis were considered, including lymphocytic infiltration, Hashitoxicosis, a "normal" stage, and subclinical and clinical hypothyroidism.

Results: Ultrasound screening was found to be highly effective in identifying Hashimoto's thyroiditis and its early stages. By detecting pseudonodes, ultrasound not only identified cases with autoimmune activity or hypothyroidism but also those that had returned to normal function or were in a "cured" state. The application of ultrasound combined with TSH determination and measuring antibodies TPO increase the number of cases of Hashimoto's thyroiditis detected. Ultrasound is safe, cost-effective, and capable of capturing a broad spectrum of Hashimoto's thyroiditis stages.

Conclusion: The results of this study support the use of ultrasound as a primary screening method for Hashimoto's thyroiditis. Ultrasound examination is non-invasive and poses no physical or psychological risks. It allows for early detection of the disease, leading to timely intervention and improved quality of life for affected individuals. Additionally, ultrasound is more affordable compared to other diagnostic tests. Integrating a comprehensive anamnesis with ultrasound screening can enhance cost-effectiveness and should be considered for future diagnostic protocols. Regular follow-up is recommended for cases with pseudonodes to monitor for changes in thyroid function and autoimmune activity.

Keywords: thyroiditis, echo, laboratory.

Hypothyroidism and Its Influence on Lipid and Glycemic Status

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Background: Hypothyroidism is a condition which results in decreased excretion of the thyroid gland hormones. Thyroid hormones control cell metabolism. They play an important role in regulating body temperature, heart beating, and the way the body burns calories. Hypothyroidism leads to slower metabolism and low energy levels.

The aim of this study is to estimate the correlation between increased TSH levels and changed levels of cholesterol, triglycerides and blood glucose, in subjects suffering from hypothyroidism.

Methods: The analysis involved 44 women with increased TSH levels (> 4.0 u/IU/ml), aged between 25 and 55 years (39 ± 3). The control group consisted of 44 healthy aged-matched women (40 ± 2), with normal TSH levels (0.4 – 4 uIU/ml).

TSH was measured with CLIA. Cholesterol and triglycerides were measured with CHOD-PAP and GPO-PAP enzymatic colorimetric method respectively. Glucose was measured with HK (hexokinase) enzymatic colorimetric method. All parameters were measured on automated analyzers (Immulite 2000 Immunoassay System, USA and Dimension EXL 200 Integrated Chemistry System, USA).

T-test and Pearson correlation test were used to analyze the data.

Results: The mean TSH for the experimental group was 5.64 ± 2.04 and the mean TSH for the control group was 1.98 ±0.89 u/IU/ml There was a positive correlation between high TSH and cholesterol levels in the blood ($r = 0.127$; $p = 0.412$), and a negative correlation between high TSH and levels of glucose ($r= - 0.053$; $p=0.734$) and triglycerides ($r= - 0.155$; $p=0.314$) in the blood. In the control group, normal TSH levels correlated positively with all three parameters (cholesterol: $r=0.003$; $p=0.986$; glucose: $r=0.121$; $p=0.436$ and triglycerides: $r=0.146$; $p=0.344$). No statistically significant difference was measured in cholesterol and triglycerides between the experimental and control group ($p=0.892$ and $p=0.056$ respectively) but a significant difference was measured in the glucose levels between the two groups ($p < 0.001$). The mean glucose was higher in the experimental group (6.348 mmol/L) compared to the control group (5.416 mmol/L).

Conclusion: The results indicated that patients with elevated TSH levels (hypothyroidism) should be considered as patients with higher levels of blood glucose, and an increased risk of developing Diabetes mellitus. This should be taken in consideration when observing patients results (patients with elevated levels of TSH).

Macroprolactinemia: Ten Years' Experience

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Background: Macroprolactinemia, as one of the common causes of hyperprolactinemia, has an important implication in managing patients with hyperprolactinemia.

The aim of this study was to determine the prevalence of macroprolactinemia among patients with hyperprolactinemia.

Methods: This retrospective, cross sectional study was conducted in Center for Laboratory Medicine, Clinical Center of Vojvodina. Data of 1399 patients, 1309 women and 90 men, collected from January 2013 until December 2022, detected to have serum prolactin > 30 ng/mL as per the upper reference limit were further screened for macroprolactin by polyethylene-glycol (PEG)-precipitation test. The macroprolactinemia was calculated as the percentage of PRL recovery. A recovery value less than 40% was taken as a cut off value to confirm significant macroprolactinemia. Prolactin was measured by chemiluminescent method (CMIA) on automated immunoanalyzer Architect i2000SR and Alinity i, Abbott, USA.

Results: In 18.0% (252/1399) patients with hyperprolactinemia, macroprolactinemia was detected. There is a statistically significantly more women diagnosed with hyperprolactinemia than men (1309 vs 90; $p<0.001$). Macroprolactinemia was significantly more frequent in women (239 vs 13; $p<0.001$).

Conclusions: Regarding the high prevalence of macroprolactinemia in patients with hyperprolactinemia, screening for macroprolactin in patients who have been detected to have hyperprolactinemia is highly recommended.

Comparison of Polyethylene Glycol Prolactin Precipitation Methods for Macroprolactin Detection

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Background: Macroprolactin (macroPRL) is a large protein complex that consists of immunoglobulin conjugated monomeric prolactin (PRL). It can be detected in patients with asymptomatic hyperprolactinemia, which suggests that anti-prolactin antibodies, binding PRL molecules, nullify the activity of hormone. The most widely used method for detecting macroPRL is precipitation of hyperprolactinemic sera with polyethylene glycol

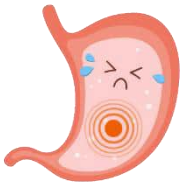
(PEG). Since there is no standard protocol for macroPRL detection and reporting of results, the aim of this study was to select an in-house algorithm for this.

Methods: The data for this method comparison study were collected from the Center for Clinical Laboratory Diagnostic of Clinical Center of Montenegro (CCM) for the period from February 2023 to May 2023, and included 102 patients with PRL serum levels higher than 1000 mIU/L. PRL concentrations were measured after each of three protocols for PEG precipitation, which differed in duration and speed of centrifugation. The first protocol implied hyperprolactinemic samples to be centrifuged at 10800 rpm for 10 minutes (Method 1), the second at 10800 for 4 minutes (Method 2) and the third at 4000 rpm for 30 minutes (Method 3). Macroprolactinemia was defined by unprecipitated PRL (mIU/L) and precipitated PRL (%) calculated from PRL in the post-PEG supernatant, with cutoff value of 50%. The Alinity i system (Abbott Laboratories, IL, USA), that utilizes chemiluminescent microparticle immunoassay (CMIA) principle, was used to measure PRL concentrations. Statistically, the data were analyzed using Passing-Bablok regression method.

Results: When comparing Methods 1 and 2, the intercept coefficient was 0,5316 with 95% confidence interval (-0,8869 to 1,6598), while the slope coefficient was 0,9832 (0,9609 to 1,0071); and for Methods 1 and 3 the intercept coefficient was 0,8978 with 95% confidence interval (-1,2381 to 2,1893), while the slope coefficient was 0,9788 (0,9522 to 1,0152). Since 0 was included in the confidence intervals for the intercept coefficients and 1 was included in the confidence intervals for the slope coefficients, the existence of a statistically significant difference between the tested methods was not established, at a cutoff value of 50% for the diagnosis of macroprolactinemia.

Conclusions: All three methods proved to be adequate and satisfied the needs for the assessment of macroprolactinemia. Considering the lack of significant differences between the tested methods, centrifugation at 4000 rpm for 30 minutes is not preferable due to the duration of sample processing. On the other hand, in some laboratories, the absence of centrifuges that reach a speed of 10800 rpm may determine this method of precipitation of hyperprolactinemic sera with PEG.

GASTROINTESTINAL DISEASES



Serum Lipase a More Sensitive Biomarker Than Serum Amylase in The Diagnosis of Acute Pancreatitis

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Background: Acute pancreatitis is a rapid onset of inflammation of the pancreas causing mild to severe life-threatening conditions.

The diagnosis of acute pancreatitis requires the presence of at least two of the three diagnostic criteria – characteristic abdominal pain, elevated serum amylase or lipase, and radiological evidence of pancreatitis.

This study aims to show that serum lipase is a more sensitive biomarker than serum amylase in diagnosing acute pancreatitis and differentiating it from nonpancreatic abdominal pain.

Methods: This observational study enrolled 160 patients of GeniusLab Clinic from March 2022 to December 2022. Of these 160 patients 80 patients were diagnosed with acute pancreatitis and 80 patients had acute abdominal pain with extrapancreatic etiology. All patients' diagnoses of acute pancreatitis were confirmed with radiological evidence of peripancreatic inflammation. The measurement of lipase and amylase in serum was performed with the absorbance photometry method using Cobas Integra 400 plus.

Normal amylase and lipase serum values were respectively 30 - 100 U/I and 0 -60 U/L.

Result: Thirty-two (40%) of 80 patients with extrapancreatic abdominal pain demonstrated an elevated admission serum amylase level with an average value of 125 units (U)/L (normal range 30-100 U/L) and a maximum value of 305 U/L. Only twelve (15%) of these 80 patients had an elevated admission serum lipase value with an average value of 98 U/L and a maximum value of 119 U/L.

Meanwhile, forty-eight (60%) of 80 patients with acute pancreatitis had an elevated amylase average value of 252 U/L and a maximum amylase value of 489 U/L. Seventy-six (95%) of 80 patients with acute pancreatitis had an elevated admission serum lipase value with an average value of 502 U/L and a maximum lipase value of 1217 U/L.

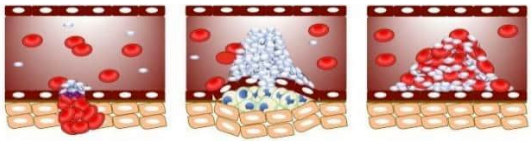
It is seen that average and maximum lipase values are significantly higher than amylase values in acute pancreatitis and even higher than lipase and amylase values from acute abdominal pain with extrapancreatic causes.

Serum amylase and lipase levels may be elevated in nonpancreatic disease processes of the abdomen, but significant elevations (greater than three times the upper limit of

normal) of lipase are uncommon in these disorders and are significant in acute pancreatitis.

Conclusion: Serum lipase offers a higher sensitivity than serum amylase in diagnosing acute pancreatitis. Lipase also offers a larger diagnostic window than amylase since it is elevated for a longer time, thus allowing it to be a useful diagnostic biomarker in the early and late stages of acute pancreatitis.

HEMOSTASIS AND THROMBOSIS



Sedentary Work Increases D-Dimer Levels in Women Regardless of Age

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Sedentary work is associated with negative health consequences independent of physical activity levels. We hypothesize that increased bouts of uninterrupted sitting are associated with a rise in D-dimer levels, especially in women. Our goal was to investigate the difference in D-dimer levels in men and women that have sedentary jobs and spend their day sitting for prolonged periods of time.

We used the Radiometer AQT90 FLEX D-dimer assay to measure the D-dimer values in healthy participants with sedentary jobs. In the assay process, the sample and the assay solution are automatically added to the cup containing the assay-specific reagents. During the incubation period, the tracer and capture antibodies form sandwich complexes with D-dimer present in the sample. After the incubation, the assay cup is washed with the assay solution and dried. Then the signal from the tracer antibody is then measured by using time-resolved fluorometry (TRF). The measured signal is converted to a concentration using the calibration curve stored in the memory of the instrument. The results are expressed in μg/L.

The average D-dimer levels in women were 298.3 μg/L ±18.3 and median 265 μg/L. Men had lower average D-dimer levels (233.6 μg/L ±15.7 and a median 232 μg/L). There was a significant difference between the means based on gender (*p=0.019*). There was a positive correlation between age and D-dimer levels (r=0.22, 95% CI: -0.09-0.49, *p=0.160).* Women had a statistically non-significant positive correlation between age and D-dimer levels (r=0.03, 95% CI: -0.36-0.40, *p=0.90*). Men had a statistically significant positive correlation between age and D-dimer levels (r=0.51, 95% CI: -0.02-0.80, *p=0.04*).

We can observe that women had a higher mean and significantly higher D-dimer levels compared to men in the same profession. Men had a significant increase in D-dimer levels with age, meaning that younger men had much lower D-dimer levels compared to older men. There was no statistically significant increase in D-dimer levels with age in women, meaning that younger women had similar D-dimer levels compared to older women. We conclude that sedentary work results in an increase in physiological D-dimer levels in all women regardless of their age.

PROS 1 Mutation and Protein S Deficiency as a Risk Factor for Poor Pregnancy Outcomes

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Introduction: Congenital protein S deficiency is an autosomal dominant disease, and the heterozygous state occurs in approximately 2% of unselected patients with venous thromboembolism. Protein S deficiency is a rare inherited thrombophilia often associated with fetal losses in pregnancy. Homozygous Protein S deficiency in neonates manifests as a catastrophic and fatal thrombotic complication termed Purpura Fulminans.

Case report: We present you a 34-year-old patient with a history of four pregnancies since 2012. y. She had one extrauterine pregnancy in 2012. y. Then in 2013. y. she had one spontaneous abortion in the first trimester, after in vitro fertilization procedure. Her third pregnancy ended in December 2014. y. with a natural delivery, in adequate terms, the estimated Apgar score of the neonate was 9. But, 8 days after delivery because of neurological symptomatology of the neonate magnetic resonance imaging pointed to thrombosis of cerebral vein sinuses, with infarct zones and elements of hemorrhage. Thrombophilia screening pointed low level of protein S (<10%) in the neonate, and also decreased level of protein S in the mother (49%) and father (52%). Despite dramatic clinical presentation, neonates survive but with serious consequences if form quadriparesis and episodes of epileptic seizures. The couple was counseled about the autosomal dominant nature of Protein S deficiency. The patient was advised early antenatal registration with thromboprophylaxis in the next pregnancy, also in all thrombosis-provocative situations. Our patient during her last pregnancy in 2017. year start LMWH prophylaxis but the proposed procedure of genetic examination she started in the last trimester of pregnancy. Taking into consideration the period of pregnancy, antenatal screening of the fetus had not been performed. Genetic examination pointed out that now 5 years old daughter is a homozygous carrier of PROS 1 mutation, also gravida, and her husband is heterozygous for PROS 1 mutation. She delivered a male baby in November 2017. y. with Cesarean section. Course of delivery and puerperium were without thrombotic complications for patient and her neonate. In addition, genetic examination confirmed that neonate is heterozygous of PROS 1 mutation.

Conclusion: Pregnant women with Protein S deficiency are typically heterozygous. Partners of women with these defects should be offered screening to identify neonates who may be homozygous or carry combined defects, in whom prenatal diagnosis can be considered. Women with genetic of Protein S thrombophilia are at very high risk of antenatal and postpartum venous thromboembolism and should receive thromboprophylaxis during pregnancy and puerperium.

Successfully Resolved Case of Acquired Hemophilia A Patient

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Introduction: Acquired hemophilia A (AHA) is a rare autoimmune disorder with an annual incidence of 1.5 cases per million. AHA is characterized by the presence of autoantibodies against factor VIII. The antibodies interfere with the coagulant effect of FVIII. It's a potentially life-threatening condition resulting in spontaneous bleeding, most commonly into soft tissues. The diagnosis should be considered with unexplained bleeding, especially in elderly patients. Treatment includes bleeding control and inhibitor eradication therapy, which should be initiated immediately. Even if bleeding is controlled, the patient remains at risk for life-threatening bleeding until the inhibitor has been eradicated.

Case: We describe the case of a 67-year-old female who presented with spontaneous soft tissue bleeding into the left leg, without a history of trauma. She had comorbidities including type 2 diabetes mellitus, hypertension, and ischemic heart disease, and had undergone a coronary arteries stent implantation. Two months before admission she had a Staphylococcus aureus infection of the right coxae endoprosthesis, implanted 3 years before. Necrotomy, incision, and drainage of the inflammation region had been performed. She had prolonged and recurrent episodes of bleeding from the surgery scratch and persistent infection, treated with an antibiotic (Fusidic acid). At that time, she was not under hematologist investigation. At her admission to the Hematology department, she had increased aPTT (63.0), also the level of factor VIII decreased (0.8 iu/dl). The level of factor VIII antibodies was 4.7 BU/mL. Diagnosis of severe Hemophilia A has been pointed out. She was treated with a high dose of factor VIII and corticosteroids (1 mg per kg body weight). After 7 days of treatment laboratory monitoring pointed normalization of factor VIII, also eradication of factor VIII autoantibodies. In further follow up we did a stepwise reduction of corticosteroid therapy. Laboratory parameters were stable, also the state of patients without new bleeding episodes. The initial bleeding presentation had been resolved, area of orthopedic surgery was without bleeding, with a sterile smear microbiology test. Fusidic acid had been stopped at admission, other screening investigations and clinical examinations did not reveal an underlying malignancy or primary autoimmune disease.

Conclusion: The diagnosis of AHA must be considered in patients with bleeding and isolated prolonged aPTT. Other clinical clues include bleeding in patients with no personal or familial history of bleeding disorders. Diagnosis is confirmed when the aPTT does not correct with plasma mixing studies, a lupus anticoagulant is excluded, and factor VIII inhibitor autoantibodies are identified in the plasma AHA is an under-recognized but

reversible acquired coagulopathy that requires early diagnosis and prompt initiation of treatment to prevent an adverse outcome.

Keywords: hemophilia A, acquired, diagnosis, treatment

Acquired Hemophilia A And Systemic Connective Tissue Disease: A Case Report

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Introduction: Acquired hemophilia A is a rare, life-threatening condition that manifests itself in spontaneous bleeding, mostly in soft tissues. The diagnosis of this disease should be considered in unexplained bleeding, especially in elderly patients.

Case presentation: An 83-year-old female patient was initially hospitalized at the vascular surgery of the Clinical Center of Montenegro due to deep venous thrombosis (DVT) of the right leg. After being discharged from vascular surgery, she noticed a bruise on the skin of her abdomen, for which she was hospitalized again. Surgical drainage of the hematoma in the anterior abdominal wall and tamponade, then new tamponade and revision were performed. During the second hospitalization, the laboratory findings showed anemia with prolonged aPTT values (Er 2.74, Hg 76, MCV 86, D dimer 3.73, aPTT 113.7, PV normal). A reduced level of factor F VIII (< 0.4 I.U./dl) is found. In a 50:50 mixing study with normal plasma, aPTT and factor VIII did not normalize. The inhibitor level was high (224 BU/mL). The patient was treated with corticosteroids, and intravenous immunoglobulins, and therapeutic plasma exchange with factor VII recombinants was performed. There was a gradual stabilization of the laboratory findings as well as the resolution of the hematoma, there had been no repeated manifestations of cowering. Screening tests were carried out in terms of malignancy and autoimmune diseases. The findings of immunoserology indicated a possible systemic connective tissue disease, which was also confirmed by a rheumatologist. She was treated with pulse doses of corticosteroids. The aPTT values remained stable. Due to previously verified DVT, treatment with rivaroxaban was started. Conclusion. Acquired Hemophilia A can be a reversible coagulopathy. Early diagnosis and early initiation of treatment can lead to the successful resolution of the disease. An adequate approach includes screening for the etiology of acquired hemophilia.

Keywords: acquired hemophilia, inhibitor, etiology, diagnosis, treatment.

INTERFERENCES IN LABORATORY TESTING

Clinical Important Interference of Heparin-Tubes on 25-OH Vitamin D Immunoassay on Maglum 4000 Plus

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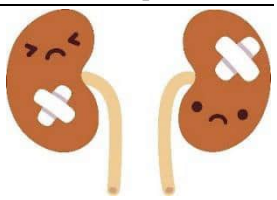
Background - aim: We report an interference in an immunoassay for 25-OH-Vit D, which could have led to a wrongful diagnosis and unnecessary treatment. An abnormal elevated 25-OH-Vitamin D was measured by our immunoassay (Maglum 4000 Plus). To confirm this suspicious diagnosis, we performed the test with another analyser (Cobas e411, Roche Diagnosis). The patient's result came back normal with Roche Eclia method. Surprisingly when measuring the patients' serum on Maglum 4000 Plus, normal 25-OH-Vitamin D levels were found. Interference was suspected when using heparin tubes to measure 25-OH-Vit D on Maglum 4000 Plus. The purpose of this work is to evaluate the impact of heparin tubes on the accuracy of Vitamin D measurement using Maglum 4000 Plus.

Methods: Plasma lithium-heparin tubes and serum tubes were collected from a healthy volunteer. Total 25-OH-Vitamin D levels were measured on Maglum 400O Plus analyzer and Cobas e411 analyzer.

Results: The Vitamin D levels were significantly higher in the sample collected in the heparin tube compared to the serum tubes on Maglum 4000 Plus analyzer, but gave comparable result on Cobas e411.25-OH-Vitamin D measured on Maglum 4000 Plus resulted in 111.9 ng/ml and on Cobas e411 16.5 ng/ml.

Conclusions: Our study showed that heparin tubes can interfere with the accuracy of Vitamin D measurements on Maglum analyser, resulting in falsely elevated levels. Further research is needed to understand the mechanism of this interference.

KIDNEY DISEASES AND TRANSPLANTATION



The Role of Soluble Transferrin Receptor in the Detection of Iron Deficiency Anemia in Dialysis Patients

Ana Cuk[1], Ivanka Mikulic[1], Vinka Mikulic[1], Kristina Ljubic[1], Ivona Cvetkovic[1], Ante Pusic[1], Andrea Prce[1], Dajana Prlic[1]

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Background: Anemia is a well-known problem in patients with chronic kidney disease (CKD), especially in patients with end-stage renal disease who require regular hemodialysis. During the course of the disease in CKD, anemia causes progression in 50-55% of patients, and indicates iron deficiency. Several parameters can be used to detect anemia, and the best diagnostic ones include iron (Fe), hemoglobin, ferritin, transferrin and soluble transferrin receptors (sTfR). Since ferritin is an acute phase reactant, its values are difficult to interpret in these patients, while soluble transferrin receptors do not respond to inflammation and are considered a new promising biomarker for the diagnosis of iron deficiency anemia (IDA). The research was conducted in the Department of Laboratory Diagnostics (DLD) of the University Clinical Hospital Mostar (UCH Mostar) in Bosnia and Herzegovina.

Methods: The subjects included in the study were patients who were treated at UCH Mostar, with signed informed consent. 69 respondents participated in the research. The concentration of Fe in the serum samples was determined by the photometric method on the automatic analyzer Beckman Coulter DxC 700 AU, while the concentration of transferrin was determined by the immunoturbidimetric method on the automatic analyzer Alinity ci. The concentration of sTfR in the serum sample was determined nephelometrically on a BN II Siemens automatic analyzer, and the ferritin concentration was determined from the serum sample by the chemiluminescence method on the Vitros 3600 analyzer.

Results: Out of 69 patients enrolled in this study, 44 (63.7%) were male, and 25 (36.2%) were female. The results of the measurements are presented with appropriate measures of centrality and dispersion, depending on the sample size and distribution. The median serum concentration of Fe was 8,10 μmol/L (6,47-9,89 μmol/L) and mean of hemoglobin was 106 ± 18,6 g/L. The median serum concentration of ferritin was 99,6 ng/mL (44,9-264 ng/mL). The mean serum concentration of sTfR and transferrin was 1,57 ± 0,51 mg/L and 174,48 ± 46,18 mg/dL.

Conclusions: It is well known that ferritin is not a good indicator of Fe in inflammation due to the confounding effect of the immune response on inflammation. Ferritin concentrations begin to rise early after inflammation and reach their maximum within a week. High concentrations of ferritin are also visible in our research. It is the inflammation that is common in patients with CKD that can underestimate and mislead

the conclusion about the iron status. This study showed that sTfR is a much more valuable diagnostic parameter of iron status in dialysis patients.

Evaluation of the Early Markers of Overweight and Obesity Related Renal Injury in Children and Adolescents

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Introduction: Recent epidemiological data suggest that overweight and obesity are associated with increased risk of renal injury in children and adolescents.

Aim: To evaluate of several markers for early detection of renal injury in overweight and obese children and adolescents.

Material and methods: In total 64 overweight and obese children and adolescents with BMI (body mass index) higher than 25 kg/m^2 and 50 healthy children and adolescents with normal body weight, 5-19 years old, matched for gender and age, were included in the study. In the second morning urine levels of KIM-1 (kidney injury molecule-1) were determined with ELISA method and the concentration of microalbumin was measured with chemiluminescent method. Serum concentrations of creatinine, urea, uric acid, fasting glucose and insulin, and parameters of lipid profile, and urine creatinine level were determined by using standard biochemical methods. Insulin resistance was estimated using homeostasis model (HOMA-IR). Electrophoretic separation of urinary protein was performed by applying of horizontal 4-22% gradient SDS-PAGE (sodium dodecyl sulfate gel electrophoresis). Total urinary proteins were measured with turbidimetric method of Meulemans.

Results: No significant differences were found between two groups of subjects for serum creatinine, glucose, urea, uric acid and for lipid profile parameters ($p > 0.05$). Urine KIM-1/Cr was found significantly higher in the group of overweight and obese children and adolescents. A modest significant correlation was detected in this group between urinary microalbumin/Cr ratio and KIM-1/Cr ($r=0.53$, $p<0.05$). Insulin resistance was detected in 53% of overweight and obese children and adolescents. No significant difference was detected between subjects with or without insulin resistance for KIM-1 and microalbumin. Analysis of electrophoretic profiles has shown the presence of selective glomerular proteinuria in two subjects from the group of overweight and obese children, one of them presented insulin resistance.

Conclusion: The results have shown that urinary KIM-1, microalbumin and SDS-PAGE profiles are promising early markers for the detection of renal injury in overweight and obese children and adolescents, that could be used in its prevention and treatment.

Status of Kynurenine in Chronic Kidney Disease

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Background: Essential amino acid Tryptophan is going through several metabolic pathways in its breakdown, resulting in the production of many biologically active components. The most common metabolic pathway is the kynurenine pathway with main metabolite kynurenine (KYN). KYN in higher concentration may cause toxic effects on the body cells related to oxidative stress, mitochondrial dysfunction, apoptosis and inflammatory process. Since chronic kidney disease (CKD) causes alterations in metabolism of several aminoacids, aim of this study was to investigate status of KYN in these patients and its relationship with parameters for assessment of kidney function.

Methods: The study included 60 patients with CKD divided into two groups: I group of 25 patients (M=11, F=14) with e GFR >60 ml/min, II group of 35 patients (M=20, F=15) with e GFR < 60ml/min. To all participants routine parameters for the assessment of kidney function were determined (urea, creatinine). Glomerular filtration rate (GFR) was measured by radionuclide clearance DiethyleneTriamine Pentaacetic Acid (DTPA) and estimated GFR by selected blood parameters related to CKD diagnosis (serum creatinine, Cys C). Serum and urine concentration of KYN were determined by using High-Performance Liquid Chromatography (HPLC).

Results: Concentration of urea and creatinine in II group were significantly higher compared with group I (urea 5.2±1.3 vs 9.5±3.8, $p<0.001$; creatinine 80.5±18.3 vs 128.0±57.5, $p<0.001$). KYN concentration in the I group was significantly lower compared to II group (3.08±0.22 vs 3.20±0.23, $p=0.014$). Significant correlation was obtained between KYN and Cys C ($r=0.3$, $p<0.05$), DTPA ($r= -0.4$, $p<0.001$) and EFBP ($r= 0.4$, $p<0.001$).

Conclusion: According to our results, the concentration of KYN increases due to CKD progression. Significant correlations with parameters of GFR make it a potential marker of evaluation of glomerular filtration rate.

Evaluation of Laboratory Parameters in Hemodialysis Patients in Vlora

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Background: Chronic kidney disease (CKD) is one of the main public health problems of the 21st century. With the increase in life expectancy and the aging of the population, chronic kidney disease has become one of the most common non transmitted diseases as

well as one of the main causes of mortality, even in Albania. Approximately more than 10% of the population is affected worldwide. Due to the high costs in the health system, especially in the advanced stages, which include kidney damage treated with dialysis as well as kidney transplantation, CKD constitutes a priority in the health system. We evaluated the impact of general and specific biomarkers on the progress of dialysis patients in Vlora and made an assessment of renal function based on the monitoring of classic parameters.

Methods: This is a retrospective study of patients with CKD on Hemodialysis. The patients were divided into groups according to age, gender and duration of dialysis. Statistical analysis was performed using SPSS. Descriptive group frequency tests were used for nominal data. Differences were considered statistically significant for values of $P<0.05$. Categorical parameters were analyzed through the Chi-square test. Between the groups taken in the study and the laboratory tests, correlations were analyzed through Pearson Correlation.

Results: The average age was 65.2 ±12.069, with a predominance of the male gender (67.4%). Almost 21.60% of the patients had been for more than 5 years in dialysis. The most common etiology was Diabetic Nephropathy (26.1%). Among the main complications, we mention mineral bone disorders (MBD) and anemia. Hypocalcemia was present in 29.4% of patients, while Hypercalcemia predominated in 25.5% of the patients. 68.6% presented with low levels of vitamin D. Hypophosphoremia was present in 5.9% of patients and 62.7% had Hyperphosphoremia. CaxFofor product <55 mg2/dL was found in 60.8% of the patients. 41.2% of patients had secondary hyperparathyroidism (HPTS) and 23.6% had severe HPTS; 47.1% of patients presented with PTH < 150 pg/mL. 13.7% of the patients had iron deficiency anemia, of which 42.8% are absolute and 57.2% had ferritin levels over 200 ng/mL with functional iron deficiency. Among the patients included in the study, 13 had ferritin levels above 800 ng/mL, which is considered inflammatory anemia.

Conclusions: Patients who perform Hemodialysis in Vlora have reference values according to the recommendations of K/DOQI as below: 45.1% for Calcemia, 31.4% for Phosphorus, 60.8% for CaxFofor product and 11.7% for PTH. Only 3.92% of patients are on target according to the recommendations of K/DOQI regarding mineral and bone disorders. Normochromic normocytic anemia and iron deficiency anemia predominate.

The Diagnostic Value of Cystatin C for Detecting the Severity of Chronic Kidney Disease

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Chronic kidney disease (CKD) is a disease characterized by progressive loss of kidney function, and can be prevented or delayed through early detection and adequate treatment. Cystatin C is a 13-kDa low-molecular-weight protein that is generated

constantly by all nucleated cells, freely filtered, reabsorbed and catabolized in the proximal tubule of the nephron. Its clinical utility in the evaluation of kidney disease has been argued for more than 30 years.

The aim of this study was to investigate the diagnostic value of cystatin C for the detection of the severity of CKD.

The study comprised 134 patients with CKD. The patients were classified into the CKD stage using eGFR from the MDRD formula. Cystatin C was measured by the immunonephelometric method (Siemens). A ROC analysis was used to determine the diagnostic accuracy of cystatin C for detection of reduced renal function in CKD stages.

The areas under the ROC curves for cystatin C for eGFR of < 30, < 60 and < 90 mL/min/1.73 m^2 were from 0.985, 0.967 and 0.919, respectively. The sensitivity values were from 87.6% to 96.3%, and the specificity values, from 87.3% to 95.3%.

The results of the study showed that Cystatin C has a good diagnostic value as a biomarker for the disease progression in patients with CKD.

LABORATORY ANALYTICAL TECHNIQUES

Types and Frequency of Errors in the Pre-Analytical Phase in the Clinical Laboratory - Single Center Study from Bosnia and Herzegovina

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Background: The complex process of obtaining laboratory test results is performed in 3 phases: pre-analytical, analytical and post-analytical.

Due to the difficulties in achieving standardized sampling procedures, the pre-analytical phase, which includes all preparatory actions to the analytical procedure, is part of the process during which there is the greatest possibility of laboratory errors. The pre-analytical phase, which includes all preparatory actions to the analytical procedure, is part of the process during which there is the greatest possibility of laboratory errors. This study was conducted to investigate the frequency and types of laboratory errors during work in the clinical laboratory as well as the frequency and types of laboratory errors in the pre-analytical phase of laboratory work.

Methods: The research was conducted as a retrospective, descriptive study for which data were collected from 5 departments (laboratories) of the Organizational Unit (OU) Clinical Chemistry and Biochemistry of the Clinical Center of the University of Sarajevo (CCUS): Regular admission, Emergency Laboratory, Emergency Medicine Clinic), Center for Heart and Cardiovascular Surgery (CHCS-lab), and Pediatric Clinic. The research covered the period from 01/01/2016 to 31/12/2016 within which the presence of 5 different indicators of quality of work, i.e., pre-analytical errors were monitored: improperly drawn blood (wrong anticoagulant), coagulated blood sample, hemolyzed blood sample, improperly marked referral for analysis (referrals with incomplete patient data), and insufficient sample for analysis (blood volume too low). Pre-analytical errors had been detected by a visual inspection of the specimen. Data from blood samples (blood samples: whole blood, plasma, and serum, using anticoagulant K2EDTA and Na-citrate) taken for biochemical analyses and hemostasis were included in the study while urine and/or other biological material samples other than blood were excluded from the study. After reporting and stating the reasons for the rejection of the inadequate sample by the laboratory staff, a visual assessment of the error and adequate marking of the samples were done by a medical biochemistry specialist in the appropriate laboratory. The research was approved by the Ethics Committee of Clinical Center University of Sarajevo.

Statistical data processing was done using the computer program Excel (Microsoft Office Excel 2003) and SPSS computer program for statistical analysis version 24 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY,). The results are presented in the

form of tables. The results of categorical variables are presented as absolute numbers and percentage frequency of individual categories. The chi-square test was used to determine the significance of the differences of the categorical variables.

Values of $p < 0.05$ were considered statistically significant.

Results: During the follow-up period, a total of 198,916 (100%) separations were performed, of which the largest number, 19,086 or 9.6%, was performed in December, 18,370 (9.24%) in January, 18,188 (9.14%) in October, and 17,956 (9.02%) in November. The same number of separations was performed in February and March, 17,406 (8.75%), in July 15,896 (7.99%), in August 15,860 (7.97%), in April 15,612 (7.85%), and in May 14,198 (7.13%), while the lowest number of separations was recorded in June, 13,936 (7.01%). The chi-squared test revealed a statistically significant difference ($p < 0.001$) in the number of separations by months.

The analysis of the presence of different types of indicators showed that the most common type of indicators were coagulated blood samples, of which there were 1,881 or 26.24%, 1,692 (23.60%) indicators were improperly drawn blood, 1,545 (21.55%) improperly marked referrals for analysis, 1,450 (20.23%) insufficient sample for analysis, and 601 (8.38%) hemolyzed samples. The chi-squared test showed that the representation of the indicators differed statistically significantly ($p < 0.001$). In the total number of separations (198,916 or 100%), there were 7,169 or 3.6% indicators. In the total number of separations, there were 1,881 coagulated blood samples (0.94%), 1,692 samples taken incorrectly (0.85%), 1,545 illegally marked referrals for analysis (0.77%), 1,450 samples insufficiently for analysis (0.70%), and 601 hemolyzed serum samples (0.30%).

Analysis of the prevalence of indicators in 5 different departments (laboratories) at OU Clinical Chemistry and Biochemistry CCUS found that the largest number of indicators was recorded in regular admission, and the smallest in CUM-lab and CHCS-lab. Of the total number of different types of indicators, 720 (43%) of improperly drawn blood, 720 (38%) of coagulated blood samples, 210 (35%) of hemolyzed samples, 700 (45%) of improperly marked referrals for analysis, and 720 (50%) insufficient sample for analysis. Using the chi-squared test, a statistically significant difference was found in the frequency of occurrence of certain types of indicators in different departments ($p < 0.005$).

Conclusions: A retrospective study conducted at OU-Clinical Chemistry and Biochemistry at CCUS provided insight into the type and frequency of five pre-analytical errors, i.e., indicators for the quality of work. Errors that occur in the pre-analytical phase are the most common errors of a methodological nature, i.e., human errors that can be prevented and minimized in a timely manner.

There is a great deal of heterogeneity within the available literature regarding the occurrence, type, frequency, and manner of minimizing preanalytical errors, which is the biggest obstacle to standardizing the pre-analytical phase of laboratory work.

The introduction and quality control of work in clinical laboratories is a relatively new interest and focus of health professionals and managers. Reducing the number of errors in the pre-analytical phase can be achieved only by joint action of experts and

international organizations, continual training of staff, as well as to following the adopted guidelines and standards.

Validation of Direct Method for Magnesium Quantification in Serum

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Background: The magnesium ion is the fourth most abundant cation in the body and the second most abundant intracellular cation after the potassium ion. Only 1% magnesium is present in the extracellular fluid. Mg^{2+} is an important electrolyte serving as a cofactor in many enzymatic processes in the body. Magnesium fractions in plasma are at equilibrium and include: a) magnesium ions (Mg^{2+}); b) protein-bound Mg, mostly bound to albumin; and c) complex-bound Mg in salt form such as magnesium bicarbonate, magnesium carbonate, magnesium acetate, magnesium phosphate and magnesium citrate. Magnesium concentrations in serum are altered in alcoholism, cardiovascular disease, acute pancreatitis, diabetic ketoacidosis, and various renal and gastrointestinal diseases. Urine magnesium levels correlate with a number of diseases as well.

Methods: Validation of the direct electrothermic atomic absorption spectrometry (ETAAS) method for magnesium quantification in serum went through several methodic points: ashing temperature optimization, atomization of water standards and serum, calibration curves, evaluation of limit of detection (LD), low limit of quantification (LLOQ), intra- and inter-assay precision, accuracy, and characteristic mass. The analysis was done on Perkin Elmer Pin Aacle 900Z. Ashing temperature optimization was performed by matrix modifier contains 0.25% $LaCl_3$ in order to effectively eliminate chemical interferences.

Results: With the modifier described and pyrolytically covered the tube with Lvov platform, were established maximal pyrolytic temperature of 1900 °C and 800 °C for ashing. For calibration curves were used liquid standards in five concentrations - 0.2, 0.4, 0.6, 0.8, and 1.0 mmol/L and standard additive method. The two calibration curves are parallel, from which it follows that the method of water calibration can be chosen which is faster and cheaper. The calibration curve for direct determination of magnesium in serum by ETAAS using the STPF concept. Every standard was measured twice, and corrected against the modifier. LD was evaluated by 22 times measured modifier; we established 0.002 μg/L. Intra-assay precision showed CV 4.2%; inter-assay repeatability – CV 4.3%. Accuracy was evaluated by control material, traced with ICP-SFMS.

Conclusions: Atomic absorption spectroscopy is an accurate, fast and reliable method for determining magnesium in blood serum. Analysis can be carried out on as little as 0.05 ml of serum and the only preliminary treatment necessary is dilution of the sample

with $LaCl_3$. The absorption of magnesium is unaffected by the presence of sodium, potassium, and calcium.

LABORATORY HEMATOLOGY

Quantification of Serum Erythroferrone in Patients with Anemia with Chronic Disease

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Background: Inhibition of hepcidin activity is performed by recently discovered erythroferrone, synthesized by erythroblasts. Inflammation in chronic diseases stimulates production of hepcidin by cytokines, which on the other side blocks iron absorption into different cells, by changing ferroportin activity. We aimed to evaluate correlation between serum erythroferrone and hepcidin in patients with anemia of chronic disease.

Methods: As an example of anemia of chronic disease (ACD), we used patients with rheumatoid arthritis. 77 patients were enrolled; the activity of the disease was evaluated by disease activity score 28; 52 were females (67.5%). Their results were compared to equal number of healthy controls, matched by gender and age. Serum erythroferrone and hepcidin were quantified by ELISA sandwich methods. Additionally iron homeostasis parameters, CBC, CRP and ESR were evaluated.

Results: Included patients with rheumatoid arthritis (RA) were divided into two groups: a) RA with iron deficiency anemia (IDA); and b) RA with ACD. In control group erythroferrone concentrations were with average value 10.1 ± 3.5 ng/mL, and average hepcidin levels 15.4 ± 7.5 μg/L. In group of patients with RA and IDA average erythroferrone serum concentrations were 12.5 ± 2.7 ng/mL, and average hepcidin levels 0.61 ± 0.4 μg/L. In group of patients with RA and ACD average erythroferrone concentrations were 31.4 ± 6.9 ng/mL, and average hepcidin levels 87.9 ± 10.7 μg/L. The significance of positive correlation between groups for erythroferrone was $r=0.899$, $P<0.005$; and for hepcidin $r=0.925$, $P<0.001$.

Conclusions: Quantification of serum erythroferrone in patients with anemia of chronic disease in rheumatoid arthritis might be useful for correct management of iron supplementation, in order to avoid intoxications.

Coagulopathy with Severe Eye Bleeding in IgM Monoclonal Gammopathy as the Presentation of Lymphoplasmacytic Lymphoma

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Introduction: Lymphoplasmacytic lymphoma (LPL) is one of the rare types of Non-Hodgkin Lymphoma (NHL), accounting for only 1 to 2% of all NHLs. It is often associated with an increased IgM protein causing several complications including interfering with the normal functioning of blood clotting factors. This often causes chronic mild nosebleeds. In rare cases, a manifestation of bleeding can be more serious.

Case report: We present a 60-year-old female patient with painless loss of vision in both eyes. Hemorrhagic retinal detachment was detected in both eyes. Due to verified leukocytosis and anemia, flow cytometry of the peripheral blood and a bone marrow biopsy had been performed. An extended activated partial thromboplastin time (aPTT 54.9) is also noted. Serum protein electrophoresis identifies IgM kappa paraprotein, with high nephelometric values (IgM 78.9). Diagnosis of lymphoplasmacytic lymphoma was established, complicated by coagulopathy and severe eye bleeding. A therapeutic plasma exchange procedure was started with the patient. After that, treatment with 6 cycles of the R CHOP (Rituximab, cyclophosphamide, doxorubicin, oncovin prednisolone) protocol was carried out, achieving remission of the disease. The ophthalmologist performed a vitrectomy on the right eye resulting in a 30% correction of vision in the right eye.

Conclusion: Acquired coagulopathy and its many causes, including malignancy, is important in the management of bleeding disorders. Discovering the underlying etiology enables the most effective treatment.

Key words: Lymphoplasmacytic lymphoma, coagulopathy, bleeding, IgM, management

Predictive Markers of Mantle Cell Lymphoma in Leukemic Phase Diagnosed with Flow Cytometry

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Introduction: Mantle cell lymphoma (MCL) is mostly present as a disseminated disease including peripheral blood involvement (leukemic phase), that has been reported with variable frequency (13 to 73%).

Objective: To do a retrospective evaluation of the laboratory and clinical characteristics in 44 patients with leukemic phase MCL, their influence on overall survival (OS), and the validity of the international prognostic index (IPI). All the patients were treated in the Clinic for Hematology, Clinical Center of Serbia for a ten years period.

Methods: Immunophenotyping by flow cytometry of peripheral blood was performed in all patients. Cox hazards model was performed to determine prognostic factors. The Kaplan-Meier method and curves evaluated the impact of all analyzed parameters on overall survival.

Results: We evaluated 44 patients with MCL. The median age was 61.5 y, male dominant (2.14: 1). Main clinical features were B symptoms (84.1%) and splenomegaly (95.5%). ECOG performance status was unfavorable (≥2) in 54.5% of patients. Patients were mostly treated with chemotherapy (90.9%), 63% achieved remission of the disease. Median survival was 31 months. Statistical analysis confirmed the validity of IPI but not s-MIPI index validity. Transformed IPI index (cut-off value 3 divides patients into the 2 prognostic groups), also transformed s-MIPI index (cut off 8 inside s-MIPI score divide patients into 2 prognostic groups) had strong predictive significance. Univariate Cox regression analysis identified prognostic markers: >1 extranodal involvement, IPI index, transformed IPI index, transformed s-MIPI index, percent of bone marrow infiltration >70%, blastoid type of MCL. Multivariate Cox regression identified >1 extranodal involvement and transformed s-MIPI index as the strongest predictive marker.

Conclusion: Patients with a leukemic phase of MCL treated with conventional chemotherapy achieved 63 % of remission but had short median survival (31 months). In our group, presentation with more than one extranodal involvement and value of s-MIPI index had the most powerful prognostic significance.

Rare Presentation of Follicular Lymphoma with Peripheral Blood Involvement Confirmed with Flow Cytometry, Clinical Characteristics, and Outcome

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Introduction: Follicular lymphoma (FL) is indolent lymphoma, mostly presented as disseminated, with bone marrow infiltration in 40-70% of patients. Peripheral blood involvement (leukemic phase) has been reported as a rare presentation (ranging from 4.5 to 23%) and can be diagnosed by immunophenotyping of the peripheral blood.

Objective: Aim was to evaluate laboratory and clinical characteristics in 10 patients with leukemic phase, their possible influence on overall survival (OS), and the validity of the international prognostic index (IPI and FLIPI). All the patients were treated in the Clinic for Hematology, Clinical Center of Serbia for 10 years period.

Methods: Immunophenotyping by flow cytometry was performed in all patients on peripheral blood specimens according to standard protocols. Statistical analyses were performed to evaluate the characteristics of FL in the leukemic phase.

Results: We evaluated 10 patients with FL in the leukemic phase. The median age was 48.9 y, male dominant (1.5:1). Histology grade I dominate (80%). Half of the patients had anemia and thrombocytopenia; median lymphocytosis was 33.43. Lactate dehydrogenase was increased in 70. Regarding clinical features ECOG performance status was favorable (<2) in 90 % of patients. The main clinical features were B symptoms and hepatomegaly (all patients), splenomegaly (50%), “bulky” tumor mass (40%). Median survival was not achieved. Distribution according to the IPI index was mostly in low-risk groups (90%), but according to the FLIPI index mostly into high-risk group (60%). Patients were mostly treated with immune chemotherapy in 70% and chemotherapy in 30 %, with remission response in 90 % of patients.

Conclusion: Leukemic phase of FL is a rare event even though FL is mostly disseminated at presentation. In our group patients were mostly treated with immune chemotherapy and achieved a good response, median survival was not achieved. In our small cohort, we did not identify some prognostic markers, but the use of multicenter studies may be a way for identifying.

Advanced Mantle Cell Lymphoma Staged by Flow Cytometry Coexisting with Colon Adenocarcinoma

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Introduction: The incidence of the coexistence of multiple malignant diseases is rare, although the prevalence rate has been increasing recently. Mantle cell lymphoma (MCL) is a B-cell lymphoma that accounts for 3-10% of non-Hodgkin's lymphomas (NHL). It is more common in the elderly, often diagnosed in an advanced stage with possible extranodal involvement of the gastrointestinal tract. In some patients, we have also found bone marrow involvement, which can be confirmed by bone marrow flow cytometry. Colon cancer and coexisting MCL are rare. There is no universal therapeutic approach.

Case report: A 67-year-old man presented with weight loss and night sweats. Physical examination and computed tomography (CT) showed massive generalized lymphadenopathy, and the blood count showed normocytic anemia. A biopsy of the axillary lymph node confirmed the diagnosis of mantle cell lymphoma (MCL). Bone marrow analysis indicated infiltration by lymphoma cells. The patient was not motivated for endoscopic exploration of the gastrointestinal tract. After the treatment, the patient was staged as NHL mantle cell CS IV BE with bone marrow involvement confirmed by bone marrow cytometry, in the prognostic group of high risk (MIPIb score 7.7). The patient is also being treated for arterial hypertension, obstructive lung disease, benign prostate hyperplasia, and polycystic kidneys. The patient's father died of colon carcinoma. The patient did not perform screening as recommended. The patient was treated with immuno-chemotherapy according to the R (rituximab)- CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) protocol, with a partial response. Treatment with a Bruton kinase inhibitor was then carried out. After three months, the radiological examination revealed a partial regression of the lymph nodes in the axillae, without significant enlargement of the lymph nodes in the abdomen and other explored regions. In the results of the blood count, microcytic anemia was noted, along with the anamnestic information about the presence of blood in the stool. The performed colonoscopy indicated an infiltrative change in the distal descending colon. Resection of the tumor change on the colon was performed and the pathohistological findings indicated adenocarcinoma. It was planned to continue hematological treatment with regular follow-ups by a gastroenterologist.

Conclusion: This case report indicates that elderly patients require careful observation during lymphoma staging and treatment. The etiological relationship between these two tumor entities, apart from male gender and older age, remains unclear. Several studies point to possible risk factors including poor immunity, common signaling pathways, and continuous exposure to toxic and carcinogenic agents.

Keywords: Mantle cell lymphoma, flow cytometry, colon adenocarcinoma, therapy, risk factors

A Case Report of EDTA Induced Thrombocytopenia

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Introduction: EDTA-dependent pseudothrombocytopenia (PTCP) is the phenomenon of a spurious low platelet count due to EDTA-induced aggregation of platelets.

This phenomenon is mostly due to the presence of EDTA-dependent antiplatelet antibodies that react optimally between 0°C and 4°C, recognize the cytoadhesive receptors gpIIb-IIIa, stimulate the expression of activation antigens, trigger activation of tyrosine kinase, platelet agglutination and clumping in vitro, which finally lead to a spuriously decreased platelet count.

Pseudothrombocytopenia is observed in 0.07-0.27% samples with tripotassium ethylenediaminetetraacetic acid (K_3EDTA) - anticoagulant.

The aggregation of platelets in EDTA-dependent PTCP usually is prevented by other anticoagulants, such as sodium citrate or heparin.

Aim and objective: The aim of this case report is to show the importance of laboratory procedures in order to identify EDTA-dependent pseudothrombocytopenia.

Case report: A 30-year-old woman was referred to our laboratory for blood smear examination because of thrombocytopenia detected incidentally from CBC. Findings from the general practitioner's laboratory in K_3EDTA tube were: platelet $35x10^3/mm^3$, WBC $6.64x10^3/mm^3$, RBC $4.05x10^6$, Hb 12.3 g/dL. There was no family history of hemorrhagic disorders. There was no history of ecchymoses, recent melena, metrorrhagia or weight loss. Microscopic examination of the peripheral blood smear showed the aggregation of platelets. We suspected that this low platelet count was due to EDTA-induced aggregation of platelets. So, we measured the platelet count in the patient's blood samples anticoagulated with sodium citrate.

Results: In sodium Citrate tube the results were: platelet $126x10^3/mm^3$, WBC $5.86x10^3/mm^3$, RBC $3.95x10^3/mm^3$, Hb 12.4 g/dL.

Conclusions: Peripheral blood smears should be examined for platelet clumping/aggregates in cases with low platelet count not correlating with clinical presentation. Alternative anticoagulants should be used for correct estimation of platelet count.

Verification of Complete Blood Count and Erythrocyte Sedimentation Rate on the MEK1305 Hematology Analyzer

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Background: The introduction of a new analytical system in a clinical chemistry laboratory requires its verification in order for routine clinical practice usage. Validation of the equipment and methods used in the biochemical laboratory is the basis of the quality control process in everyday practice. Verification is a selection of several necessary validation procedures that must be carried out in every laboratory when introducing a new method and a new analyzer given by the CLSI (Clinical and Laboratory Standards Institute, USA) organization according to the IFCC recommendations. MEK1305 hematology analyzer (Nihon Kohden Corporation Tokyo, Japan) is distinct because of an innovative method for erythrocyte sedimentation rate (ESR) determination based on photometry that records the light intensity under conditions of controlled shear pressure of the created erythrocyte aggregates.

Methods: For the verification procedure, control materials (high and low controls) and patient samples from Nova Med+ (Belgrade, Serbia) were used. The verification procedure was carried out through the following steps: reproducibility and intermediate precision, trueness, linearity, and carry-over. Statistical methods used for these analyses were: Student's t-test, ANOVA, linear regression analysis, and Passing and Bablok, as well as Bland-Altman analysis.

Results: Coefficient of variation (CV) values within the series for high concentrations controls ranged from 0.1% for MCV to 9.6% for the monocytes % (0.4% for MCV to 9.6% for the granulocytes' number between series), while for the low concentration control CV were from 0.3% for MCV to 16.7% for the monocytes % (0,6% for MCV to 16,7 % for the monocytes % between series) in the total leukocyte formula. Bias ranged from 0.99% for erythrocyte count to 50.11% and 37.43% for ESR corrected for temperature and hematocrit, respectively. The results of the Passing-Bablok analysis showed excellent agreement between the tested analytical systems (MEK 1305 and comparison system routinely used in the laboratory) for the majority of the parameters, i.e., systematic and percentage errors absence). Linearity for the number of blood cells, hemoglobin concentration, and hematocrit value showed good linearity for the declared range, with satisfactory correlation coefficients' values (r >0.990 for all tested parameters). Carry-over values (in %) for the blood cell count, hemoglobin, hematocrit, and ESR ranged from -1.9 to 2.7%.

Conclusion: The verification procedure of the MEK1305 hematology analyzer showed its good analytical characteristics and its suitability for regular routine use in clinical chemistry laboratories. The high bias for ESR determination could be explained by the different analytical principles usage and possible significant environmental influences on

Westergren's system, which is avoided in this new ESR determination method employed in the MEK1305 machine.

The Challenge of Diagnosing Promyelocytic Leukemia in the Hematology Laboratory: A Case Report

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Acute myeloid leukemia (AML) implies a clonal expansion (more than 20%) of myeloid blasts in the bone marrow, peripheral blood and other tissues. Complete blood count (CBC), differential, bone marrow smear and flow cytometry data are used for diagnosis, with confirmation by cytogenetic FISH methods.

A 75-year-old female patient was admitted to the hematology clinic with suspected acute leukemia. Laboratory tests were performed, including complete and differential blood counts, using a Sysmex XN-3000 analyzer. The findings indicated pancytopenia: WBC 1.19 $x10^9$/L, RBC 1.43 $x10^{12}$/L, PLT 4 $x10^9$/L. Differential blood count showed monocytosis with 32.8% monocytes. The presence of immature granulocytes of 13.4% was registered. The device also issued a flag for the presence of blasts or abnormal lymphocytes. These findings were confirmed on the Beckman Coulter DxH 900 analyzer, with almost identical CBC values and a higher percentage of monocytes of 45.0%. The second part of the in-line system, Sysmex DI-60, recorded only 3.6% of monocytes in the blood smear, while 23.6% of the cells were listed as unspecified blasts. Both analyzers gave falsely elevated values of monocytes within the differential blood count, instead of atypical blasts. However, suspicious cells were observed, and their differentiation required additional analyses. Also, it is important to note that immature leukocyte forms were observed at a very low WBC count. By optical examination of the May-GrünwaldGiemsa stained blood smear under a microscope, the analyst observed the following: segmented neutrophils 5%, eosinophils 1%, lymphocytes 13%, monocytes 3%, promyelocytes 14%, myelocytes 3% and blasts 11%.

The patient's bone marrow sample was analyzed by flow cytometry on a BD FACSCanto™II analyzer. Immunophenotypically, up to 70% of cells with expression on CD33+, cyMPO+, CD13+, and CD117+ were present in the sample. Considering that the cells had a slightly higher SSC-A/CD45+ position (in the granulocyte gate) andare losing the expression of CD34 and HLA-DR, acute promyelocytic leukemia AML/M3 is phenotypically considered. To confirm or exclude the diagnosis of M3, it is necessary to correlate the findings with cytogenetic FISH methods and myelogram.

Due to the specificity of the structure of certain types of leukocyte lineage, the error of the hematological analyzer rarely occurs in the analytical phase. Both devices registered pseudo-monocytosis in this case, and promyelocytes were counted as monocytes. The

possibility of error is reduced by the complementary capabilities of modern instruments, automatic preparation and microscopy of blood smears with standard optical blood count. Additionally, a microscopic examination performed by the analyst is indispensable for a more detailed finding and confirmation of the diagnosis.

The cooperation of hematology and cytology laboratories is necessary to establish a correct diagnosis of acute promyelocytic leukemia M3.

Is Na-Citrate an Alternative Anticoagulant in Conditions of EDTA-Dependent Pseudothrombocytopenia?

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Background: Pseudothrombocytopenia (PTCP) is still an insufficiently researched phenomenon manifesting itself in a falsely reduced number of platelets analyzed on an automatic blood cell counter. A relatively common finding in clinical laboratories, it can lead to diagnostic errors, excessive treatment and further (even invasive) redundant testing. Consequences of unrecognized PTCP can be potentially life-threatening (unnecessary platelet transfusion, inappropriate treatment including splenectomy or corticosteroids). A practical laboratory approach, based on current knowledge of PTCP, is proposed to overcome falsely low platelet counts.

The aim of this study is to compare platelet and MPV values in samples with different anticoagulants in patients with suspected PTCP.

Methods: The study was conducted at the Clinical Center University of Sarajevo, and included 17 patients with suspected PTCP. The number of platelets and MPV in the blood sample with EDTA and Na-citrate as anticoagulants was determined for patients. The analysis was performed on a Sapphire (Abbott Diagnostics) hematology analyzer, after which all samples were optically examined using Fonio's method. Results were presented as mean and SD-standard deviation, and statistical significance ($P<0.05$) was determined by t-test for independent samples.

Results: By comparing mean values of the number of platelets analyzed on the hematology analyzer with EDTA and Na-citrate, following values were obtained: $\underline{x}_{EDTA}$=75.6; SD=47.8; $\underline{x}_{Na\text{-}citrate}$=245.7; SD=95.4). A statistically significant difference ($P<0.001$) was found in platelet numbers between the two anticoagulants. Samples with EDTA and Na-citrate were also analyzed using Fonio's method, and it was determined that only EDTA samples had groups of platelets. By comparing mean values of MPV ($\underline{x}$=10.2; SD=1.4 and $\underline{x}$=8.0; SD=1.1) in the same samples, an independent t-test also revealed a statistically significant difference ($P<0,001$). By comparing mean values of

platelets (PLT) and MPV between sexes in samples with EDTA, no statistically significant difference was found for any parameter (PPLT=0.298; PMPV=0.219). However, the results for the number of platelets in samples with Na-citrate are at the limit of statistical significance (P=0.059), and the MPV results are comparable to EDTA samples (P=0.400).

Conclusion: To accurately and timely determine PTCP, it is necessary to optically examine the number of platelets using Fonio's method and if necessary, correct a false positive result of thrombocytopenia by using another anticoagulant. Namely, results of this study show that in order to determine PTCP, it is necessary to perform an examination according to Fonio, and that Na-citrate is a better choice of anticoagulant compared to EDTA. It would be significant to determine PTCP at the level of primary health care. In this way, patient would be spared from additional tests, which notably burden clinical laboratories and the entire healthcare system.

Hemoglobin H disease in Albania. A Case Report

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Background: Hemoglobin H (HbH) is a rare variant of hemoglobin, the incidence of which is not very common in Albania. This disease is characterized by deletion mutations of three of four alpha-globin genes, causing a deficiency of the alpha-globin chains of hemoglobin and an excess production of beta-globin chains (formation of beta-4 tetramers, HbH.) HbH tetramers have a high affinity for oxygen, and are highly unstable, precipitating as toxic Heinz bodies which predominate in mature red blood cells, leading to premature hemolysis.

Methods: The 34-year-old male was admitted to hospital suffering of fever for 10 days and history of progressive fatigue, pallor and jaundice for the past 2-3 years. His physical examination showed mild pallor and a palpable spleen. Blood sample from the patient was collected in an K3-EDTA tube and was analyzed on a Sysmex XN-1000 Hematology analyzer. A serum sample collected in a BD vacutainer gel tube was used for measuring Lactate Dehydrogenase, Iron, Ferritin, Liver function tests, Bilirubin, Total Protein and Albumin on Alinity C modular system. Hemoglobin electrophoresis was performed on the hemolysate of whole blood samples collected in tubes containing K3-EDTA as anticoagulant, using capillary electrophoresis in alkaline buffer (pH 9.4) with the Sebia capillarys flex-piercing instrument. The peripheral blood smear was prepared using Giemsa stain 2.5%.

Results: On admission the blood tested showed: Hb 7.6 g/dL, Hct 26.6 %, MCV 66.7 fL, MCH 19.0 pg, MCHC 28.6 g/dL, RDW 23.2 %, WBC 2,500/mm^3, platelet 102,000/mm^3. Reticulocyte preparations showed 61% reticulocytes. The peripheral smear showed presence of moderate hypochromia, anisocytosis (microcytes, polychromatophilic macrocytes), poikilocytes (elliptocytes, tear drop cells. schizocytes, a lot of target cells) and Heinz bodies.

In biochemical investigations: Total Bilirubin 2.7 mg/dL, Direct Bilirubin 0.52 mg/dL, serum LDH 872 U/L, Iron 160 µg/dL, Ferritin 385 ng/mL, SGOT 96 U/L, SGPT 33 U/L, Total Protein 7.0 g/dL, Albumin 4.3 g/dL and Alkaline phosphatase 54 U/L.

Hemoglobin electrophoresis using capillary electrophoresis in alkaline buffer, showed a band which moved ahead of the adult HbA band and was highly suggestive of HbH. The quantitative analysis of the hemoglobin fractions result was: HbH 29%, Hb Bart 0.5%, HbA 70% and HbA_2 0.5%.

Conclusion: Mostly clinically silent, the HbH may present with hemolysis and mild to moderate anemia. Capillary electrophoresis has a high resolution and an accurate measurement of the hemoglobin fractions. A molecular genetic analysis should be performed to obtain a definitive identification for this pathology. This case triggers us to think of HbH as a potential cause when sorting out a differential diagnosis for any case of anemia.

Analytical Verification and Comparability of Differential Counting Values on the Hematology Analyser Celltac G

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Background/Aim: The aim of the paper was to carry out an analytical verification of the automated hematology analyzer Celltac G (Nihon Kohden) and a comparison with the analyzer that uses a different method of measurement for the parameters of the complete blood count (CBC) as well as measuring a five-part differential blood count.

Methods: The verification was carried out in accordance with the EP15-A2 procedure (CLSI), and included the following tests: within-run precision (repeatability), between-run precision, and accuracy by using commercial control samples at three levels; the comparison of results of patient samples with hematology analyzer Advia 2120i (Siemens). All 40 blood samples for method comparison were collected from hospitalized and ambulatory patients in tubes containing K3-EDTA.

Results: By examining repeatability, the lowest coefficient of variation (CV) was obtained for the hemoglobin concentration (Hb) (g/L) and the mean corpuscular volume (MCV) (fL) (0.3%), whereas the highest CV was obtained for the absolute monocytes count (10^9/L) (20.1%) and the absolute eosinophils count (10^9/L) (24.3%). The smallest deviation from the target value (bias) was found for erythrocytes (Rbc) (10^{12}/L), while the highest deviation was found for the absolute basophils count (10^9/L). With method comparison or by measuring patient samples, the value of the correlation coefficient obtained was $r > 0.90$, except for the mean platelet volume MPV (fL) and absolute and relative monocytes, the eosinophils and basophils count. There was no statistically significant difference (95% CI of the mean difference contains 0) for MCV, Plt, absolute and the relative neutrophils and lymphocytes count.

Conclusions: The obtained results confirm that the tested automated hematology analyzer Celltac G meets the set analytical criteria for precision and accuracy. Hence, it can be used in routine work to determine a complete blood count with a five-part differential blood count.

Hemoglobin Levels: A Comparative Study of Female Textile Workers and Bank Office Workers in North Macedonia

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Background: Hemoglobin levels are an important indicator of overall health and can be influenced by various factors, including occupational conditions and socioeconomic status. By comparing two distinct occupational groups (female textile workers and female bank-office workers), we seek to gain insights into the potential impact of working conditions, and income disparities, on the health of female workers in different work environments. This research study aims to explore the possible differences in hemoglobin levels between these two groups in North Macedonia.

Methods: In the study, total of 418 women were included, divided into two groups: textile workers N=209, subsequently divided into two subgroups, 50 years old and under, N=122 and above 50 years, N=87; and bank-office workers N=209 subsequently divided into two subgroups, 50 years old and under, N=151 and above 50 years, N=58.

Hemoglobin levels were measured using a standardized procedure, such as a complete blood count test on an automated analyzer.

The data was analyzed using unpaired t-test ($p<0.05$ was considered significant).

Results: In the group of textile workers the average values of hemoglobin level are 12.8±1.1 g/dL (median=12.7 g/dL), 50 years old and under 12.5±1.1 g/dL (median=12.5 g/dL), above 50 years 13.3±0.9 g/dL (median=13.2 g/dL). In the group of bank-office workers the average values of hemoglobin are 13.4±1.1 g/dL (median=13.4 g/dL), 50 years old and under 13.2±1.1 g/dL (median=13.2 g/dL), above 50 years 13.8±1 g/dL (median=13.9 g/dL).

The results showed statistically significant lower levels of hemoglobin ($p<0.05$) in the textile workers group compared to the bank-office workers group. There is a statistically significant lower hemoglobin level ($p<0.05$) in the subgroups of 50 years old and under from the textile workers group in comparison to the same subgroup from the bank-office workers group. Hemoglobin levels are slightly lower in the subgroup of above 50 years from the textile workers group, but there is no statistical significance ($p>0.05$) compared with the same group in the bank-office workers. Comparing the subgroups in the textile workers group, hemoglobin levels are slightly higher in the above 50 years subgroup compared with the textile workers 50 years old and under, which is statistically significant ($p<0.005$). In addition, the same subgroup comparison in the bank-office

workers showed the same statistical significance ($p<0.005$); the hemoglobin levels are slightly higher in the above 50 years subgroup than the subgroup of 50 years old and under.

Conclusion: The findings in this research study show apparent differences between the hemoglobin levels among Macedonian female textile and bank office workers. Therefore, bigger awareness about anemia is needed among women to improve the hemoglobin status of the Macedonian women population, particularly in lower-income occupations including implementation of extension of health and nutrition education and welfare programs.

MOLECULAR DIAGNOSTICS

Acquired EGFR T790M Resistance Mutation Identified from Liquid Biopsy in a Patient With EGFR-Mutated Lung Cancer: A Case Report

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Background: Activating mutations of the epidermal growth factor receptor gene (EGFR) are present in about 10-15% of Caucasian patients with advanced non-small-cell lung cancer (NSCLC). These mutations are predictive biomarkers of response to first- and second-generation tyrosine kinase inhibitors (TKIs). Unfortunately, patients that initially respond to these drugs develop resistance and progression of their cancer after 8-13 months. The most important molecular resistance mechanism is the acquired T790M mutation in EGFR exon 20, occurring in 50-60% of cases of resistance. Liquid biopsy-based analysis of circulating tumor biomarkers, such as cell-free tumor DNA (cfDNA) in blood, has opened a new minimally invasive way for the early screening, diagnosis, and treatment of lung cancer, especially when tissue samples cannot be obtained.

Methods: A patient data and informed consent were extracted from the patient record database of the Institute for Pulmonary Diseases of Vojvodina. Genomic DNA was isolated from the representative cytological specimen and cfDNA was isolated from the K2EDTA blood plasma using the cobas® DNA and cfDNA Sample Preparation Kits, respectively. Real-time PCR analysis was based on the cobas® EGFR Mutation Test V2.

Results: A 72-year-old never-smoking woman presented with dry cough and chest pain in November 2019. Computed tomography (CT) of the chest revealed tumorous lesions in the right upper and middle lobe. Pleural fluid cytology was positive for lung adenocarcinoma, stage IVA. EGFR testing detected the presence of deletion in exon 19. Gefitinib (first-generation TKI, 250 mg/day) was chosen as a therapy. During the treatment the patient showed a partial radiological response, but after 12 months of follow-up CT scan indicated progression of the disease. A liquid biopsy was performed and confirmed deletion in exon 19 and T790M mutation. Due to its steric hindrance „gatekeeper“ T790M mutation creates an obstacle for the binding of first- and second-generation TKIs to the ATP-binding site of EGFR. In January 2021 the patient was administered osimertinib (third-generation TKI, 80 mg/day) which binds irreversibly to

EGFR with T790M mutation. Our patient is still on osimertinib therapy (28 months), with good quality of life and with confirmed a partial radiological response.

Conclusions: Repeated tissue biopsy is the gold standard for molecular profiling, but there are significant limitations related to challenging biopsy locations, patient's comorbidities, inadequate tissue for molecular testing and tumor heterogeneity, particularly in patients progressing on treatment. Because of that, liquid biopsy-based cfDNA analysis is able to provide an alternative approach for investigating tumor-derived alterations. Understanding the resistance mechanisms of EGFR-mutant NSCLC could guide future drug development and improve personalized therapy.

The Emerging Power of Mirnas in Management of Acute Coronary Syndrome

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Background: Acute coronary syndrome (ACS) is one of the leading causes of death worldwide and a continuous burden to the health systems and the human society overall. Despite major diagnostic advancement based on highly sensitive Troponins and imaging techniques, precise early rule-in/rule-out is still challenging especially in unstable angina and so is the accurate risk stratification which is deemed instrumental for adequate and effective therapeutic approach. Laboratory medicine research promises identification of novel ACS biomarkers like micro-RNA molecules (miRNAs) to be implemented in early diagnosis, prognosis, monitoring of the therapeutic effectiveness or serve as targets for designing novel therapeutics.

Methodology: Comprehensive literature survey and critical review aiming to assess the potential and rationale for applying miRNAs as cutting-edge laboratory biomarkers or novel ACS drug targets.

Results: The analysis of recently published data revealed great discrepancy among studies but a definite involvement of miRNAs in many mechanisms associated with the pathogenesis of ACS including inflammation, lipid and glucose homeostasis pathways, renin-angiotensin-aldosterone axis, genesis, progression and instability of atherosclerotic plaque etc. It is now known that many drugs treating myocardial ischemia, heart failure, interstitial fibrosis, arrhythmia, diabetes, and hypertension, actually affect miRNAs by up- or down-regulation.

Circulating miR-22-5p and miR-150-3p have demonstrated overexpression while miR-132-5p is reduced in early stages of acute myocardial infarct (AMI). It has been suggested that early detection of AMI can be facilitated with miR-208 measurement only one hour after infarction, while diagnosis several hours after the onset of symptoms involves assessment of miR-499 and miR-133.

Upregulation of miR-92a-3p and miR-206, as well as downregulation of miR-939, miR-181-a-3p and 181-a-5p have been detected among ACS cases suffering from chronic coronary artery disease, hence can be useful parameters for risk stratification and ACS predictive markers in these patients.

It has been demonstrated that serum exosomal miR-126 might serve as a diagnostic biomarker, as well as indicator of the severity of coronary artery stenosis in patients with ACS. Serum exosomal miR-21 might also be a candidate diagnostic biomarker for ACS, but studies are underway for confirmation of its application as a therapeutic target. Another promising drug target is miR-145, which overexpression may affect endothelial injury and abnormal inflammation through targeting FOXO1.

Conclusion: Current data reveal the applicative potential of miRNAs, but improvement in the laboratory profiling of ACS based on research investigating miRNAs as diagnostic and prognostic markers, merit their validation within further, larger studies based on protocols standardized in regards to type and preparation of samples, analytical methodology and data processing.

Prevalence of Thrombophilia-Associated Genetic Risk Factors in Reproductively Challenged Female Population in Montenegro

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Objective: Thrombophilia is a multifactorial condition, usually categorized into two types – acquired and inherited. People with inherited thrombophilia tend to form clots due to a genetic predisposition passed to them by their parents. The role of these inherited disorders in pregnancy loss and vascular gestational disorders has been investigated in several studies, and the results seem to be contradictory. Aim of this study was to evaluate the frequency of thrombophilia-associated genetic risk factors in Montenegrin reproductively challenged female population with recurrent pregnancy losses.

Material and methods: We have investigated blood samples from reproductively challenged, otherwise healthy females with recurrent pregnancy losses. Genomic DNA was extracted using Bosphore Genomic DNA Extraction Spin Kit v2 (Anatolia Geneworks). The detection of mutations in factor V Leiden (*FVL G1691A*), prothrombin (*FII G20210A*), methylenetetrahydrofolate reductase (*MTHFR C677T* and *MTHFR A1298C*) was performed using the Bosphore Thrombophilia Panel Kit v1 kit (Anatolia Geneworks) in 164 samples. Polymorphism in plasminogen activator inhibitor type I (*PAI-I -675 4G/5G* and *PAI-I -844G/A*) were examined using Bosphore PAI-I 4G/5G Detection Kit v1 and Bosphore PAI-I 844 Detection Kit v1 (Anatolia Geneworks)

according to the manufacturer's instructions. PAI-I 4G/5G and PAI 844 polymorphism were tested in 135 and 100 women, respectively.

Results: The calculated frequencies of each genotypic variant in heterozygosity were: 0,6% for the FV gene, 38,41% (*C677T*) and 44,51% (*A1298C*) for methylenetetrahydrofolate reductase gene. Mutated homozygotes *FII G20210A* and *FVL G1691A* were not found. The genetic variants of MTHFR were found in homozygosity, with frequencies of 14,63% and 15,85% (*C677T* and *A1298C*).

Regarding the frequencies of genetic variants in plasminogen activator inhibitor type I they were present in both - heterozygosity and homozygosity. Heterozygous genotypes were present in 55.56% for -675 4G/5G and 57% for -844G/A, while homozygous mutant genotypes were present with frequency of 24,44% and 31%, respectively.

Conclusion: Frequency of F II and FV gene variants in our group was not significantly different compared to general population in Montenegro, as well as MTHFR hetero- and homo-zygosity. PAI-1 heterozygotes were present at a higher rate in our group than in healthy regional population, while homozygous mutant genotypes had similar prevalence. Our results suggest that PAI-I polymorphism could be involved in reproductive health (recurrent pregnancy losses).

NUTRITION AND NUTRIGENOMICS

The Impact of Macedonian Orthodox Christian Fasting During Lent on Lipid Status

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Background: Orthodox fasting (OF) is a dietary regime that can be characterized as a vegan diet. During Lent, which lasts for 48 days before Easter, food consumption excludes meat, milk and dairy products, eggs and cooking oils, except for weekends when cooking oils are allowed. Fasting individuals are allowed to consume fish only for two days during the 48-day fasting period.

Methods: The study involved 58 participants (with an average age of 49.8±14.9 years; males=13, females=45) who visited the laboratory twice, first time 3 days before the start of the OF, and second time on the 46th day of the OF. The measure of every parameter was made after overnight fast ≈ 12h.

Total cholesterol was measured the with CHOD-PAP enzymatic colorimetric method. Triglycerides were measured with the GPO-PAP enzymatic colorimetric method. HDL cholesterol was measured with a direct enzymatic method without precipitating. LDL cholesterol was calculated using the Friedewald equation. All parameters were measured on an automated analyzer.

Paired t-test and Wilcoxon sign-rank were used to analyze the data. (p-values <0.05 were taken as statistically significant.)

Results: The results indicate that the OF has a significant impact on the lipid profile of individuals, as evidenced by the reduction in Total cholesterol from 5.91±1.13 mmol/L to 5.38±1.02 mmol/L (p=0.0095), a reduction in LDL cholesterol from 3.87±0.99 mmol/L to 3.35±0.84 mmol/L (p=0.0026), a slight reduction in HDL cholesterol levels from 1.44±0.38 mmol/L to 1.35±0.33 mmol/L (p=0.0002), and a slight elevation in Triglyceride levels from 1.28±0.73 mmol/L to 1.47±0.80 mmol/L (p=0.0134).

Conclusion: Meat, eggs, milk and dairy products significantly affect the lipid status. These findings could be useful in promoting the benefits of OF for individuals seeking to improve their cardiovascular health. However, it is questionable if total absence of meat, eggs, milk and dairy products is the right way of alimentation. Further studies are needed to confirm and expand upon these results.

The elevated levels of triacylglycerols may be due to mobilization of depo-lipides. Also, it is known that excessive intake of carbohydrates elevates the levels of triacylglycerols, and the main macronutrients in fasters alimentation during Lent are carbohydrates.

Non-Celiac Gluten Sensitivity (NCGS) vs Celiac Disease (CD): The Trend in The Albanian Population

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Background: Non-Celiac Gluten Sensitivity (NCGS) belongs to the group of gluten-related disorders with many symptoms similar to those of celiac disease (CD) or wheat allergy. Coeliac disease is a multisystemic chronic autoimmune inflammatory disease where the immune system reacts abnormally to gluten. NCGS, frequently known as gluten intolerance in the Albanian population, is characterized by intestinal and extraintestinal symptoms but without damage to the lining of the small instestine. It is important to differentiate CD from NCGS to establish a strict gluten-free diet for individuals who are at risk of complications.

Methods: This is a Retrospective observational study conducted for a period of nine months at the FitHealth clinic. During this period, we followed a group of 120 patients with abdominal pain, bloating, diarrhea, fatigue, mental fogginess, muscle aches, etc. Serology tests were used: tissue transglutaminase (tTG) immunoglobulin A (IgA) and tTG immunoglobulin G (IgG), endomysial antibody (EMA)-IgA test, and small bowel biopsy in the suspected cases. In the patients with negative serology results, we used the cytotoxic method, observing alterations in leukocytes under the microscope.

Results: From 120 patients included in the study, 2 (1.6%) were confirmed with CD after serologic and histologic evaluation; 45 (37.5%) of the total number of patients presented with symptoms were diagnosed with NCGS with a high level of intolerance to gluten; and 73 patients (60.9%) had no evidence of CD or NCGS.

Conclusion: Digestive issues are frequent in our country, and for many years, the Albanian population has been a great consumer of foods containing gluten. It is necessary to perform a prompt evaluation of the symptomatic patients to prevent CD and further damage. In both cases, we advised a gluten-free diet for the patients, and most of the symptoms diminished in a few weeks, improving their quality of life.

PRENATAL AND NEONATAL TESTING

Newborn Screening in Albania

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Background: Neonatal screening tests are performed during the first days of a newborn's life upon medical request and with the consent of the parents. These tests are intended for the early detection of specific health conditions like congenital hypothyroidism, phenylketonuria, etc. Early intervention prevents the appearance of symptoms and reduces the risk of complications and mortality in the following years of life. If the screening test results are out of the normal range, additional tests can be performed. Further evaluation comes from the collaboration between the laboratory team and the neonatologists.

Following successful screening programs in industrialized countries, we have implemented in our laboratory newborn blood spot screening panel since 2016. In the past 6 years, approximately 10223 infants underwent screening for a panel of 5 disorders: Congenital Hypothyroidism (hTSH), Phenylketonuria (PKU), Glucose-6-Phosphate Dehydrogenase deficiency (G6PD), Congenital Adrenal Hyperplasia and screening for Cystic Fibrosis (CF).

Methods: A few drops of blood from the heels of the newborns were collected on specific filter papers provided. The dried blood spot samples were assessed in our laboratory using the fluorometric immunoassay intended for the quantitative determination of thyrotropin, phenylalanine, 17-OH progesterone, glucose-6-phosphate dehydrogenase and trypsinogen. The results were then evaluated to determine if they were within the normal ranges provided by the analyzer kit or had pathological results.

Results: From January 2017 until December 2022, interest in neonatal screening tests increased by more than 54.9%. From the newborns tested, approximately 1.43% had pathological results carrying the deficiency of glucose-6-phosphate dehydrogenase, an enzymopathy affecting a significant number of babies in Albania. Congenital hypothyroidism was found with pathological results in 0.015% of the newborns; deficiency of the hepatic phenylalanine hydroxylase was seen in only 0.049% of the patients; 0.28% of babies were detected with elevated 17-hydroxyprogesterone levels; and 0.23% had elevated immunoreactive trypsinogen levels, indicating the possibility of Cystic Fibrosis.

Conclusions: Neonatal screening panels continue to have enormous benefits in early intervention after detecting serious but, in most cases, treatable diseases ensuring the healthy physical and neurological development of the affected babies. Though neonatal screening tests have rapidly evolved in recent years, early detection of these disorders in Albania has provided a great approach for clinician evaluations and decisions.

QUALITY CONTROL OF THE CLINICAL LABORATORY

Evaluating Assay Precision of Glucose on Cobas C311 Roche Analyzer: "The Importance of Verification in Clinical Management"

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Introduction: The verification of examination procedures is essential for biochemical laboratories in order to evaluate the analytical characteristics of automated analyzers. This process supports laboratory quality service and clinical decision making.

The aim of our study was to perform verification process for glucose assay for Cobas c311 following the CLSI EP15-2 guidelines.

Methods: The verification was performed using two levels of quality control (QC) materials (normal and pathological) provided by the manufacturer, which were run three times a day, as three replicates for 5 consecutive days using Roche/Hitachi Cobas c311 analyzer. We compared the intra-assay coefficient of variation (CV), and the inter-assay coefficient of variation (CV) between replicates with the manufacturer's claim.

Results: The average of all results for QC material with normal values was 5.35. The intra-assay CV value was 0.24, compared with the manufacturer's claimed value which is 0.9. The inter- assay CV value was 0.25, compared with the value claimed from the manufacturer being 1.5. For the pathological QC pool, the average of all results was 12.49. The intra-assay CV value from our measurements was 0.35 in comparison to the manufacturer's intra-assay CV value of 0.8. As for the inter-assay CV value, our measurements showed 0.18 and the manufacturer claimed value is 1.4.

Conclusions: Our data have confirmed that calculated values have been consistent with the manufacturer's claimed values. This study allowed the initial training and familiarization with the instruments and the identification of operational issues. It also represented an opportunity to evaluate the QCs and to obtain analytical performance information for quality assurance.

Professionals are advised to adequately standardize their verification procedure to ensure laboratory competence and patient safety.

Keywords: analytical performance; precision; verification; laboratory standardization;

C-Reactive Protein Assay Precision Evaluation on Cobas C311 Roche Analyzer

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Background: C-Reactive Protein (CRP) is released by the liver into the bloodstream as a response to inflammation in the body. The CRP test has its everyday use aiding the diagnosis of a myriad of conditions such as severe bacterial or fungal infections, autoimmune diseases, pelvic inflammatory disease, osteomyelitis, inflammatory bowel disease and others. Measuring changes in the concentration of CRP provides useful diagnostic information about how acute and how serious a disease is. It also allows judgements about the disease genesis. Our aim was to evaluate the assay precision of the C-Reactive Protein assay on the Cobas c311 analyzer.

Methods: We used two pools of control serums, provided by the manufacturer, one with normal values and the other with pathological values. Our protocol consisted of triplicate measurements of the serums, three times a day, five days in a row. The particle-enhanced immunoturbidimetric method on Roche/Hitachi Cobas c311 analyzer was used. We compared the intra-assay coefficient of variation (CV), and the inter-assay coefficient of variation (CV) between replicates with the manufacturer's claim.

Results: The average of all results for the pool with normal values was 5.203. The intra-assay CV value was 0.99, compared with the manufacturer's claimed value which is 1.3. The inter-assay CV value was 1.16, compared with the value claimed from the manufacturer being 1.7. For the pathological pool values, the average of all results was 48.928. The intra-assay CV value from our measurements was 0.47 in comparison to the manufacturer's intra-assay CV value of 2.0. As for the inter-assay CV value, our measurements showed 0.36 and the manufacturer claimed value is 2.2.

Conclusions: In conclusion, the results demonstrate that the C-Reactive Protein assay on the ROCHE Cobas c311 platform is a reliable immunoturbidimetric assay, with the measured and calculated values being consistent with the manufacturer's claimed values with the intra- and inter-assay coefficients of variation being in the target range of 5% and 10% respectively.

SEPSIS

Determination of NEUT-RI and NEUT-GI Values in Septic Patients

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Introduction: Sepsis is a life-threatening organ dysfunction caused by an inadequate host response to infection. New hematological parameters, called extended inflammatory parameters, can be indicators of the activity of immune cells during active inflammation. Neutrophil - Reactive - Intensity (NEUT–RI) and Neutrophil - Granularity - Intensity (NEUT–GI) are indicators of neutrophil activity, that is, the early innate immune response. The aim of this research was to determine the values of these parameters in septic patients.

Materials and methods: This study included 104 patients, of whom 65 had established and proven diagnosis of sepsis, as well as 39 patients who did not have a systemic inflammatory response at the time of blood sampling. The examined group of septic patients was subsequently divided into two subgroups depending on the treatment outcome: the first subgroup of 31 patients with a favorable treatment outcome and a subgroup of 34 patients who had a fatal outcome. All patients are tested for following laboratory parameters: total number of leukocytes (Le), number of neutrophil granulocytes (Neut), number of lymphocytes (Lymph), number of immature granulocytes (IG), neutrophil reactive intensity (NEUT-RI), neutrophil granularity intensity (NEUT-GI) and C-reactive protein (CRP).

Results: The examined group of septic patients had statistically significantly higher Le values: Le [13.39x10^9/l (7.72 - 20.41) 95% CI 2.44 vs 6.92 x10^9/l ±1.52; p=0.000], Neut [12.06 x10^9/l (6.92-18.04 95% CI 2.19) vs 4.30±1.10 x10^9/l; p=0.000], IG [0.18 x10^9/l (0.07-0.51 95% CI 0.528) vs 0.02 x10^9/l (0.01-0.03 95% CI 0.004); p=0.000] NEUT-GI [156.39±5.38 SI vs 151.75±3.57 NE; p=0.000], NEUT-RI [62.64±13.24 FI vs 46.38±2.39 FI; p=0.000] and CRP [159.1mg/l (91.1-301.6 95% CI 31.68) vs 1.1mg/l (0.5-1.9 95% CI 0.392); p=0.000) compared to the control group. Subgroup of septic patients with an unwanted outcome had significantly higher NEUT-RI values (59.02±10.40 FI vs 65.95±14.77 FI, p=0.029) in relation to the subgroup of septic patients with a favorable outcome (survivors).

Conclusion: Patients who developed an unwanted disease outcome had significantly higher values NEUT-RI at the time when the diagnosis of sepsis was made.

SMART TECHNOLOGY LABS

Awareness of Medical Students About Green Chemistry, Green Labs and Sustainability

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Backgrounds: Green laboratories are the primary term for programs that help laboratories reduce footprints without compromising research quality or safety. Green chemistry, on the other hand, provides solutions to universal problems such as climate changes, sustainable agriculture, energy, toxicants and the destruction of natural resources, by designing chemical products and processes that do not involve the production and use of harmful substances. It is imperative that current and future scientists consider human health and ecological issues in their professional lives. This research was carried out to examine the knowledge of undergraduate students about green chemistry, labs and hospital whether taking or did not take the related courses.

Methods: The research group consists of 110 students in total, 57 female and 53 male studying in the field of medicine in the 2022-2023 academic year. The data were collected by using a 30-question questionnaire including "Personal Information Form" and "Opinions on Green Chemistry and Labs". Since the data was distributed normally in statistical analysis, t-test analysis was used. Descriptive statistical methods such as frequency, percentage, mean and standard deviation were used to reveal student personal characteristics.

Results: 53,64% of the students who took green chemistry course stated that their knowledge status about green chemistry was partially adequate. While 70% of those who took the course knew what green chemistry was and its purpose and principle, 30% of those who did not take the course knew. Students who took courses knew 80% of the factors that played a role in the benefits of the green hospital. As a result of the survey, there was a significant difference between the students who took the course and those who did not ($p<0.05$).

Conclusions: In order to increase the awareness of green chemistry and labs new courses should be opened that emphasize applications of both principles. Web-based resources and interactive training modules should be developed, workshops and informative meetings should be organized.

Keywords: Green Chemistry, Sustainability, Green Lab, Green Hospital

THERAPY DRUG MONITORING

Cyclosporine And Tacrolimus Use in Montenegrin Patients From 2020 To 2023

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Background: Cyclosporine (CsA) and tacrolimus (TAC) are potent immunosuppressant drugs (ISDs) administered to patients after solid organ transplantation, to prevent and treat allograft rejection. However, there are other indications of ISDs, such as endogenous uveitis, nephrotic syndrome, rheumatoid arthritis, psoriasis, atopic dermatitis. This study aims to evaluate demographic data of patients who use CsA and TAC and to what degree these medicines are used for transplantation and non-transplantation indications.

Methods: The data on the use of ISDs in the Montenegrin population were collected from L@B-IS® - Laboratory Information System used in the Clinical Center of Montenegro (CCM). Oracle Database and Microsoft Excel were used in the data processing. The Center for Clinical and Laboratory Diagnostics of CCM is the only government laboratory that monitors CsA and TAC in whole blood. ISDs monitoring is performed on the Alinity i analyzer (Abbott Laboratories, IL, USA), using the chemiluminescent microparticle immunoassay (CMIA).

Results: Between January 2020 and May 2023, 2741 analyses of ISDs were performed in the Center for Clinical and Laboratory Diagnostics in 214 patients, of which 137 (64.0%) were men, and 77 (36.0%) were women. Slightly more than two-thirds of patients (76,2%) were in the age group 19-64, followed by 28 (13.1%) patients in the age group 65-100, 12 (5.6%) and 11 (5.1%) patients in the age groups 13-18 and 1-12 respectively. The level of TAC was analyzed three times more often than CsA level; 2093 (76.4%) and 648 (23.6%) respectively. As many as 158 (73.8%) patients were referred for laboratory testing as allograft recipients, and only 1 (0.5%) patient was diagnosed with psoriasis. In 2021 and 2022, there was a significant increase in number of ISDs analyses compared to the previous year, namely 29.8% and 28.3%, respectively.

Conclusions: The study shows that over the past three years there was a growing need for monitoring ISDs concentrations, which may indicate a growing number of patients with transplanted organs. It also highlights the importance of monitoring ISDs in non-transplant patients and represents the basis for a more detailed study of ISDs.

Infliximab and Adalimumab Use in Montenegrin Patients

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Background: Infliximab (IFX) and Adalimumab (ADA) comprise the main part of Tumor Necrosis Factor (TNF) Inhibitors. TNF inhibitors are drugs that help stop inflammation and are used worldwide to treat inflammatory conditions such as rheumatoid arthritis (RA), psoriatic arthritis, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), that includes Crohn's disease and ulcerative colitis, ankylosing spondylitis and psoriasis. This study aims to evaluate demographic data of patients who use IFX and ADA.

Methods: The data on the use of IFX and ADA in the Montenegrin population were collected from L@B-IS® - Laboratory Information System used in the Clinical Center of Montenegro (CCM). Oracle Database and Microsoft Excel were used in the data processing. The Center for Clinical Laboratory Diagnostics of CCM is the only government laboratory that monitors IFX and ADA in serum, and antibodies on these medicines if they are present. ADA and IFX monitoring are performed on the Quantum Blue® 3rd generation, based on lateral flow technology, offering results within minutes using the Quantum Blue® reader to analyze the signal intensity from the test and control line to give a quantitative value.

Results: Between March 2022 and May 2023, 684 analyses of IFX, anti-IFX, and ADA, anti-ADA were performed in the Center for Clinical Laboratory Diagnostics of which 72,2% were for determination of IFX and anti-IFX, and 27,8% for ADA and anti-ADA. Out of a total of 126 patients, 84 (66,7%) were on IFX therapy, and 47 (33,3%) of them were on ADA therapy, with 5 patients belonging to both groups considering that they were switched from one drug to another. Out of a total of 84 patients on IFX, 38 (45,2%) were women and 46 (54,8%) were men. We had approximately the same percentages in 47 patients who were on ADA therapy, 26 (55,3%) of them were women and 21 (44,7%) were men. Out of a total of 84 patients on IFX, 4 (4,8%) and 8 (9,5%) patients belonged to the age groups of 1-12 and 13-18 years, respectively. While the largest number of patients were in the 19-64 age group, 70 (83,3%) of them, only two (2,4%) were between 64 and 100 years old. There were no patients in group 1–12 years old receiving ADA and almost all were in group 19-64, 43 (91,5%) patients. Two patients each from group 13-18 and 65-100 were on therapy with ADA. Although for 20 (15,9 %) patients we did not have data on the diagnosis for which they were on these therapies, for 64 (50,8%) of them, based on the data, we knew that they suffered from Crohn's disease, and 42 (33,3%) of them from ulcerative colitis.

Conclusions: Based on the data obtained for a period of 14 months, we can conclude that in Montenegro we have approximately the same number of women and men who are being treated with IFX and ADA, also that in the vast majority of them TNF inhibitors are used for management of IBD. As well as the fact that the largest number of patients are adults, which coincides with data reported in literature.

The Importance of the Value of the Total Allowable Error When Calculating the Sigma Metric for Determining the Concentration of Lithium and Tacrolimus

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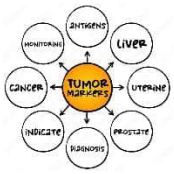
Introduction: The Sigma metric measures the quality of analyte determinations in a medical laboratory objectively and quantitatively, combining the total allowable error (TEa), bias and precision. The main problem of calculating the sigma metric is the lack of a reliable source for TEa. The aim of the work was to calculate the sigma metric for determining the concentration of lithium and tacrolimus.

Methods: To calculate the sigma metric for determining the lithium concentration, the CV value was obtained by determining two levels of control samples in a period of nine months from May 2021 to January 2022. To calculate sigma, values for TEa taken from two different literature sources (20% and 15%) were used, and the value for bias from the external quality control "Randox International Quality Assessment Scheme" (RIQAS), which was determined once a month in the same period. The calculated sigma values for determination of lithium concentration are satisfactory (values are > 3) for all months of determination, as well as for the entire period of nine months for a TEa value of 20% for both levels of control samples.

Results: If a TEa value of 15% is used for the calculation, in that case the calculated sigma value is < 3 for four of the nine months for the first level of the control sample and for three of the nine months for the second level of the control sample, respectively. To calculate the sigma metric for determining tacrolimus concentration, the CV value was obtained by determining two levels of control samples in duplicate over a period of 10 days in June 2022. To calculate the sigma, the value for TEa of 15% was used, and the value for bias from the external control of the quality of work RIQAS from June 2022. The calculated sigma value is < 3 for the first level of the control sample, which is unsatisfactory, and for the second level of the control sample, it is minimally acceptable and is 3.2.

Conclusions: The calculation of the sigma metric depends on which value of TEa is used for the calculation, and the data sources for TEa are different.

TUMOR MARKERS



Investigation of the Possible Relationship Between Galectin and Apoptosis in Gastric Cancer Patients

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Cancer is a type of disease that occurs as a result of the uncontrolled growth of cells, which has been very common in recent years, and some species have a poor prognosis. Galectin-3 is a multifunctional protein and is associated with the developmental process of tumors, including cell growth, adhesion, proliferation and metastasis. Galectin-3 has a broad effect on tumor development, including cell proliferation, apoptosis, cell adhesion, invasion, angiogenesis, and metastasis. Members of the Bcl-2 family are anti-apoptotic molecules required for the proteolytic degradation of the cell by caspases, which is the ultimate drive of programmed cell death, which plays a very important role in the regulation of the apoptotic pathway, ensures the integrity of the mitochondrial membrane and prevents the release of cytochrome C from the mitochondria. NF-kB, which is one of the important factors in cancer formation, is found in the cytoplasm, and there is a correlation between the protein levels of proinflammatory cytokines such as interleukin (IL)-1b, and IL-8, and the high incidence of cancer. To examine this mechanism in more detail, we aimed to examine the difference between Galectin, BCLF-2, Caspase3, Caspase 8, Nfkb levels before and after treatment in operable gastric cancer patients with the Elisa test. In this study, we observed a statistically significant increase in Galectin, BCLF-2, Nfkb levels in the preoperative group compared to the control. There was a statistical difference in Caspase 3, Caspase 8 levels. We observed a statistical difference in Galectin, BCLF-2, Caspase 3, Caspase 8, Nfkb levels in the postoperative group compared to the control group. Although there was no statistical difference in Galectin, BCLF-2, Caspase3, Caspase 8, Nfkb levels between pre and postoperative groups, we observed a significant decrease in Galectin, BCLF-2, Nfkb levels, and a very slight increase in Caspase 3 and Caspase 8 levels. In conclusion, we think that Galectin-3 Bcl-2 and NF-kB may be early detection markers for gastric cancer patients. We think that it is appropriate to conduct this study with more patient groups and a longer period.

Keywords: Galectin, BCLF-2, Caspase 3, Caspase 8, Nfkb

The Associations Plasma Cathepsin S with Redox Status Parameters and HDL Subclasses in Non-Hodgkin's Lymphoma Patients

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Background: Cathepsin S (Cat S) is lysosomal protease that plays an important role in intracellular protein catabolism, usually with an activity optimum at an acidic pH. Cat S is important in degrading the extracellular matrix (ECM), and is also associated with antigen- presenting cells localized in lymph and spleen, as well as other immune cells such as macrophages. Studies have shown that cat S is involved in cancer progression, invasion, angiogenesis and metastasis, particularly through its ability to degrade the ECM. Additionally, oxidative stress plays a significant role in the pathogenesis of carcinoma. The aim of the study was to examine the association of plasma cathepsin S with redox parameters and HDL subclasses in non-Hodgkin's (NHL) lymphoma patients.

Methods: 47 newly diagnosed NHL patients over the age of 18 were consecutively enrolled in the study, which was conducted at the University Clinical Centre of the Republic of Srpska, Banja Luka (Bosnia and Herzegovina) from July 2020 to April 2022. Cat S was determined by Invitrogen ELISA kits (Thermo Fisher Scientific, Inc, Waltham, MA, USA). Total oxidative status (TOS) was measured by a spectrophotometric method using o-dianisidine, and total anti-oxidative status (TAS) was measured by a spectrophotometric method using ABTS as a chromogen. Prooxidative-antioxidative balance (PAB) was determined by a modified PAB test using 3, 30, 5, 50 - tetramethylbenzidine as a chromogen. Serum paraoxonase 1(PON1) activity was measured kinetically using paraoxon and diazinon-O-analog as substrates. Advanced oxidation protein products (AOPP) were determined using a reaction with glacial acetic acid and potassium iodide. HDL particle sizes and subclass distributions were measured by gradient gel electrophoresis.

Results: Significantly higher value of Cat S was found in the NHL patients, as compared to the control group: 12.20 (10.57–14.64) vs 9.97 (8.44– 10.99). TOS and PAB were significantly higher in NHL patients as compared to healthy subjects: 12.85 (8.80-18.00) vs 9.30 (5.60-12.80), p=0.049 and 127.00 (106.00-148.00) vs 99.0 (87.00-108.00), p<0.001, respectively. The TAS was significantly reduced in NHL patients: 994 (776.0-1136) vs 1143 (999-1220), p=0.012. There was no significant difference in values of PON1 and AOPP between the lymphoma and the healthy subjects.

The correlations between Cat S and redox parameters were: PAB, rho=0.324 and p=0.023; AOPP, rho=0.301 and p=0.037; HDL3a, rho=0.421 and p=0.003; HDL3b,

rho=0.295 and p=0.040. There was no correlation Cat S with other parameters: TOS, rho=0.243 and p=0.097; TAS, rho=0.165 and p=0.264; PON1, rho=0.014 and p=0.925.

Conclusion: The results of the study contribute to a better understanding of the pathology of lymphoma, and indicate that cathepsin S could be a useful therapeutic target in the treatment of lymphoma.

Cervical Cancer: Knowledge, Attitudes, Practices of Women and Feasibility of Screening for Precancerous Lesions in a Health District of Cameroon

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Background: Cervical cancer (UCC), because of the frequency of advanced forms and the problems of adequate management that it poses, constitutes a real public health problem for developing countries in general and Cameroon in particular. Faced with this, screening using effective and inexpensive methods appears to be the best solution for these countries. The objective of this study was to assess the impact of women's knowledge, attitudes and practices on cervical cancer prevention and to study the feasibility of screening in one of the health districts of Kribi.

Methods: We conducted a descriptive cross-sectional study in six hospitals in the Kribi health district during the period April to July 2021. Our study population consisted of women aged between 21 and 65 years residing in the Kribi health district. Sampling was probability based and the Lorenz formula was used to calculate the sample size. We opted for VIA screening and FCV to optimize management. Smear slides were stained according to Papanicolaou and results were reported according to the Bethesda 2014 system. The data obtained was entered into Microsoft Excel software, SPSS software was used for data analysis, chi-square test was used for rate comparison, a variable was considered significant if $p<0.05$.

Results: Out of a total of 386 women interviewed, 239 (61.92%) were aware of the existence of UCC and 147 (38.08%) were unaware of its existence; the causes, means of transmission, risk factors, symptoms as well as prevention methods of this cancer remain poorly known. Attitudes and practices are affected by the level of knowledge, only 22.42% of women had ever used screening. A total of 330 VIA tests were performed, the prevalence obtained was 24/330 (7.27%), in which 79.17% were LSIL (low-grade squamous intraepithelial lesion) and 20.83% were HSIL (high-grade squamous intraepithelial lesion). Statistically, an association was found between the level of education and knowledge about UCC.

Conclusion: Cervical cancer is a major public health problem in Cameroon. Women in general are aware of its existence, but their knowledge of how to combat the disease is limited; their attitudes and practices are also inadequate.

Provided that a number of difficulties affecting screening practices are resolved, VIA appears to be a reliable test that is affordable and easy to set up, and can be used in areas where technical facilities are poor.

It is also important to take care of the organizational side of things, with a good reception service, proper handling of appointments and well-organized referrals.

Under these two conditions we will be able to offer a bright future for opportunistic screening using visual methods while waiting for organized screening, and thus reduce the number of advanced forms of cervical cancer.

Pretreatment Neutrophil-to-Lymphocyte Ratio as a Prognostic Biomarker in Patients with Lung, Pancreas, and Colorectal Cancer

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Introduction: The increasing incidence of cancer impels the necessity of identifying prognostic indicators associated with cancer progression and outcome. It is evident that chronic inflammation plays a crucial role in the initiation, progression, and metastasis of cancer. In the last decade, many studies have explored the association between neutrophils and cancer prognosis. Due to the easy way of calculating from patients' blood cell counts, the neutrophil-to-lymphocyte ratio (NLR) has appeared to be a convenient biomarker of cancer prognosis. However, the presence of several types of cancer, stages, and survival outcomes often makes interpretation of NLR difficult.

The aim of the study was to determine the utility of the NLR as a prognostic biomarker in patients with lung, pancreas, and colorectal cancer.

Material and method: In this retrospective study 152 patients (67 women and 85 men) with lung, pancreas, and colorectal cancer were enrolled. Their mean age was 64.86 ± 10.6 years. The patients were diagnosed and treated in the Complex Oncology Center – Burgas, Bulgaria, between March 2022 and April 2023. TNM classification, stage, previous therapy, last PET-CT for metastasis assessment, dynamics of tumor markers, complete blood count (CBC) and cancer outcome were extracted from medical documentation. CBC values were determined on a Dymind DH76 5-Part Hematology Analyzer (Shenzhen Dymind Biotechnology Co., LTD, China). The absolute number values of neutrophils and lymphocytes were obtained from CBC measured before treatment to calculate the pretreatment NLR. Standard statistical analysis including descriptive, correlation, and ROC analysis was performed. $P < 0.05$ was considered statistically significant.

Results: Patients were divided according to the disease stage: 68 were in clinical stage IV, 38 – in stage III, 26 – in stage II, and 20 – in stage I. The NLR of patients in stage IV was 6.64±3.31, statistically significantly higher compared to NLR of patients in stage III (4.91±2.51, p=0,0045), in stage II (3.91±1.19, p< 0,0001) and in stage I (2.82±0.68, p< 0,0001). Over the period assessed, 45 patients died, including 35 in stage IV and 10 in stage III. Their overall survival was 10.5 months (IQR 7.0÷16.25) and the NLR was significantly higher than that of all other patients (6,361 vs 4,290, respectively; p<0.0001). A moderate and statistically significant positive correlation of NLR to mortality was observed (Spearman r=0.3899, p<0.0001) as well as with clinical stage or progression (Spearman r=0.3708, p<0.0001). The ROC curve analysis (area under curve (AUC)=0.895, p<0.0001 at a cut-off value of NLR ≥3.0) established a high diagnostic reliability of the NLR.

Conclusion: Elevated pretreatment NLR indicates poor prognosis for patients with lung, pancreas, and colorectal cancer. NLR can be easily determined by CBC and, at a certain threshold, appears to be an accessible and reliable biomarker that can help clinicians predict the outcome of the disease.

Comparison of CA125, HE4 and ROMA Index in Predicting Ovarian Cancer

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Background: Ovarian cancer causes more deaths than any other cancer within a woman's reproductive system. Diagnosing ovarian cancer is that much more difficult due to the fact that the symptoms are rather unspecific which leads to ovarian cancer primarily diagnosed in its late stages. Tumor markers are substances that are created by cancer tissue or created in a host as a result of tumor presence. Heightened concentrations are created before a cancerous state occurs. The most important tumor markers used for diagnosis are CA125 and HE4. The aim of this work is to determine the specificity and sensitivity of markers CA125 and HE4 in the diagnosis of ovarian cancer as well as to compare the two markers. In addition, the goal of this work is to attain a ROMA index, through the combination of measured concentrations of both markers, which will point to the probability of the start of the malignant process. The research was conducted in the Department of Laboratory Diagnostics DLD) of the University Clinical Hospital Mostar (UCH Mostar) in Bosnia and Herzegovina.

Methods: Measuring the concentration of HE4 within 29 patients whose serum sample was taken beforehand, through the chemiluminescent method in two steps, via the Architect 2000i analyzer. The subjects included in the study were patients who were treated at UCH Mostar, with signed informed consent.

Results: In this work, the results of measuring the concentrations of markers CA125, HE4 and ROMA index show sensitivity of 100% and specificity of 85,7% for marker CA125, for marker HE4 sensitivity is 100% and specificity 66,7%. Results for ROMA index are the same as results for CA125. Positive predictive value (PPV) for marker CA124 is 85%, and negative predictive value (NPV) is 92%. Marker HE4 has PPV of 66%, and NPV 81%. ROMA index has the best results, PPV 86% and NPV 95%.

The ROMA index value is statistically higher in the serum of patients with ovarian cancer compared to the subgroup without ovarian cancer. This makes the ROMA index the most optimal option for the analysis of ovarian cancer.

URINE ANALYSIS - QUALITATIVE AND QUANTITATIVE

Novel Urinary Biomarkers with Potential for Clinical Application

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Background: Containing a variety of biomolecules and being easily available with noninvasive and cost-effective procedure, urine is a valuable biological sample and potential source of novel biomarkers for early diagnosis, prognosis and monitoring of many diseases including kidney diseases, prostatic and urinary bladder cancer, diabetes mellitus, autoimmune conditions, even Alzheimer's disease.

Methods: Review of studies published in Pubmed in the last 5 years with an aim to identify and highlight novel urinary biomarkers with clinical potential.

Results: Our critical evaluation of the recent literature, pointed to biomarkers like interleukin-18, NGAL (neutrophil gelatinase-associated lipocalin) to be elevated in urine of patients with acute renal failure, earlier than serum creatinine. NAG (N-acetyl-β-D-glucosaminidase) has demonstrated higher sensitivity and specificity for tubular renal injury than serum creatinine, especially its isoenzymes and combinations with biomarkers such as NGAL and KIM-1. Urinary Cystatin C has shown as a good indicator of the function of proximal tubules, strongly correlated with the need of renal substitutional therapy, and its outcome.

Urinary fibronectin and laminin, with their high sensitivity and high negative predictive values have proved useful as markers of early diabetic nephropathy and can substitute microalbuminuria in routine evaluation of patients with DM II. Urinary α1-microglobulin is associated with the longevity, severity and the treatment of DM and being directly associated with albuminuria, combined with other urinary biomarkers it is a promising biomarker of the renal damage severity in these patients. Higher levels of urinary angiotensin in patients with DM II and albuminuria were proven as useful in detecting patients with increased risk of renal or cardiovascular complications. Some biomarkers of oxidative stress such as 8OHdG (8-Oxo-7,8 dihydro-2-deoxyguanosine) and Fetuin A, are candidate markers for predicting progression and development of diabetic nephropathy.

Apart from urinary fibronectin, proven as a diagnostic biomarker for urinary bladder cancer (BC), BLCA-4 is expressed in the urothelium of affected bladder, even before cancer presentation. Aurora A kinase (AURKA) has shown good performance in discriminating low-grade BC from normal urothelium, especially among patients with hematuria. The analyses utilizing panels of urinary proteins have demonstrated increased sensitivity but suboptimal specificity for BC diagnosis.

Conclusion: The advancement in LC-MS/MS methodology has enabled large scale proteomic analysis for identification of novel urinary biomarkers, which along with bioinformatics and establishment of comprehensive urinary proteome databases, provide effective guidance for further research in this field. Still, standardization of employed preanalytical and analytical procedures is instrumental for comparing results from independent studies and drawing relevant general conclusions.

OTHER TOPIC

Presentation of the Results of HCV Testing for a 5-Year Period from 2018 to 2023 in the Bitola Clinical Hospital, North Macedonia

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Background: Hepatitis C virus (HCV) infection is a significant health problem worldwide, and is the leading cause of cirrhosis, hepatocellular carcinoma, and liver transplantation. It is estimated that approximately 130-170 million people worldwide are infected with hepatitis C virus.

Methods: This is a retrospective study conducted in Clinical Hospital Dr. Trifun Panovski Bitola, North Macedonia 4591 patients were included during a period of 5 years from 2018 to 2023 year. Individuals 5 years and older were included in this study. Assays were performed on an Abbott Architect CI 4100 platform.

Results: We detected 157 positive patients and 4434 tests were nonspecific. We analyzed positive results and we concluded that most of them were drug abuser patients. 35 of the positive patients were females age 5 to 79 years old with mean age 42.3±15.22. Concentration of anti HCV antibodies in their samples was 2.35 to 19.38 with mean of 11.81±4.79.

We detected 122 males with HCV infection with positive anti HCV antibodies, their age was 24 to 85 years with mean 39.35±9.35 years, and their concentration of anti HCV antibodies was 2.45 to 22.88 with mean 13.85±4. We can conclude that male's patients were more affected compared to females.

Conclusions: The majority of HCV infected patients are young, and injection drug use is the most common risk factor. Other, less commonly reported ways of acquiring HCV are occupational exposure to blood, high-risk sexual activity, tattooing, body piercing, and other forms of skin penetration. HCV prevention relies on identifying and counseling uninfected individuals at risk of hepatitis C infection.

Keywords: anti HCV antibody, Bitola, drug abuser, tattooing, body piercing.

The Importance of Serum Protein Electrophoresis: A Case Report

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Background: Serum protein electrophoresis (SPEP) is a test that measures specific proteins in the blood. Electrophoresis separates proteins based on their physical properties. The main clinical value of an SPEP is to determine which of the four globulin proteins is elevated, and to differentiate between monoclonal and polyclonal gammopathies. The differentiation is vitally important, as monoclonal gammopathies, which are indicated by evidence of a monoclonal (M) band on the SPEP, may indicate malignant or premalignant conditions.

Methods: During our daily routine in the laboratory, we casually found an elevated total protein. In this case we decided to perform an SPEP. We used cellulose acetate electrophoresis system ADALYA IFE.

Results: K.Z, female, 57 years coming to the laboratory for a general checkup. Blood sugar 121 mg/dl, urea 40 mg/dl, creatinine 0.89 mg/dl, alkaline phosphatase 90UI/L, ALT 53 UI/L, AST 33 UI/L, total bilirubin 0.4 mg/dl, GGT 40 UI/L, total protein 9.8 g/dl, albumin 3.8 g/dl, sodium 138 mmol/l, potassium 4.1 mmol/l, chloride 99 mmol/l, normal prothrombin time, erythrocyte sedimentation rate 94 mm/h and a normal complete blood count, except platelets 127 x 10^3/µL.

We performed SPEP and we found a monoclonal gammopathy (34.7 % gammaglobulin). After that we did immunoelectrophoresis: IgA 31 mg/dL, IgM 26 mg/dL, IgG 3518 mg/dL, kappa 0.170 g/L, lambda 10.186 g/L, and kappa/lambda ratio 0.02. Considering the importance of these results we referred the patient to a hematologist for further investigation, diagnosis, treatment and follow up.

Conclusions: SPEP is an easy, inexpensive test that should always be done in patients with elevated levels of total protein.

Clinical Justification of Extensive and Too Frequent Laboratory Retests of Oncological Patients

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Introduction: The aim of this research is to objectively consider and factually determine the disadvantages of frequent subjecting of the patient to extensive and unnecessary laboratory processing, in relation to the determination of the necessary

targeted parameters, which have unambiguously enormous importance to the ordinarius in disease monitoring and treatment.

Elaboration: In laboratory diagnostics, oncological patients themselves are one of the most vulnerable categories, malignancy often means immunocompromising and anemia, therefore the amount of blood that we subtract from such a patient during sampling is not negligible. The amount of blood taken depends on the number of tests required, while most often this implies sampling of the entire biochemical, hematological and hemostasis panel, which means all 5 test tubes. This outflow is approximately 20 mL of blood at one arrival to the laboratory.

If we take into account the low values of hemoglobin and the fact that this is repeated every 7 to 14 days, there is a real possibility that only for this reason we can endanger the general health condition of the patient. In addition to the aspect of blood volume reduction, we must not neglect the equally important aspect of difficult venipuncture, since oncology patients' veins are exhausted by long-term treatment and frequent poor general health condition, the psychological moment is also important, because frequent exposure to unpleasant and invasive venipuncture methods demoralizes these patients additionally.

Objective: This issue leads us to consider the possibility of making new recommendations regarding the parameters that are really necessary for monitoring the disease course, as well as the method of blood sampling. One of the recommendations could be the use of microvacutainer in order to sample as small volume of blood as possible and create the most comfortable feeling for the patient.

Circulating Progranulin Levels in Newly Diagnosed Obstructive Sleep Apnea Syndrome Patients

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Background: Obstructive sleep apnea syndrome (OSAS) is associated with repeated episodes of upper airway obstruction during sleep. Obstruction of the upper airway can lead to a decrease in blood oxygenation which is mainly associated with metabolic diseases. Progranulin is a glycoprotein consisting of tandem repeats of 12- cysteine module called granulin and an epithelial domain. Recently, progranulin was also found to be abundantly expressed in adipose and brain tissues. Deficiency of the secreted protein progranulin in the central nervous system causes neurodegeneration but in the peripheral tissues, progranulin excess is linked to obesity and insulin resistance. In this study we hypothesized that circulating progranulin levels are changed during sleep in OSAS patients. The aim of this study was to determine the progranulin levels in newly diagnosed OSAS patients.

Methods: OSAS patients (n=20) and age/gender matched healthy subjects (n=30) are added in the study. After polysomnographic recording, whole blood was collected. Progranulin levels were determined using commercially available ELISA kits in serum samples. Blood biochemical parameters and PSG results were correlated. Results were given as mean ± SD and $p<0.05$ were identified as significant.

Results: The apnea/hypopnea index (AHI) was significantly higher in OSAS patients (28,5 ± 21,8 vs 1,9 ± 0,9; $p<0.001$) with respect to the control groups. CRP levels are 0,34 ±0,28 mg/L in control subjects and 1,25 ± 0,70 mg/L in OSAS patients; $p<0,001$. Serum triglyceride, total cholesterol and LDL-cholesterol levels are significantly higher in OSAS patients, because HDL-cholesterol and total lipid and fasting glucose levels were not changed. The progranulin levels are significantly higher in OSAS group (245,2 ± 16,4 ng/mL) with respect to the control group (138,6 ± 24,6 ng/mL; $p<0.001$).

Conclusion: In conclusion, we demonstrated that progranulin concentrations are higher in OSAS patients which suggests that progranulin may contribute to the pathogenesis of sleep apnea.

Correlation between Automated Semen Analysis SQA VISION® and Manual Semen Assessment

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Background: Semen analysis is a very important tool that medical practitioner uses to investigate the male infertility, fertility monitoring after cancer therapy, preparation of seminal samples for ICSI/IVF, forensics, etc. The standard microscopic examination of semen samples shows high analytical variability and is time-and material-consuming. Therefore, it is important to find an adequate automated system that will replace manual methods. The aim of this study was to compare the conventional manual method of sperm analysis with the outcomes of the SQA Vision® instrument regarding sperm concentration and motility.

Methods: Semen samples collected from 79 male patients. Semen samples were collected by masturbation after four to five days of sexual abstinence. Ejaculate volumes of > 2.0 mL were included in this trial. Sperm concentration and sperm motility (% rapidly progressive, RP; % slowly progressive, SP; % non-progressive, NP and immotile, IM) were analyzed simultaneously on the SQA Vision instrument and manually using microscope.

Results: The pH (mean ± SD) of ejaculate samples were 7,6 ± 0,2. Volume of the samples was 3,3 ± 1,1 mL. The sperm concentration ($\times 10^6$/ml) (median and interquartile range (25th, 75th percentile)) determined by the manual approach and the SQA Vision®

machine were 16.0 (4.5, 52.0) vs 21.7 (6.2, 68.4). Sperm motility (RP, SP, NP and IM) (mean ± SD) analyzed manually vs analyzer were 14,76± 9,48% vs 13,99 ± 8,67%; 13,75 ± 7,09% vs 16,96 ± 9,62%; 11,68 ± 6,52% vs 10,38 ±6,87% and 48,90 ± 18,64% vs 52,02 ± 19,74%, respectively. Strong correlation of SP, NP and IM sperm motility and very strong correlation of sperm concentration and RP motility between the results obtained by manual approach and the SQA Vision was obtained.

Conclusion: SQA Vision automated semen analyzers' results of sperm concentration and motility were in good accordance with manual semen analysis.

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