UNDER THE AUSPICES OF

EFLM
EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

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International Federation of Clinical Chemistry and Laboratory Medicine

XXVIII

BALKAN CLINICAL LABORATORY FEDERATION MEETING

AND

XIII

NATIONAL CONFERENCE OF CLINICAL LABORATORY

SOFIA 2021

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XXVIII BALKAN CLINICAL LABORATORY FEDERATION MEETING

XIII NATIONAL CONFERENCE OF CLINICAL LABORATORY

WEDNESDAY, SEPTEMBER 8

15:00 - 19:00 **Registration**

THIRSDAY, SEPTEMBER 9

8:30 - 9:00 **OPENING CEREMONY**

Moderators: J. Coric, D. Svinarov, I. Paskaleva, M. Velizarova

9:00 - 9:45 **OPENING LECTURE**

Moderators: D. Svinarov, M. Velizarova

Technological Advances in Laboratory Medicine: Predicting the Lab of the Future.

Khosrow Adeli, Canada

Symposium 1.
Quality control, Neurologic diseases

Moderators: T. Deneva, A. Ruseva, J. Laleva

9:45 - 10:15 **Biochemical and Genetic Markers in Dementias.**

Christos Kroupis, Greece

10:15 - 10:30 **The Usefulness of Neurofilament Light Polypeptide Measurements in the Diagnosis of Neurological Diseases.**

Burak Arslan, Turkey

10:30 - 10:45 **Quality control paradigms: conventional QC to patient based QC.**

Deniz Ilhan Topcu, Turkey

10:45 - 11:00 **A state-of-the-art internal QC: incorporating traditional QC, EWMA and CUSUM with machine learning.**

Hikmet Can Cubukcu, Turkey

11:00 - 11:30 **Posters/ Coffee Break / Industrial Exhibition**

Industrial Workshops.

Session 1

11:30 - 12:00 **GREINER BIO-ONE: Laboratory management – people and processes in the new normal.**
Dunja Rogić

12:00 - 12:30 **BIOMEDICA/DIASORIN: The impact of CLIA methodology on the COVID-19 pandemic management in the laboratory routine – Diasorin perspective.**
Massimo Rosa, Gian Antonio Ginipro

12:30 - 13:30 **Lunch Break / Poster session/ Industrial Exhibition**

Plenary Lecture 1

Moderators: D. Svinarov, A. Tzoncheva, M. Boncheva

13:30 - 14:15 **Expanding Space for Next Generation Sequencing Clinical Applications.**
Maurizio Ferrari, Italy

Symposium 2.
New Demands in Laboratory Management

Moderators: D. Svinarov, Y. Bocheva

14:15 - 14:45 **Medical Diagnostics Data Management: Emerging Role of Artificial Intelligence (AI)-Powered eApps and Machine Learning.**
Khosrow Adeli, Canada

14:45 - 15:15 **Is There the Perfect Recipe for Leadership?**
Katerina Tosheska-Trajkovska, North Macedonia

15:15 - 15:30 **Risk Management in Medical Laboratories.**
Irini Leimoni, Greece

15:30 - 16:00 **Posters/ Coffee Break / Industrial Exhibition**

Plenary Lecture 2

Moderators: Y. Bocheva, M. Genova

16:00 - 16:45 **Preanalytical Variability: The Dark Side of the Moon in Laboratory Diagnostics.**
Giuseppe Lippi, Italy

Industrial Workshops.

Session 2

16:45 - 17:15 **ELTA 90 M: Tumor diagnostics – new perspectives from Thermo Fisher Brahms & Sysmex**
Borislav Arabadzhiev

17:15 - 17:30 **SNIBE: COVID-19 pandemic: why partnership between laboratory professionals and manufacturers is essential to control the outbreak**
Mario Plebani

17:30 - 17:45 **INTERBUSINESS: SEBIA electrophoresis: applications, challenges and advances**
Sebastien Sammut

Symposium 3.
New Psychoactive Drugs / Mass Spectrometry

Moderators: D. Svinarov, D. Gerova

17:45 - 18:00 **New psychoactive substances.**
Abuelgasim Elrasheed A. Al Hassan, UAE

18:00 - 18:15 **Determination of uracil in human plasma by a validated LC-MS/MS method for prevention of 5-fluororuracil toxicity.**
Dobrin Svinarov, Bulgaria

18:15 - 18:30 **LC-MS/MS method for the quantification of aripiprazole and dehydroaripiprazole.**
Duygu Eryavuz Onmaz, Turkey

18:30 - 18:45 **Development of liquid chromatography tandem mass spectrometric method for gliclazide.**
Sedat Abuşoğlu, Turkey

19:00 - 21:00 **Networking event**

FRIDAY, SEPTEMBER 10

Plenary Lecture 3

Moderators: Y. Bocheva, M. Genova

8:30 -9:15 **An Update on the Establishment and Utility of Biological Variation Data.**
Sverre Sandberg, Norway

Symposium 4.
Endocrinology and Metabolism

Moderators: A. Tzoncheva, D. Gerova, D. Tersieva

9:15 - 9:45 **Reliability of Laboratory and Dynamic Tests in Endocrinopathies.**
Oytun Portakal, Turkey

9:45 - 10:15 **Association between Hematological Parameters and T2DM Complications - Prospective Study.**
Dragana Milošević, Serbia

10:15 - 10:45 **Analysis of Genetic Variants Associated with Reduction of Insulin Secretion and Type 2 Diabetes Risk in Population of Bosnia and Herzegovina.**
Tamer Bego, Bosnia and Herzegovina

10:45 - 11:15 **Bone Markers, Bone Fracture and Obesity.**
Aylin Sepici Dinçel, Turkey

11:15 - 11:45 **Posters/ Coffee Break / Industrial Exhibition**

Industrial Workshops.

Session 3
ROCHE: Roche as a partner for improving healthcare

11:45 - 12:00 **Roche as a partner for improving healthcare – opening**
Hesham Sabry

12:00 - 12:10 **Roche commitment in Women's Health**
Ivana Kasanski-Braikovic

12:10 - 12:25 **Fertility and Pregnancy care biomarkers to provide meaningful results to clinicians and add value to decision-making process**
Poliya Penkova

12:25 - 12:45 **Assays for the management of emicizumab-treated patients with haemophilia A**
Yana Bocheva

12:45 - 13:45 **Lunch Break / Poster Session**

Plenary Lecture 4

Moderators: Y. Bocheva, A. Ruseva

13:45 - 14:30 **Navigating Between Technology and Professionalism: The Future of Laboratory Medicine**
Mario Plebani, Italy

Symposium 5.
Fertility, Pregnancy, Porphyria

Moderators: A. Tzoncheva, M. Genova, D. Terzieva

14:30 - 14:45 **Interdependent role of dyslipidemia and inflammation in early pregnancy with risk of late-onset preeclampsia development**
Aleksandra Stefanović, Serbia

14:45 - 15:00 **Nephrinuria as a Predictive Tool for Preeclampsia in Women with High Risk Pregnancy.**
Irena Kostovska, North Macedonia

15:00 - 15:15 **Laboratory Role in Early Pregnancy Screening for Preeclampsia.**
Aleksandra Atanasova Boshku, North Macedonia

15:15 - 15:45 **Porphyria - an Example of a Disease with Close Interactions between Laboratorians and Clinicians.**
Sverre Sandberg, Norway

15:45 - 16:15 **Posters/ Coffee Break / Industrial Exhibition**

Industrial Workshops.

Session 4
16:15 - 17:15 ABBOTT DIAGNOSTICS - Your Partner for a Healthier Tomorrow

hsTnI - an Ambulatory cardiac marker
Blagovesta Pencheva

The use of hsTnI in asymptomatic patients as a risk predictor and indication for therapy
Assen Goudev

Antibody testing for SARS-CoV-2 infection, quantitative determination, response to vaccines
Ivanova Irena Dimitrova

Symposium 6.
Cancer Diagnostics and Therapy.

Moderators: D. Svinarov, T. Deneva, V. Koleva

17:15 - 17:45 **Utilization of Liquid Profiling for Cancer Management.**
Maurizio Ferrari, Italy

17:45 - 18:15 **Basic Principles of Epigenetics and Epigenetic Regulation in Cancer therapy.**
Tomris Ozben, Turkey

18:15 - 18:30 **The Value of Prostate-specific Antigen for Prostate Cancer and Benign Prostatic Hyperplasia.**
Nafija Serdarević, Bosnia and Herzegovina

18:30 - 20:00 **BCLF Board Meeting**

20:00 - 23:00 **Working Dinner**

SATURDAY, SEPTEMBER 11

Symposium 7.
Haematology, Inflammation, Varia
Moderators: M. Velizarova, J. Ialeva, V. Tzoneva

8:30 - 9:00 **Application of targeted next-generation sequencing in BCR-ABL1-negative myeloproliferative neoplasms**
Cristina Mambet, Romania

9:00 - 9:15 **Pharmacokinetic interactions between DOACs and antiepileptic drugs.**
Ivanka Paskaleva, Bulgaria

9:15 - 9:30 **Clinical laboratory evaluation of coagulation and fibrinolysis in cancer patients.**
Snezhana Stoencheva, Bulgaria

9:30 - 9:45 **Reconsidering the Role of HDL in Cancerogenesis: Current Knowledge and Future Perspectives.**
Aleksandra Zeljković, Serbia

9:45 - 10:00 **Noninvasive Methods for Diagnosing Hepatic Fibrosis in Chronic Hepatitis C.**
Irisa Pjeci. Albania

10:00 - 10:30 **Posters/ Coffee Break / Industrial Exhibition**

Symposium 8.
Cardiology
Moderators: V. Tzoneva, D. Gerova, J. Ialeva

10:30 - 11:00 **High Sensitive Cardiac Troponin: Biological Variation, Cardiac Rhythm and Diagnostic Algorithms.**
Mario Plebani, Italy

11:00 - 11:30 **High-sensitivity Cardiac Troponin Immunoassays beyond the Diagnosis of Myocardial Infarction.**
Giuseppe Lippi, Italy

11:30 - 12:00 **The value of MMP-9 as a predictor of left atrial reverse remodeling following catheter ablation of atrial fibrillation.**
V. Koleva, Bulgaria

12:00 - 12:30 **CLOSING LECTURE**
Moderators: D. Svinarov, M. Velizarova

Current and Future Advances and Challenges in Laboratory Medicine
Tomris Ozben, Turkey

12:30 - 13:00 **CLOSING CEREMONY**
Moderators: D. Svinarov, M. Velizarova, J. Coric

13:00 - 15:00 **AGM of BSCL**

Poster Session Program

ADVANCED ANALYTICAL TECHNIQUES (N = 6)

A1

ICP-MS MULTI-ELEMENT DETERMINATION – POTENTIAL APPLICABILITY IN CLINICAL LABORATORY PRACTICE

Delyana M. Davcheva, Todorka Z. Tsvetkova, Gergana K. Kirova, Dora D. Terzieva, Maria M. Orbetzova, Veselin J. Kmetov

Bulgaria

A2

DETECTION OF MIANSERIN BY TANDEM MASS SPECTROMETRIC METHOD

Gulsum Abusoglu, Duygu Eryavuz Onmaz, Sedat Abusoglu, Fatma Humeyra Yerlikaya, Ali Unlu

Turkey

A3

FAST DETERMINATION OF ITRACONAZOLE BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Abdullah Sivrikaya, Duygu Eryavuz Onmaz, Oguzhan Tok, Hamiyet Kose, Fatma Humeyra Yerlikaya

Turkey

A4

DEVELOPMENT OF A SIMPLE LC-MS/MS METHOD FOR THE QUANTIFICATION OF LOSARTAN LEVELS

Ali Unlu, Duygu Eryavuz Onmaz, Firdevs Sak, Menekse Kuzu

Turkey

A5

THE EFFECT OF WET CUPPING THERAPY ON THE BLOOD LEVELS OF METHYLARGININE DERIVATIVES

Fatma Humeyra Yerlikaya, Duygu Eryavuz Onmaz, Berivan Unat, Hayriye Alp, Abdullah Sivrikaya, Firdevs Sak

Turkey

A6

VALIDATION OF ELISA METHOD FOR SERUM ERYTHROFERRONE EVALUATION

V. Manolov, O. Georgiev, V. Vasilev, V. Pencheva-Genova, I. Petrova, E. Hadjiev, T. Kunchev, K. Tzatchev

Bulgaria

MOLECULAR DIAGNOSTICS / MOLECULAR BIOLOGY (N = 5)

M1

ASSOCIATION OF VITAMIN D RECEPTOR SINGLE NUCLEOTIDE POLYMORPHISMS WITH ALZHEIMER'S DISEASE IN THE GREEK POPULATION

Efthimios Dimitrakis, Martha Katsarou, Maria Lagiou, Vasiliki Papastefanopoulou, Evangelia Stanitsa, Socratis Papageorgiou, Paraskevi Moutsatsou, Katerina Antoniou, Christos Kroupis, Nikolaos Drakoulis

Greece

M2

PLASMA PROGRANULIN LEVELS IN FRONTOTEMPORAL DEMENTIA (FTD) PATIENTS

Christos Kroupis, Vasiliki Papastefanopoulou, Aimilios Simoudis, Christos Koros, Petros Stamatellos, Roubina Antonellou, Evangelia Stanitsa, John D. Papatriantafyllou, Sokratis G. Papageorgiou

Greece

M3

DETERMINATION OF HLA B27 POSITIVITY - COMPARISON OF TWO METHODS

Antoaneta Mihova, Rossen Mihaylov, Blagovesta Pencheva, Stanislava Zlateva, Zhasmina Kyurkchieva, Ekaterina Teneva, Eugeni Dantchev Pentchev

Bulgaria

M4

EFFECTS OF CYP2C9 AND VKORC1 POLYMORPHISMS ON ACENOCOUMAROL SENSITIVITY AND RESPONSIVENESS DURING THE POSTOPERATIVE PERIOD AFTER CARDIAC SURGERY

Milena Velizarova, Julieta Christova, Dobrin Svinarov, Philip Abedinov

Bulgaria

M5

MOLECULAR LABORATORY TECHNIQUES USED IN RESEARCH AND MEDICAL PRACTICE: APPLIED TRAINING COURSE PRETEST/POSTTEST EVALUATION RESULTS

Rabia Semsi, Aylin Sepici Dincel

Turkey

ENDOCRINOLOGY AND METABOLISM (N = 5)

E1

SEX DIFFERENCES IN LEPTIN AND ITS CORRELATION WITH C-REACTIVE PROTEIN IN PATIENTS WITH LONG-STANDING TYPE 1 DIABETES MELLITUS

G. Chausheva, S. Shefket, Y. Bocheva, V. Iotova , K. Tsochev , S. Galcheva, I. Yotov, G. Valchev, N. Usheva, M. Boyadzhieva

Bulgaria

E2

RHYTHM OF MELATONIN, LEPTIN AND GHRELIN IN WOMEN WITH METABOLIC SYNDROME WITH AND WITHOUT IMPAIRED FASTING GLUCOSE

Vania M. Peneva, Dora Terzieva, Mitko Mitkov

Bulgaria

E3

MYELOPEROXIDASE (MPO) ACTIVITY STUDY IN OBESE AND SUBJECTS WITH METABOLIC SYNDROME

Dragana P.S., Bojana K., Andjela M., Olivera C., Vladimir C.

Bosnia and Herzegovina

E4

LABORATORY FINDINGS OF INSULIN RESISTANCE IN POLYCYSTIC OVARY SYNDROME

Anisa Daka, Ndok Marku, Hamide Shllaku, Irisa Pjeci, Emilda Belortaja

Albania

E5

COMPARISON OF PARATHYROID HORMONE (PTH) IMMUNOASSAYS ON VITROS 3600® AND COBAS-E411® ANALYZERS

A. Ćuk, I.Mikulić, V.Mikulić, K.Ljubić, Ante Pušić, Ivona Cvetković

Bosnia and Herzegovina

GASTROINTESTINAL/CARDIOVASCULAR/ CSF BIOMARKERS/DECISION LIMITS (N = 4)

G1

SALIVARY SIGA AND NITRITE LEVELS IN PATIENTS WITH HELICOBACTER PYLORI CHRONIC GASTRITIS

Mariana G. Yordanova, Daniela I. Gerova

Bulgaria

G2

FECAL CALPROTECTIN - A USEFUL LABORATORY MARKER IN GASTROENTEROLOGICAL PRACTICE

Irena I. Gencheva, Adelaida L. Ruseva, Desislava L. Pavlova, Iren A. Angelova

Bulgaria

G3

ANALYTICAL CHARACTERIZATION OF THE HIGH-SENSITIVE CARDIAC TROPONIN I ASSAY ON THE SIEMENS DIMENSION EXL SYSTEM

Y. Patokova, A. Tzenkova , I. Petrov, K. Petkova

Bulgaria

G4

CSF BIOMARKER METHOD COMPARISON IN DEMENTIA PATIENTS

Aimilios Simoudis, Kostas Valkanas, Athanassios Akalestos, Vasiliki Papastefanopoulou, Ioanna Tsantzali, Aikaterini Foska, Aikaterini Theodorou, Georgia Papagiannopoulou, Eirini Tsilipounidaki, Roubina Antonellou, Petros Stamatellos, Konstantinos Voumvourakis, Georgios Tsivgoulis, Paraskevi Moutsatsou, Sokratis G. Papageorgiou, Georgios P. Paraskevas, Christos Kroupis

Greece

HAEMATOLOGY AND HAEMOSTASIS (N = 7)

H1

REFERENCE RANGES FOR SERUM ERYTHROFERRONE IN BULGARIAN POPULATION

V. Manolov, V. Vasilev, O. Georgiev, R. Emilova, I. Petrova, V. Pencheva-Genova, E. Hadjiev, T. Kunchev, K. Tzatchev

Bulgaria

H2

MYELODYSPLASTIC SYNDROMES, REFRACTORY ANEMIA WITH RING SIDEROBLASTS AS AN IMPORTANT ENTITY OF MYELODYSPLASTIC SYNDROMES

Emilda Belortaja, Teuta Dedej, Irisa Pjeci, Anisa Daka, Adela Buzo

Albania

H3

ASSESSMENT OF PLATELET INDICES IN PATIENTS WITH LONG-STANDING TYPE 1 DIABETES MELLITUS

G. Chausheva, Y. Bocheva, V. Iotova, S. Galcheva, Y. Yotov, B. Balev, M. Boyadzhieva, N. Usheva, R. Pancheva

Bulgaria

H4

COMPARISON OF TWO DIFFERENT HAEMATOLOGICAL MODULES FOR ANALYSIS OF THE NUMBER OF PLATELETS

Marina Cukovic, Saska Djekic

Bosnia and Herzegovina

H5

INTRODUCTION OF HEPARIN-INDUCED MULTI-ELECTRODE AGGREGOMETRY METHOD FOR HEPARIN-INDUCED THROMBOCYTOPENIA TESTING

Dobrinka Dineva, Evelina Doncheva, Iva Paskaleva

Bulgaria

H6

ANTITHROMBIN DEFICIENCY IN PEDIATRIC PATIENT WITH MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C)

Evelina Doncheva, Iva Paskaleva, Dobrinka Dineva

Bulgaria

H7

HYPOCHROMIC MICROCYTIC ANEMIA IN OUTPATIENT

Hamide Shllaku Sefa, Ndok Marku

Albania

INFLAMMATION, INFLAMMATORY AND INFECTIOUS DISEASES (N= 7)

I1

IMMUNOASSAY SARS COV-2 AG QUANTITATIVE TEST IN SALIVA V.S PCR TEST IN NASOPHARYNGEAL SWAB FOR DIAGNOSIS OF COVID-19

Boncheva M., Petrov B., Lukova S., Hristova Z., Pencheva 1, Rukova 2, Nachev G.

Bulgaria

I2

SARS COV-2 PATIENTS AT HOSPITAL ADMISSION AND PLASMA LEVEL OF KL-6 FOR PREDICTING LUNG DAMAGE

Boncheva M., Lukova S., Hristova Z., Pencheva T., Nachev G.

Bulgaria

I3

HEMATOLOGICAL INFLAMMATORY INDICES ROLE IN PREDICTING THE NEED FOR INTENSIVE CARE DURING COVID-19 HOSPITALIZATION

Helena Lame, Etleva Refatllari, Valbona Tole, Arba Coraj, Adela Kryeziu, Nevila Heta, Irena Korita, Anyla Bulo

Albania

I4

THE CONTRIBUTION OF COVID – 19 SEROPREVALENCE TO COVID – 19 CONVALESCENT PLASMA COLLECTION

Margarita Kurti

Albania

I5

SEROPREVALENCE OF ANTIBODIES AGAINST COXIELLA BURNETII AMONG YOUNG CHILDREN AND ADOLESCENT WITH FEVER OF UNKNOWN ORIGIN (FUO): A PROSPECTIVE STUDY

Petia D. Genova-Kalou , Dragana Angelov , Snezhana M. Dikova, Stefka K. Ivanova , Radostina S. Stefanova , Veselin D. Dobrinov , Konstantin Simeonov

Bulgaria

I6

VIRAL INFECTIOUS AND ANEMIC SYNDROME IN PREGNANCY

I. Andonova , R. Stefanova , S. Dikova , P. Genova-Kalou , St. Krumova

Bulgaria

I7

DIFFERENCES IN BIOCHEMICAL & HAEMATOLOGICAL PARAMETERS OF NON-SURVIVE AND SURVIVE PATIENTS HOSPITALIZED FOR COVID 19

Biljana Ilkovska, Bisera Kotevska Trifunova, Sandra Hristovska

North Macedonia

PREGNANCY AND LABORATORY (N = 3)

P1

THE SERUM SELENIUM STATUS IN BULGARIAN PREGNANT WOMEN AND ITS RELATIONSHIP WITH GLUCOSE METABOLIC INDICES

M. Genova , B. Atanasova , I. Ivanova, K. Tosheva

Bulgaria

P2

FIRST TRIMESTER COMBINED SCREENING TEST: CONCENTRATION OF SERUM B-HCG I PAPP-A IN WOMEN WITH INSULIN-DEPENDENT DIABETES MELLITUS

V.Mikulić, I.Mikulić, K.Ljubić, A.Ćuk, Ante Pušić, Ivona Cvetković , Andrea Prce, Nikolina Penava, Igor Karaban, V.Tomić

Bosnia and Herzegovina

P3

CARDIOVASCULAR BIOMARKERS AND THEIR CHANGES IN HYPERTENSIVE DISORDERS OF PREGNANCY

D. Gencheva, F. Nikolov, E.Uchikova, R. Mihaylov, B. Pencheva, K. Hristova, G. Yamakova, M. Vasileva

Bulgaria

TRACE ELEMENTS/ VITAMINS/ ELECTROLYTES (N = 3)

T1

VITAMIN D LEVELS IN ADULT OUTPATIENTS FOR A PERIOD OF 8 MONTHS

Monika T. Todorova, Daniela I. Gerova

Bulgaria

T2

SLEEP APNEA INVOLVES IRON HOMEOSTASIS REGULATION

V. Manolov, O. Georgiev, V. Pencheva-Genova, V. Vasilev, R. Emilova, I. Petrova, K. Tzatchev, S. Hadjidekova, L. Traykov

Bulgaria

T3

SERUM CALCIUM DIFFERENCE IN GERIATRIC PATIENTS WITH ALZHEIMER AND VASCULAR DEMENTIA

Jordan Petrov

North Macedonia

VARIA TOPICS (N = 4)

V1

PLASMA KL-6 AS A POTENTIAL BIOMARKER FOR BPD IN NEONATES

Boncheva M., Radulova P., Slancheva B., Nachev G.

Bulgaria

V2

KLIPPEL-TRENAUNAY-WEBER SYNDROME: A CASE PRESENTATION

Irini Leimoni, Marilena Stamouli, Sofia Kougioumtzidou, Antonia Mourtzikou, Anastasios Skliris

Greece

V3

LABORATORY FINDINGS OF A DECOMPRESSION SICKNESS CASE

Irini Leimoni, Marilena Stamouli, Sofia Kougioumtzidou, Antonia Mourtzikou, Anastasios Skliris

Greece

V4

EVALUATION OF TAT FOR BIOCHEMISTRY ED SAMPLES

Irini Leimoni, Marilena Stamouli, Antonia Mourtzikou, Evaggelia Marasidi

Greece

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ABSTRACTS

OPENING LECTURE

Technological Advances in Laboratory Medicine: Predicting the Lab of the Future

Khosrow Adeli

Clinical Biochemistry, Pediatric Laboratory Medicine, the Hospital for Sick Children, University of Toronto, Toronto, Canada

Laboratory medicine is the branch of medicine that provides objective data to clinicians and other healthcare workers to guide appropriate clinical decision making. Laboratory medicine is integral to many clinical decisions on prevention, diagnosis, treatment, and disease management. It supplies health care professionals with evidence-based data necessary to provide high-quality, safe, effective and appropriate care to patients. The past 50 years have witnessed notable achievements in the field of laboratory medicine and clinical laboratory diagnostics. There have been enormous advances in clinical laboratory technology as well as its clinical applications through the identification of a growing number of laboratory biomarkers of acute and chronic disease. These technological advances have augmented the important role of laboratory medicine in healthcare delivery clearly establishing it as a vital part of the continuum of patient care. Technological innovations in analytical methodology have also had a major impact on enhancing the efficiency and quality of clinical laboratory service. Improved assay technology combined with the advent of automation have contributed to increased productivity and reduced laboratory error. Automation has particularly had a direct impact in the field of clinical pathology including clinical chemistry, immunology, serology, and hematology. Automated analyzers combined with the use of track technology has allowed the processing of thousands of samples in a single day and significant improvements in laboratory turnaround time in both hospitals and community reference laboratories. In addition to technological advances in routine chemistry and hematology, the introduction of advanced analytical instrumentation such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) into the clinical laboratory has revolutionized complex and specialized areas of laboratory testing particularly in the areas of special chemistry, therapeutic drug monitoring (TDM), toxicology, microbiology and metabolic disease screening. Another major breakthrough in laboratory medicine has been the miniaturization of assay systems and the advent of point of care testing (POCT) at patient bedside. POCT is a fast growing area and is likely to have an immense impact on the future delivery of laboratory services. During my over 30 years as a laboratory scientist, I have witnessed enormous advances in laboratory medicine and the science of clinical laboratory diagnostics. However, it has also been my experience that many clinical laboratories do not take full advantage of technological advancements as well as emerging scientific evidence in test selection and test result interpretation. What is critically needed in the field of laboratory medicine is building a culture

of innovation and adopting the concept of evidence-based laboratory medicine across the continuum of the laboratory testing process including post-analytical interpretation of laboratory test results using the latest evidence-based reference intervals.

PLENARY LECTURES

Expanding Space for Next Generation Sequencing Clinical Applications

Maurizio Ferrari

Vita-Salute San Raffaele University, Milan; Synlab Italia, Monza, Italy

The recent advances in the genomic field and the development of new technologies for DNA testing started the revolution of the diagnostic laboratory. For the diagnosis DNA-based diagnostics provide a sensitive alternative to protein-based diagnostics and the mutation detection is one of the most important areas of molecular diagnostics today. Advances in DNA analysis to develop methods, which are increasingly specific, sensitive, fast, simple, automatable, and cost-effective are considered paramount. These demands are currently driving the rapid evolution of a diverse range of newer technologies. Researchers have discovered hundreds of genes that harbour variations contributing to human illness, identified genetic variability in patients' responses to dozens of treatments, and begun to target the molecular causes of some diseases. In addition, scientists are developing and using diagnostic tests based on genetics or other molecular mechanisms to better predict patients' responses to targeted therapy. For the future of genomics is demanding the rapid evolution of high-throughput genotyping technologies (next generation sequencing) toward increased speed and reduced cost. The speed, accuracy, efficiency, and cost-effectiveness of DNA sequencing have been improving continuously since the initial derivation of the technique. With the advent of massively parallel sequencing technologies, DNA sequencing costs have been dramatically reduced. The recent introduction of instruments capable of producing millions of DNA sequence reads in a single run is rapidly changing the landscape of genetic diagnostics, providing the ability to answer questions with heretofore unimaginable speed.

Preanalytical Variability: The Dark Side of the Moon in Laboratory Diagnostics

Giuseppe Lippi

Section of Clinical Biochemistry, University of Verona, Italy

Medical errors can be traditionally clustered into 4 categories, which include errors of diagnosis, errors of treatment, errors of prevention, and an 'other miscellaneous' category. Owing to the volume and complexity of testing, and considering that laboratory error is defined as any defect from ordering tests to reporting results and appropriately interpreting and reacting on these, it is

not surprising that mistakes in the total testing process occur with frequency, have connections to all four types of medical errors and represent a serious hazard for patient health. Throughout the laboratory diagnostics, preanalytical problems prevail. Remarkable advances in instrument technology, automation and computer science have greatly simplified many aspects of previously tedious tasks in laboratory diagnostics, creating a greater volume of routine work, and significantly improving the quality of results of laboratory testing. Following the development and successful implementation of high-quality analytical standards, analytical errors are no longer the main factor influencing the reliability and clinical utilization of laboratory diagnostics. Therefore, additional sources of variation in the entire laboratory testing process should become the focus for further and necessary quality improvements. Errors occurring within the extra-analytical phases are still the prevailing source of concern. Accordingly, lack of standardized procedures for sample collection, including patient preparation, specimen acquisition, handling and storage, account for up to 93% of the errors currently encountered within the entire diagnostic process. The profound awareness that complete elimination of laboratory testing errors is unrealistic, especially those relating to extra-analytical phases that are harder to control, highlights the importance of good laboratory practice and compliance with the new accreditation standards, which encompass the adoption of suitable strategies for error prevention, tracking and reduction, including process redesign, the use of extra-analytical specifications and improved communication among caregivers.

An Update on the Establishment and Utility of Biological Variation Data

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There are many sources of variation in numerical results generated by examinations performed in laboratory medicine. Some measurands have biological variations over the span of life and others have predictable cyclical or seasonal variations. Most measurands in an individual display random variation around homeostatic set points and this is termed within-subject biological variation. The homeostatic set points vary between individuals and the variation between the set points of different individuals is termed between-subject biological variation. Estimates of analytical, within-subject, and between-subject biological variation are usually generated by prospective studies; series of specimens from a cohort of individuals are examined, followed by statistical analysis to identify and quantify the different types of variation. An EFLM Task Group has developed an evidence based database containing data on biological variation as well as estimates of analytical performance specifications and reference change values – www.biologicalvariation.eu. Applications biological variation include the ‘index of individuality’ and ‘reference change value’ where the latter is used to determine whether changes

in serial results from an individual can be explained by analytical and within-subject biological variation only. Additionally, models for setting analytical performance specifications for imprecision, bias, total error and measurement uncertainty as well as how to generate personal reference intervals will be dealt with in the present lecture.

Navigating between Technology and Professionalism: the Future of Laboratory Medicine

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The role of laboratory medicine is essential in healthcare, since in vitro diagnostic testing represents now an unavoidable part of reasoning and clinical decision making. Laboratory tests are an essential part of most care pathways, aimed at optimizing resource utilization and improving patient outcome. The activity of laboratory professionals is interconnected with all medical disciplines, and provides a crucial support for ordering the right test, for the right patient and at the right time, but also helps interpreting and using laboratory data. Although recent advancement in laboratory medicine, catalyzed by technical innovations and development of innovative tests, have promoted a substantial revolution in the organization of clinical laboratories, the future of this profession seems still ambiguous. I have hence developed a "manifesto" of laboratory medicine, meant to promote an innovative prospect of our discipline and encouraging the establishment of a new generation of laboratory professionals and managers. This manifesto has been updated taking into consideration the lessons from the COVID-19 pandemic.

ORAL PRESENTATIONS

Biochemical and Genetic Markers in Dementias

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Neurodegenerative dementias are becoming an increasingly significant burden in the developed world; a battery of clinical, imaging, psychological and laboratory tests are used to diagnose cognitive impairment and mood/behavioral/language disturbances and differentiate from other non-degenerative causes. They comprise a spectrum of diseases; mainly, are classified in one of four categories such as Alzheimer's (AD, the majority of cases ~60-70%), Frontotemporal dementia (FTD), Lewy-body dementia and Vascular dementia (although mixed cases are not uncommon). Key events in these diseases are the extracellular deposition of amyloid plaques and

the presence of neurofibrillary tangles of p-tau protein that lead to neurotoxicity and neuronal cell death. They form the basis for the latest AT(N) 2018 classification that evaluates amyloid aggregation, tau aggregation and neurodegeneration. Assessments can be performed either with CSF lab investigations or with significantly more expensive imaging techniques (PET amyloid etc.). CSF amyloid, tau and p-tau measurements are nowadays shifting from manual ELISA methods to automated immunochemical analyzers that provide improved precision; also efforts are underway for standardization and harmonization of results worldwide. A typical AD CSF profile includes reduced amyloid and increased tau and p-tau concentrations while the other dementias vary significantly. Additional biomarkers are examined in CSF in order to increase diagnosis accuracy (such as neurogranin, neurofilaments etc.) and in blood, in order to provide earlier detection in a less-invasive way. While early-onset AD refers to genetic analysis of highly-penetrant amyloid precursor protein and preselins genes, many genes confer slight increased risk for late-onset AD. A well-established intermediate-risk genetic marker for all dementias is the APOE4 allele that can be easily-assessed in most clinical labs. For FTD, genes such C9orf72, PGRN, MAPT, TARDBP, FUS etc. are examined. With the advent of Next Generation Sequencing, a panel of 25-30 genes can be examined simultaneously and detect genetic causes of all dementias. Those patients that will be identified could benefit from precision medicine novel treatments (e.g. C9orf72 and PGRN mutation positive patients).

Medical Diagnostics Data Management: Emerging Role of Artificial Intelligence (AI)-Powered eApps and Machine Learning

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Laboratory medicine is a domain which offers a unique opportunity to analyze objective patient laboratory data and enable ready communication to both healthcare workers as well as patients. In recent years, an increasing number of web-based and mobile applications has been developed to improve access to laboratory test information and test result interpretation. Computational methods, such as big data analysis, are an emerging domain that offers a unique opportunity to analyze rich and complex laboratory test result data. This data is being used to develop powerful artificial intelligence (AI) tools for clinical diagnostics. The growing number of eApps range from simple apps that provide reference lab value information to complex medical diagnostics data management. As examples, the “eLab” developed by Tru-Solutions Inc. is a comprehensive medical diagnostic center and lab management software that provides a user friendly interface and access control. It is linked iMedDx.com to allow flexible patient search and selection and includes an eLab Dashboard on mobile/tablet, allowing patients and labs/hospitals access to lab reports online. The Davis’s Laboratory & Diagnostic Tests medical app provides another useful

app with a wide-breadth of tests, as well as guidance on how to counsel and collect tests. The app is available on multiple platforms including the iPhone/iPad, Android and Blackberry. The "LabGear" is a medical lab reference app providing a pocket tool for medical laboratory test and is integrated with MedCalc with normal lab value reference information for over 200+ lab tests. There are several other medical apps that provide reference lab values including CALIPER, MedRef, Normal Lab Values, and Lab Tests. The CALIPER App has been developed in our laboratory for paediatricians, family physicians, and other healthcare workers worldwide. It is a user friendly and easy tool to assess a child's laboratory test results using the latest reference value database developed based on a study of thousands of healthy children and adolescents. The CALIPER apps allow pediatricians & family physicians to interpret laboratory test results for over 180 medical laboratory tests in children and adolescents using a comprehensive database of pediatric reference standards. WEB App: https://caliper.research.sickkids.ca/#/. In this presentation, I will review some of the key web and mobile resources in laboratory medicine and will discuss the critical importance of electronic apps in management of medical diagnostics data.

Is there the Perfect Recipe for Leadership?

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What is a Leadership? It is the process through which leaders influence the values, behavior and attitude of others. Leadership qualities can either be innate or also can be acquired. A Leader is someone who shows a direction, influences, motivates and inspires. A Leader is a person who can bring constructive change. Core values of a Leader are moral courage, integrity, decisiveness and assertiveness. Good Leader has to have: knowledge and skills, sense of priority, focus, vision, judgment, charisma, trust and emotional intelligence. A Leader motivates the team members in any situation, if they perform well or in case of failure. A good Leader applies the following approaches to lead: strategic approach, human assets approach, expertise approach, unbox approach, change approach. A Leader does the right thing at the right time, in right place. Manager does things right. A Manager is someone who plans, organizes and allocates resources, controls and solves problems. Manager administers while Leader innovates. Manager focuses on system processes, a Leader focuses on people; Manager relies on control, a Leader inspire trust; Manager has a short range view; a Leader has a long-range perspective. A Leader knows the way, goes the way and shows the way. All Managers are not Leaders but all Leaders can be Managers. **Keywords:** Leadership, Leader, Manager

Reliability of Laboratory and Dynamic Tests in Endocrinopathies

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In the last two decades, more sensitive and specific methods have been developed in diagnosis and follow-up of endocrine-disorders. Endocrinopathies may due to over/under-functioning of the gland, target cell resistance, by endocrine tumors or iatrogenic. Diagnosis needs to comprehensive clinical evaluation and differentiation from non-endocrine diseases, and baseline-laboratory tests. Today, high-sensitive methods reduced the need for dynamic-tests in diagnosis of endocrine disorders, such as HbA1C-assay for diabetes mellitus has been replaced OGTT. However, dynamic tests are important to evaluate the response of the hormonal axis and to determine the exact location of the disorder. Patient with Cushing's syndrome the basal cortisol levels may be within the reference range; however, dexamethasone suppression test (DST) is more useful for diagnosis. TRH testing is important in isolated TSH-deficiency/thyroid-hormone resistance. Dynamic-testing has been used for a long time and is a fundamental in endocrinology. Briefly; an exogenous-agent is given externally and hormonal response is observed. Dynamic-tests is not important only in diagnosis/follow-up of endocrine disorders, but also monitoring growth and pubertal-development in children. Clinicians may use different agents, test-protocols and interpretations. The response to stimulation/suppression with that agent may be different. The methods/standards used in the laboratory may be different; therefore dynamic tests are not homogeneous. They are time-consuming and risky methods; including strict-rules to be obeyed such as determining appropriate protocol, proper sampling time, pre-test patient preparation, and post-test interpretation. Selection of the proper method is important; for example, in the low-dose DST, the free-cortisol measurement by immunoassays give cross-reaction with synthetic-steroids that patient had taken, whereas mass spectrometry does not. Since, dynamic tests play a role in clinical decision-making, specific decision-limits should be selected to improve sensitivity and specificity depends on methods. Furthermore, interpretation is very critical in dynamic testing; thus, the clinician and laboratory-specialist should work together to achieve optimum-test results.

Association between Hematological Parameters and T2DM Complications - Prospective Study

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Background: Diabetes mellitus (DM) is the leading global epidemic of the 21st century, a complex disease characterized by metabolism disorders and chronic hyperglycaemia, that leads

to the development of microvascular and macrovascular complications. The aim of the research is to determine possible changes in the complete blood count (CBC) parameters in patients with Type 2 diabetes mellitus (T2DM). **Methods:** The study included a total of 137 subjects, 90 with T2DM and 47 healthy, of both gender over the age of 40 years, from the Health Care Center "Dr Milorad Mika Pavlović" Indjija, Serbia. The subjects were divided into T2DM and control group. We analysed CBC parameters, parameters of glycoregulation, lipid status using standard biochemical methods, performed anthropometric measurements and collected patients data by questionnaire and electronic patient card. **Results:** There were statistical difference for WBC (T2DM 6.98±1.47; control 6.48±1.5; p=0.045), eos (T2DM 3.32±1.78; control 2.72±1.57; p=0.048), Hgb (T2DM 143.13±11.24; control 147.8±13.39; p=0.032), MCH (T2DM 29.93±1.55; control 30.64±2.0; p=0.023), MCHC (T2DM 327.35±8.27; control 331.72±10.78; p=0.009), and neutro (T2DM 53.53±6.79; control 57.07±7.58; p=0.009), mono (T2DM 6.81±1.5; control 6.08±1.55; p=0.022), RDW(T2DM 14.26±0.66; control 13.93±0.52 p=0.006), PDW(T2DM 54.75±6.23; control 57.41±5.77; p=0.012). **Conclusion:** Based on the results of our research, it can be concluded that there is an association between particular hematological parameters and glycoregulation, diabetes mellitus, in patients with T2DM. **Keywords:** T2DM, CBC parameters, HbA1c

Analysis of Genetic Variants Associated with Reduction of Insulin Secretion and Type 2 Diabetes Risk in Population of Bosnia and Herzegovina

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Type 2 diabetes (T2D) is a chronic, complex, polygenic disease, characterized by hyperglycemia, which occurs as a result of reduced insulin secretion, inadequate response of pancreatic β cells to the progressive development of insulin resistance in peripheral tissues, or impaired glucose

regulation in the liver. Numerous studies have identified different genetic loci involved in the pathophysiology of T2D. This is the first study that investigates the impact of genetic variants (CDKAL1 (rs7754840 and rs7756992); CDKN2A / B (rs10811661); TCF7L2 (rs7903146) and KCNJ11 (rs5219)), on the reduction of insulin secretion and risk of developing T2D, and their association with the most important clinical and biochemical indicators of disease in the populations from Bosnia and Herzegovina and R. Kosovo. The study included 638 patients with T2DM and prediabetes and 360 healthy subjects as a control group recruited at the Clinical Center University of Sarajevo, University Hospital of Clinical Center in Banja Luka, General Hospital in Tešanj, and Health Center in Prizren, both sexes, aged from 40 up to 65 years. Genotyping of analyzed polymorphisms was performed by MassArray Sequenom iPlex platform in cooperation with Diabetes Center in Lund University (Malmo, Sweden), and RT-PCR method in cooperation with the Clinical Center of Charles University (Hradec Kralove, Czech Republic). The results of the study confirmed the association of analyzed genetic variants with reduction of insulin secretion and the most significant clinical and biochemical indicators but also with the higher risk of developing T2D. The most important correlations of analyzed genetic variants were with glycemic control markers, markers of insulin resistance and status of pancreatic β-cell, and also with markers of inflammation and obesity.

Bone Markers, Bone Fracture and Obesity

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A wide range of biochemical markers provide information on bone cells known as bone turnover markers which can be divided as markers of bone resorption and formation. Also bone remodeling is characterized by temporal and spatial coupling of bone formation and resorption that is necessary for normal bone structure maintenance and skeletal growth. Imbalance of bone remodeling can cause metabolic bone disorders such as osteoporosis that is characterized by the deterioration of bone mineral content and bone micro-architecture compromises bone strength leading to fractures. Osteoporotic hip fractures occur as the results of osteoporosis and decrease in bone strength by aging in both genders. The pathogenesis of osteoporosis is complex and determined by the interaction of genetic, metabolic and multiple environmental factors. In addition to all bone fragility directly increases with diabetes duration, with poor glucose control, microvascular complications and need of insulin. Although obesity has traditionally been known as a positive regulator of bone strength, recent studies have shown that obesity is an important risk factor for osteoporosis. Although low fat mass and low bone mineral density (BMD) is associated with an increased risk of fracture, conversely, overweight and obese patients also have an increased risk of fracture despite their greater BMD. Obesity primarily regulates the osteoclast, osteocyte, osteoblast and bone microcirculation; which is associated with apoptosis of osteocytes, induces the MSCs to generate more adipocytes rather than osteoblasts, and thereby increase bone marrow cavities followed by increases in bone fragility and decreased bone

microcirculation. Thus, it is of great importance to evaluate the potential cellular mechanisms how obesity may facilitate osteoporosis and bone fractures. Identification of different biomarkers for bone with other organ associations may improve the success and treatment of diseases, in an attempt to meet large groups in which specific functions of bone tissue can be assessed.

Porphyria - an Example of a Disease with Close Interactions between Laboratorians and Clinicians

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The porphyrias are mainly inherited diseases with enzymatic defects in the haem synthetic pathway. There are two main categories of porphyrias: porphyrias with cutaneous symptoms and porphyrias with acute neurovisceral symptoms. Laboratory medicine is essential to diagnose and monitor porphyrias. In the lecture an overview of laboratory aspects of porphyria includes EQA. The audience will learn how to give advice on what samples to send for porphyria diagnosing and monitoring, select the correct constituents for analysis given the clinical history, perform the analyses with high analytical quality, diagnose the different types of porphyria, give expert comments on the results, give advice on the further follow up and monitoring of the patient. The European Porphyria Network Association (EPNET) promote fundamental and clinical research in the field of porphyrias, improve knowledge of the porphyrias and facilitate best practice in their treatment and diagnosis. An overview of EPNET will be given including the activity of the Balkan countries.

Utilization of Liquid Profiling for Cancer Management

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Advanced genetic diagnostics based on circulating molecular markers requires innovative methods for the detection of minority mutant alleles. This is particularly true in the case of mixed samples, where mutations are present at a low concentration among a background of wild-type sequences. Most of the molecular alterations found in ctDNA and CTCs in plasma reflect the genetic and epigenetic changes found in primary tumors and, thus, the analysis might be valuable for tumor diagnosis and monitoring. Highly sensitive methods are required to detect those alterations among larger quantities of non-altered molecules. The molecular analysis of genetic biomarkers in plasma has also a huge significance from a cancer therapeutic perspective. The

analysis of ctDNA and CTCs in the plasma of cancer patients allow the potential opportunity to perform mutation testing without a biopsy specimen. It has also been argued that liquid biopsy analysis, compared to the analysis of tumor tissue DNA, might yield real-time information about all subclones within the tumor and about the presence of any genotypic changes responsible for development of drug resistance. The clinical value of ctDNA and CTCs in plasma is already more than a theoretical idea, since the characterization and the quantitation of such nucleic acids have been shown to be complementary tools. It is therefore expected that in the coming years, an improved understanding of the relationship between circulating nucleic acids and the molecular biology of cancer will lead to better diagnosis, management, and treatment.

Basic Principles of Epigenetic Regulation in Cancer Therapy

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Heritable phenotype changes in gene expression not involving alterations in the DNA sequence are defined as epigenetics. Epigenetic changes occur naturally during whole life span, and can be influenced by several factors including age, environment, diet, lifestyle, and disease states. Epigenetic modifications play important role in cell differentiation and may cause damaging effects leading to diseases. DNA methylation, chromatin remodeling, histone modifications, and non-coding RNAs are the major modifications causing epigenetic changes. DNA methylation commonly refers to the covalent addition of a methyl group from the s-adenosylmethionine to the fifth carbon of the cytosine base catalyzed by DNA methyltransferases resulting in 5-methylcytosine (5-mC), known as the “fifth base” of DNA. Methyl groups project into the major groove of DNA and inhibit transcription. Tissue-specific DNA methylation patterns exist in cancer, metabolic, autoimmune, and neurological diseases. Differentially methylated regions (DMRs) identified by genome-wide methylation profiling may be used as biomarkers or potential targets of epigenetic therapy. Chromatin remodeling is highly implicated in epigenetics. Histones are principle components of chromatin and covalent post-translational epigenetic modification (PTM) to histone proteins including methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation can alter the structure of chromatin resulting in transcriptional activation or repression. Global histone modification patterns have been shown to be associated with various types of cancers, many of which are regulated by natural components. Histone deacetylase (HDACs) inhibitors are currently being developed as anticancer agents. A non-coding RNA (ncRNA) is a RNA molecule transcribed from DNA, but not translated into proteins. Non-coding RNAs can be short or long and are classified by their genomic origin and mechanism of action. Given the key importance of non-coding RNAs in cell biology, therapeutic approaches based on their targeting via diverse tools are now under development and offer many advantages over classical protein-targeting therapies.

Application of Targeted Next-generation Sequencing in BCR-ABL1-negative Myeloproliferative Neoplasms

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Background: Classic *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs), represented by essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), are chronic hematopoietic stem cell disorders associated with a well-characterized profile of somatic driver mutations in *JAK2*, *CALR* and *MPL* genes. The subset of MPNs that lacks genetic alterations in the three genes is referred to as "triple-negative" (TN)-MPNs. In addition to classic MPNs, less frequently encountered entities, such as chronic neutrophilic leukemia (CNL) or MPN-unclassifiable (MPN-U) may pose diagnostic difficulties in clinical settings where only molecular tests for classic MPNs are available. In this preliminary study we aim to assess the utility and limitation of targeted next-generation sequencing (NGS) for molecular characterization of TN-MPNs and non-classic MPNs. **Methods:** A targeted NGS panel of 54 genes involved in myeloid disorders was employed to identify somatic mutations of potential clinical significance in 14 MPN patients with negative results at routine molecular tests. We used archived DNA samples obtained from peripheral granulocytes and matched CD3+ cells as a non-tumoral control. **Results:** Using this approach we were able to provide molecular characterization of 5 out 6 TN-PMF, 2 out 4 TN-ET, 3 MPN-U and establish diagnosis of 1 CNL case. We identified both pathogenic and unreported likely pathogenic somatic mutations (according to prediction software) in *ASXL1*, *TET2*, *IDH2*, *EZH2*, *SRSF2*, *U2AF1*, *TP53*, *CBL*, *SH2B3 (LNK)*, *KRAS*, *RUNX1*genes. **Conclusions:** Targeted NGS proves to be a useful tool to confirm disease clonality in MPNs with negative results at routine molecular testing, however with some limitation, especially in ET-TN cases. **Keywords**: targeted NGS, classic MPNs, TN-MPNs, non-classic MPNs, somatic mutations

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High Sensitive Cardiac Troponin: Biological Variation, Cardiac Rhythm and Diagnostic Algorithms

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The availability of high-sensitivity cTnI/CTnT (hs-cTnI/cTnT) assays has improved the accuracy of cTn measurements at concentrations around and below the 99th percentile, allowing the evaluation of biological variation. The biological variability and circadian rhythm of cardiac troponins are still debated issues. Troponin I demonstrates a diurnal rhythm with decreasing values throughout daytime and the peak concentrations in the morning. The circadian variability is statistically significant, but not relevant from a clinical viewpoint. The intra-individual variation (CVI) is lower than that reported in the literature and the index of individuality lower than 0.6 suggests a scarce value of reference interval. Other data confirm the need for further research to better understand the biological variation and circadian rhythm of both cTnI and cTnT.

High-sensitivity Cardiac Troponin Immunoassays beyond the Diagnosis of Myocardial Infarction

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Although the measurement of cardiac troponin I (cTnI) and T (cTnT) has now become the cornerstone for diagnosing cardiac injury, both ischemic and non-ischemic, recent evidence has become available that many patients display extra-cardiac causes of cTn elevations and carry a considerably enhanced risk of future mortality. The current literature data suggests that cTn elevations may be equally common in patients with cardiac and extra-cardiac diseases. Among the latter cohort of patients, the leading extra-cardiac diseases which may be responsible for either cTnI or cTnT elevations include infectious diseases/sepsis, pulmonary disorders, renal failure, malignancy, as well as gastrointestinal, neurological and musculoskeletal diseases. What also emerges rather clearly from the current literature data, is that the risk of dying for extra-cardiac diseases is higher (i.e., between two to three-fold) in patients with extra-cardiac cTn elevations than in those with cardiac pathologies, and that the most frequent cause of death

would then be infections/sepsis, followed by malignancy, respiratory disorders, myocardial infarction, gastrointestinal and neurological diseases, heart failure, stroke, cardiac arrhythmias, renal failure, psychiatric, metabolic, urogenital and musculoskeletal disorders. These figures would lead to conclude that there is a considerable risk that the underlying pathology causing cardiac injury and cTn elevation would then become the cause of death in these patients. This important evidence shall lead the way to defining appropriate and effective strategies for managing patients with extra-cardiac cTn elevations, so that their risk of future death could be prevented or limited.

The Value of MMP-9 as a Predictor of Left Atrial Reverse Remodeling Following Catheter Ablation of Atrial Fibrillation

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Background: Left atrial (LA) reverse remodeling following catheter ablation (CA) of atrial fibrillation (AF) is associated with improved procedural outcomes. We aimed to study the value of matrix metalloproteinase 9 (MMP-9) as a predictor of LA reverse remodeling in a mixed population of AF patients undergoing CA of AF. **Methods:** We report preliminary results from a single center observational study enrolling 52 patients (38 males, 73.1%) with AF (persistent in 27 cases, 42.9%) undergoing radiofrequency CA of AF. Serum levels of MMP-9 were studied preprocedurally in all patients and they all underwent transthoracic echocardiography studying LA dimensions (LA volume index, LAVI) and function. LA reverse remodeling was defined as LAVI reduction ≥ 15% assessed by echocardioraphy at 3 months after the procedure. **Results:** The mean age of the studied patients was 58.7±9.8 years. Twenty-seven of them (42.9%) had persistent AF. Mean duration of arrhythmia history was 47.1±52.7 months. LAVI at baseline was 37.4±8.5 ml. Serum MMP-9 level at baseline was 487.2±247.9 pg/ml. LAVI assessment at 3 months was available for 21 patients. Five of them (24%) demonstrated LA reverse remodeling and they had lower preprocedural levels of MMP-9: 248.1±57.9 pg/ml vs. 554.7±293.6 pg/ml in those who did not demonstrate LA reverse remodeling, P=0.013. ROC analysis showed that a preprocedural MMP-9 value of 203.5 pg/ml was able to predict LA reverse remodeling with a sensitivity of 93.8% and a specificity of 80% (AUC = 0.86). **Conclusion:** Our preliminary data shows that preprocedural levels of MMP-9 are able to predict LA reverse remodeling in patients undergoing CA of AF with a reliable diagnostic accuracy.

The Usefulness of Neurofilament Light Polypeptide Measurements in the Diagnosis of Neurological Diseases

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In the management of neurological diseases, reliable and easily accessible biomarkers are necessary for establishing the diagnosis, evaluating the prognosis, and monitoring the response to treatment. Ideally, these should be applicable not only to certain, but to various central nervous system (CNS) disease groups, such as inflammatory, neurodegenerative, traumatic, and vascular diseases. In this context, the neurofilament light chain (NfL) is a promising biomarker. The NfL is a subunit of neurofilaments, which are cylindrical proteins found in the neuronal cytoplasm that maintain axonal stability. Although neurofilaments are present in dendrites and neuronal soma, their expression is particularly high in axons. Because the NfL forms the backbone of neurofilaments and is the most soluble and abundant subunit, it has become possible to reliably measure its levels in biological fluids. NfL release dramatically increases as a result of axonal damage, regardless of the cause. NfL is a promising biomarker that would be able to be used in routine clinical laboratories for screening axonal damage.

Quality Control Paradigms: Conventional QC to Patient Based QC

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Background: Internal quality control is the primary quality control tool to evaluate the analytical phase. Recently, patient-based real-time QC (PBRTQC) has become the novel approach for quality control in association with conventional paradigms. PBRTQC essentially functions as a monitor of real-time patient results. The average of normal, moving average and EWMA are recognized as useful QC instruments in the practice of PBRTQC. PBRTQC is a cost-effective tool that helps to detect errors better than conventional QC procedures. However, a shortcoming of PBRTQC is that it is time-consuming because of the lack of guidelines and requires advanced laboratory statistics. The aim of this study is to develop an open-source web application for simulation, evaluation, and optimization of EWMA based PBRTQC. **Methods:** In this study, web-based QC data management software was developed by using the R programming language. Shiny R package was used for a user-friendly interface. Multiple test and day data can be uploaded easily. Mean and standard deviation can be entered manually or calculated dynamically using a user-selected section of data. Smoothing factor (lambda) and control limits can be adjusted in real-time. To perform a validation study of the tool, readily

available datasets were used. **Results:** The developed application can be accessed at https://denizt.shinyapps.io/tool_ewma/. Users can upload laboratory results using spreadsheets or they can be directly entered into the corresponding field. After the calculation of the EWMA, users are able to evaluate and observe the results using interactive control plots. Utilizing these plots allows users to simulate and optimize EWMA limits. The obtained results can be easily exported as a spreadsheet or PDF document. **Conclusions:** Patient-based QC procedures are valuable for clinical laboratories, however, they have demonstrated challenges. This application can be the first step to overcome these difficulties. **Keywords** Patient-based QC, EWMA, R

A State-of-the-art Internal QC: Incorporating Traditional QC, EWMA, and CUSUM with Machine Learning

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Background: Internal quality control (IQC) results are generally evaluated using conventional Westgard rules, including 1-2s, 1-3s, 2-2s, R-4s, 1-4s, and 10x. Exponentially weighted moving averages (EWMA) and cumulative sum (CUSUM) charts are other tools to assess out-of-control IQC results. The present simulation study aimed to investigate the Random Forest (RF) machine learning (ML) model, utilizing conventional IQC rules, EWMA, and CUSUM results to assess the IQC process. **Methods:** 16.000 in-control IQC results apply to the Gaussian distribution were generated using the Numpy package of the Python program. Systematic errors corresponding to 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.50, 2.75, 3.0, 3.25, 3.50, 3.75, and 4.0 standard deviations were added to the novel Gaussian distributed 16.000 IQC results. The Westgard rules, EWMA, and CUSUM charts were implemented on the data. The outcome of the IQC evaluation models was used as input variables for training our RF ML model. Then, all models' performances were evaluated on other 170.000 IQC results comprised of in-control and out-of-control IQC results by simulation. The probability of false rejections, probability of error detection, and average run length were calculated. **Results:** RF ML model had the highest probability of error detection (mean: 0.882). The lowest probability of error detection was observed for the 1-3s rules (mean: 0.277). The highest and the lowest value for the probability of false rejections belonged to 1-2s and 12x rules, respectively. The lowest and highest average run lengths were detected for 1-2s and 12x, respectively. The power chart showed that the best model for error detection was the Random Forest model. **Conclusions:** RF model was shown to be an efficient candidate for IQC result assessment. The combined utility of this new model with the rules having low ARL as an alert will enable a more effective evaluation of IQC results.

Risk Management in Medical Laboratories

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The clinical laboratory is integrated with patient care, assisting diagnosis, monitoring therapies and predicting clinical outcomes. Clinical laboratory tests ensure approximately 70% of the medical decisions, so that the time until the release of the results and its accuracy are critical for the diagnosis and the efficiency of the treatment. There are many procedures and processes that are performed in a laboratory which are highly complex and each of these must be carried out correctly in order to assure reliability and accuracy of testing. Risk is the combination of the probability of occurrence of harm and the severity of that harm. In order to manage the risks in medical laboratories we plan for risk, identify the risk, examine for risk impact, develop risk mitigation strategies, monitor and control risk outcome. Risk analysis is the systematic use of available information to identify hazards and to estimate the risk in pre-Analytical, Analytical and post-Analytical phase. Once the risks have been identified, the laboratory must implement control processes and continuously monitor and modify them to make certain that risk is maintained at a clinically acceptable level. Risk management is the systematic application of management policies, procedures and practices to the tasks of analyzing, evaluating, controlling and monitoring risk. The European Committee of Experts and Management of Safety and Quality in Health Care proposed to use the quality indicators to identify the critical stages of each process, thus being possible to assess continuously the medical processes with the aim of identifying the errors when they occur. The most common tool in order to estimate risk is known as Failure Modes and Effects Analysis, or FMEA. A universe of literature exists on FMEA and related tools like Fault-Tree Analysis and Failure Mode Effects and Criticality Analysis (FMECA).

New Psychoactive Substances

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Background: New Psychoactive Substances (NPS) and New Emergent Substances (NES), are subversive and withering substances that invaded all communities around the globe without exceptions or immunity. They are being sold as recreational drugs containing synthetically designed substances, despite the tag label states “Not for Humans Use (e.g. bath salts, synthetic cannabinoids).The aim of this paper is to enhance and add to the huge efforts of different international and national organizations, government and law enforcement authorities to combat

the spread and highlight the risks and serious implications of these substances on the health of humans and their wellbeing. **Methods:** Literature review was undertaken, with especially focus on the published data related to the objective of the paper. Some reference textbooks were also consulted, especially for the information regarding the laboratory techniques. Literature search was conducted using PubMed, EMBASE, PsycInfo, and Internet underground and governmental websites using the following keywords alone or in combination: designer drugs, club drugs, party drugs, GHB, synthetic cathinones, mephedrone, methylone, flephedrone, MDAI, and MDVP. **Results:** NPS present global health problems, side effects, dependence, and severe intoxications. Safety data on toxicity and carcinogenic potential of many NPS are not available or are very limited, long-term adverse effects or risks are still largely unknown. Purity and composition of products containing NPS are often not known, which places users at high risk as evidenced by hospital emergency admissions and deaths associated with NPS. **Conclusion:** These substances pose a global threat that will affect young generations due to the health risks associated with the consumption of these drugs. This phenomenon requires the collaboration between the international organizations, the national reference laboratories, legislation authorities, law enforcement officers and political systems in the different countries to make the necessary plans and measures capable of stopping the production and distribution of these substances. **Keywords:** New Psychoactive Substances, health problems, side effects, dependence intoxications.

Determination of Uracil in Human Plasma by a Validated LC-MS/MS Method for Prevention of 5-fluororuracil Toxicity

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Background: Plasma concentrations of 5-fluorouracil (5-FU) exhibit wide inter-individual variability depending mainly on the activity of dihydropyrimidine dehydrogenase (DPD), the enzyme that catabolizes pyrimidines. Patients with low or absent DPD activity are at increased risk of severe, sometimes lethal 5-FU toxicity. The aim of this study was to develop and validate a sensitive and selective phenotyping of DPD for prevention of 5-FU toxicity via the analysis of endogenous uracil (U) in human plasma by LC-MS/MS. **Methods:** U and d2-U (internal standard) were extracted with a mixture of ethyl-acetate and isopropanol from 100 µL of human plasma after protein precipitation with $ZnSO_4$. Chromatographic separation was performed on C18 analytical column under gradient elution with mobile phases consisting of methanol (phase A) and 0.2 mM ammonium fluoride/methanol (97.5/2.5, v/v, phase B). Positive electrospray ionization and multiple reaction monitoring were used to follow the predominant transitions: collision energy 18, m/z 113→70 (qualifier for U), m/z 113→96 (quantifier for U), m/z

115→72, and m/z 115→98 for d2-U respectively. Raw data of mass chromatograms were collected and processed by specialized software, and equal linear regression was performed to determine the concentration of U. Validation strategy was adhered to current industrial and clinical guidance. **Results**: Selectivity was assessed with 12 individual native matrices of human plasma applying the technique of standard additions at 10 µg/L and 162 µg/L, and associated with normalized matrix effect averaging 92–101%, percent matrix bias between 0 and -6%, and imprecision within 20%. Inaccuracy tested at three levels ranged from -5.7 to 12.4 % within runs and from -7.7 to 11.6 % between runs. Imprecision was billow 3.4% within-runs, and up to 7.0% between-runs. Linearity was assured in the range 6.0÷220 µg/L, R2>0.996. Freeze-thaw stability was determined for three cycles each lasting 24 h, post-preparative stability was documented for 24 h at 4ºC, short-term stability at room temperature was proven for 2 h at daylight and 2 h in the dark; stock solution stability and long term stability in plasma were documented for 98 days at -20ºC. With run time of 12 min, a throughput of over 40 samples per standard working day was achieved. **Conclusion**: The method allows an accurate and precise phenotyping of DPD via the determination of U in human plasma. **Keywords**: DPD phenotyping, uracil, LC-MS/MS

LC-MS/MS Method for the Quantification of Aripiprazole and Dehydroaripiprazole

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Background: Aripiprazole is a second generation atypical antipsychotic commonly used in the treatment of schizophrenia, major depressive disorder, bipolar disorder and autism. Its major metabolite, dehydroaripiprazole, is pharmacologically active and has similar pharmacodynamic profile to aripiprazole. The recommended therapeutic range for aripiprazole is between 150-210 ng/mL. Therapeutic drug monitoring of aripiprazole and dehydroaripiprazole is important, as serum levels of aripiprazole and dehydroaripiprazole are highly variable between individuals and distinct ranges are associated with good therapeutic response and minimal side effects. Our aim in this study was to develop an LC-MS/MS method for aripiprazol and dehydroaripiprazole. **Methods:** Briefly; 250 µL of sample was taken into eppendorf tubes and 100 µL of carbamazepine (100 ng/mL) and 800 µl acetonitrile were added. Then, the mixture was vortexed for 30 seconds and centrifuged at 2000xg for 10 minutes. The supernatants were taken into clean glass tubes and dried under nitrogen gas at 40°C. The dried residues were dissolved in 200 µl acetonitrile: water (10:90, v/v) and 25 µL were injected for analysis. **Results**: The assays for aripiprazole and dehydroaripiprazole were linear over the ranges from 2.5 to 5000 ng/ml and 0.98 to 1000 ng/ml, respectively. Total run time was 5 min. Inter-assay imprecision values were less than 9.5% and accuracy ranged from 87.4% to 112.2% for aripiprazole and dehydroaripiprazole. The extraction recoveries ranged from 91.1 to 108.7% and matrix effect values were less than 11% for the two analytes. In the freeze-thaw and long term (frozen at -20 °C for 45 days) stability studies, analytes

were found to be within ± 15% of the actual concentrations. **Conclusion**: A rapid, cost-effective, simple and robust measurement method has been developed for the quantification of aripiprazole and its active metabolite, and the method can be used for the quantification of aripiprazole and dehydroaripiprazole levels. **Keywords**: Tandem mass spectrometry, aripiprazole, dehydroaripiprazole

Development of Liquid Chromatography Tandem Mass Spectrometric Method for Gliclazide

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Background: Gliclazide is a second generation sulfonylurea medication that increases insulin secretion by binding to sulfonylurea receptors in pancreatic β cells. The common adverse effects of gliclazide are hypoglycemia, gastrointestinal disturbances, dermatological reactions such as rash, transient itching and elevated serum creatinine, alkaline phosphatase and AST levels. Therefore, monitoring of serum gliclazide levels during treatment is important for effective and safe treatment. The aim of this work was to develop a simple, rapid and accurate tandem mass spectrometric method for quantitation of gliclazide. **Methods:** 100 µL of the internal standard (carbamazepine) and 500 µL of acetonitrile with 0.1% formic acid was added on a sample of 250 µL, then vortexed for 30 s. This mixture was centrifugated at 12 000 rpm for 10 min. The supernatants were taken into glass tubes and evaporated with nitrogen gas. The residue was dissolved in 200 µL of a mixture of acetonitrile:water (50:50, v:v) then injected into the LC-MS/MS system. **Results**: The standard curves for gliclazide was linear within the range of 1.46-6000 ng/ml with a correlation coefficient (R2) better than 0.998. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.76 and 1.46 ng/ml, respectively. Total run time was 5 minutes. The intra- and inter-assay CVs were less than 9.7%. The accuracy values from the intra-day analysis were 93.5% – 104.5% while the values for the inter-day analysis were 91.2% − 109.4%. The mean recovery was 97.2% and matrix effect values were less than 12.6%. **Conclusion**: A rapid, economic, simple, accurate and sensitive method was developed for gliclazide, which could be used for routine analysis. **Keywords**: therapeutic drug monitoring, LC-MS/MS, gliclazide.

Interdependent Role of Dyslipidemia and Inflammation in Early Pregnancy with Risk of Late-onset Preeclampsia Development

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Background: The role of the altered lipid profile and inflammation in the pathogenesis of endothelial dysfunction in preeclampsia has become an important area of research during the last decades. However, the role of these parameters in preeclampsia risk assessment is still incompletely clear. The aim of this research was to investigate metabolic changes in lipids and inflammation parameters in the first trimester of high-risk pregnancies, to assess their possible association with late-onset preeclampsia development. **Methods:** We recruited 91 pregnant women with a risk for preeclampsia development. 21 developed preeclampsia (PEC), while 70 women did not develop preeclampsia despite being at risk (HRG). General biochemical parameters were measured in serum using commercially available kits. The Atherogenic Index of Plasma (AIP) was calculated as the base 10 logarithm of the ratio of the plasma concentration of triglycerides (TG) to the plasma concentration of high density lipoprotein cholesterol (HDL-C). The separation of plasma low density lipoprotein (LDL) particles and HDL particles were done by using non-denaturing polyacrylamide gradient gel (3–31%) electrophoresis. Resistin concentration was evaluated using the Human Resistin ELISA (the Enzyme-linked immunosorbent assay) development kit. **Results:** The results have shown that lipid indices, especially AIP, were significantly higher in the first trimester in PEC group ($p<0.001$), accompanied with significantly lower LDL diameter ($p<0.05$). We did not find significant changes in HDL diameter. Resistin concentration in the first trimester was statistically higher in the PEC when compared to the HRG ($P<0.001$). Analysis of the Receiver Operating Characteristics (ROC) curves indicated AIP and resistin included in the research model have very good diagnostic accuracy for preeclampsia (AUC = 0.856) and (AUC=0.870) respectively, and improved diagnostic accuracy of the basic model. **Conclusions:** We speculate that a multi-marker model which includes clinical, metabolic and inflammatory markers might be a better approach for late-onset preeclampsia risk assessment.

Nephrinuria as a Predictive Tool for Preeclampsia in Women with High Risk Pregnancy

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Background: Pre-eclampsia (PE) is characterized by new-onset hypertension and proteinuria. Damage of podocyte cells has been reported in women with PE, thus shedding of podocyte specific protein through urine such as nephrin. The aim of study was to investigate the role of

urinary nephrin (u-nephrin) levels in prediction of PE in women with a high-risk pregnancy. **Methods:** In this study were included 101 pregnant women and classified into three groups: pregnant women at high risk of developing PE (n=41), pregnant women with PE (n=30) and healthy pregnant women as controls (n=30). Urine samples were used for measurement of nephrin levels by immunoenzyme assay, creatinine and microalbumin. Blood samples were collected for biochemical analyses. **Results:** U-nephrin levels were elevated in 96.7% of women with PE, and 73% of women with a high-risk pregnancy. U-nephrin levels were significantly increased in women with a high-risk pregnancy and women with PE compared with a control group ($p<0.001$). A significant difference among subgroups of pregnant women classified according to gestational age regarding u-nephrin levels was found. There was a significant positive correlation between levels of u-nephrin and glomerular filtration rate, and significant negative correlation between levels of u-nephrin and gestational age. ROC analysis revealed that cut-off value of 304.6 ng/ml u-nephrin had sensitivity (Se) of 96.7%, specificity (Sp) of 96.7%, in distinguishing women with PE and healthy pregnancies. **Conclusion:** U-nephrin levels could be useful predictive tool for PE in women with a high-risk pregnancy. **Keywords:** high-risk pregnancy, nephrin, pre-eclampsia

Laboratory Role in Early Pregnancy Screening for Preeclampsia

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Preeclampsia (PE) is the most frequent medical complication in pregnancy and the most important cause of maternal and perinatal morbidity and mortality which affects about 3 to 5% of pregnant women. Preeclampsia is a multisystem disorder in pregnancy with a specific collection of signs and symptoms as a result of serious dysfunction of multiple systems. The timely and accurate recognition and management of PE are often challenging because diagnostic criteria are still based on nonspecific clinical signs and symptoms primarily because severity criteria correlate poorly with adverse maternal and fetal outcomes. During the past three decades, numerous clinical, biophysical, and biochemical screening tests have been proposed for the early detection of preeclampsia. There are large discrepancies in the sensitivity and predictive value of several of these tests. No single screening test used for preeclampsia prediction has gained widespread acceptance into clinical practice. Instead, its value seems to be in increasing the predictive value of panels of tests, which include other clinical measurements. Here we have evaluated some of the most frequently used biochemical markers in routine laboratory examination for timely diagnostic of preeclampsia. However, up today the most promising predictive models seems to be the mathematical models which incorporate panels of tests evaluating different aspects of maternal susceptibility and placentation, such as maternal risk

factors, mean arterial blood pressure, uterine artery Doppler, and biomarkers. There is sufficient data on some combinations of markers to make reasonable estimates of PE risk. Prospective studies are necessary to evaluate risk prediction in different populations. Furthermore, we need to evaluate the potential of novel biomarkers, generated by novel research strategies, in order to try to improve the predictive performance of the existing models.

The Value of Prostate-specific Antigen for Prostate Cancer and Benign Prostatic Hyperplasia

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Background: Prostate specific antigen (PSA) is a serine protease produced at high concentrations by normal and malignant prostatic epithelium. Only minor of PSA leak out into circulation from the normal prostate, but the release of PSA is increased in prostatic diseases. The tPSA is marker in diagnosis of carcinoma of prostate (PCa), and fPSA (free prostate specific antigen)/tPSA can improve its specificity in diagnosis of PCa. **Methods:** The serum samples of 300 patients have been analyzed using VITROS®5600 (Ortho-Clinical Diagnostic) for tPSA, fPSA and f/tPSA. The investigation include 110 patients with prostate cancer, 90 patients with benign prostatic hyperplasia (BPH) and a control group of 100 males. **Results:** Differences in the distribution of the biomarkers were seen as follows: tPSA and fPSA were significantly higher in the PCa group, and f/tPSA was significantly lower than in the BPH group. The analysis showed statistically significant differences in the values of the parameters tPSA ($x2 = 64.962$, $p = 0.000$), fPSA ($x2 = 28.421$, $p = 0.000$) and f/tPSA ($x2 = 100.825$, $p = 0.000$) between the groups. The sensitivity of tPSA between prostate cancer group and the control group was 77.5% with the specificity of 97.5%, while the sensitivity of fPSA was 57.5% with a specificity of 12.5%. AUC for tPSA was 0.866, while for fPSA was 0.680. Determination of the proportion of free PSA has become widely used to improve the cancer specificity of PSA especially in men with PSA values in the `grey zone' (4–10 μg/l). **Conclusions:** One of the earliest serum tumour biomarker predicting a subsequent diagnosis of prostate cancer is ratio of free to total PSA. Measurement of the free to total serum PSA ratio would appear to reduce false positive results among men without prostate cancer. **Keywords:** PSA, FPSA/TPSA, prostate cancer, benign prostatic hyperplasia

Pharmacokinetic Interactions between DOACs and Antiepileptic Drugs

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Background: Over the last decade, the use of DOACs (direct oral anticoagulants) has demonstrated their clinical efficacy and safety without requiring routine coagulation monitoring. However the fixed dose strategy of DOACs may not be optimal for patients taking concomitant drugs with an intensive effect on metabolic pathways. Strong inducers of CYP3A4 and p-glycoprotein (p-gp) efflux activity such as antiepileptic drugs, might increase the metabolism of anti-Xa DOACs, reducing their plasma levels and resulting in a decrease of therapeutic effect. **Methods:** A total of 120 patients on DOACs for deep vein thrombosis or pulmonary embolism were enrolled, 50 patients on apixaban (5 mg twice daily), 50 patients on rivaroxaban (20 mg once daily) and 20 of them were on dabigatran (2x150 mg daily). Blood was drawn at trough level for dabigatran or apixaban and at peak level for rivaroxaban within the first 3 months. Diluted thrombin time was performed with Hemoclot (Hyphen BioMed) for dabigatran and anti-FXa measurements for apixaban and rivaroxaban was calibrated with Hyphen DiXaI on CS 2500i. **Results:** We found marked inter-individual variability in plasma levels of DOACs expressed as median (5-95th perc) as follows for apixaban 73 ng/ml (45 – 105), for rivaroxaban 248 ng/ml (130 – 400) and for dabigatran 74 ng/ml (43 – 180). We are reporting 2 cases of a 30-years man and 61-years woman, with femoro-popliteal and ileofemoral thrombosis, both were taking sodium valproate due to underlying conditions. On this background very low level of anti-Xa activity was measured below 10-20 ng/ml after 2 x 15 mg rivaroxaban for 21 days, followed by 20 mg/d and 26 ng/ml after taking 2 x 5 mg apixaban without significant clinical improvement. We decided to switch to vitamin K antagonist treatment with acenocoumarol and received a good anticoagulant response. **Conclusions:** The pharmacokinetic interaction between valproate and rivaroxaban/apixaban, significantly reduces their plasma levels and antithrombotic efficacy. **Keywords:** direct oral anticoagulant, plasma levels, drug interactions, anti-Xa measurement

Clinical Laboratory Evaluation of Coagulation and Fibrinolysis in Cancer Patients

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Background: Prothrombotic tendency is characteristic of tumors. The aim of the study is to investigate the changes in the laboratory parameters for coagulation and fibrinolysis – fibrinogen, thrombin-antithrombin complex (TAT), Tissue factor (TF), prothrombin fragment

(F1+2), Antithrombin III (AT III), D-Dimer and tissue plasminogen activator (t-PA) in cancer patients treated with chemotherapy and radiotherapy. **Methods:** The current investigation included 80 patients, divided into 2 patient groups: breast cancer (n = 38), lung cancer (n = 42) and a control group of 65 healthy volunteers. We measured the levels of F1+2, fibrinogen, TAT, AT III, TF, D-Dimer и t-PA baseline – before treatment (visit 1); visit 2 - after the 4th course of treatment and after termination of treatment (visit 3). These parameters were investigated only once in the healthy controls. TF, TAT, F1+2, t-PA were measured by ELISA assay, AT III, D-Dimer and fibrinogen were measured by automated coagulation system Sysmex CS 2000i. **Results:** It was found that the levels of F1+2, fibrinogen, TAT, AT III, TF, D-Dimer и t-PA in cancer patients were significantly higher than these of subjects in the control group, while the levels of ATIII activity were significantly lower ($P<0.001$). During the follow-up the levels of F1+2, TAT, TF and D-dimer decreased, and baseline levels were significantly higher vs visit 2 and visit 3 ($P<0.05$). We did not find statistically significant difference in the levels of fibrinogen during the follow-up. **Conclusion:** Higher levels of TF, TAT, F1+2, t-PA, fibrinogen and D-dimer and lower activity of AT III in cancer patients support our hypothesis of association between malignant disease and coagulation disorders. Chemotherapy influences significantly the dynamic of the measured parameters. Cancer patients are at an increased risk of thrombosis and antithrombotic prophylaxis can be considered. **Keywords:** cancer, coagulation, fibrinolysis

Reconsidering the Role of HDL in Cancerogenesis: Current Knowledge and Future Perspectives

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Background: Although the association of high-density lipoprotein (HDL) with the development of malignant diseases has been largely explored, definitive conclusions are still lacking. Studies have mainly reported decreased HDL-cholesterol levels in cancer patients, but the opposite results have also been observed. Moreover, it is still unclear whether changes in HDL-cholesterol are merely a reflection of cancer-related metabolic alterations, or impaired HDL metabolism could be intrinsically involved in the development of various types of cancer. Therefore, we analyzed qualitative and quantitative characteristics of HDL particles, as well as modulators of HDL structure and functions in a cohort of patients with colorectal cancer (CRC). **Methods:** This research involved 121 patients with CRC and 101 healthy volunteers. We analysed HDL particles size and distribution by gradient-gel electrophoresis, levels of cholesterol synthesis and absorption markers within HDL particles by liquid chromatography/tandem mass spectrometry, as well as activities of lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP) and paraoxonase-1 (PON-1) by kinetic enzyme assays. **Results:** We observed

decreased HDL-cholesterol levels in CRC patients, followed by redistribution of HDL subclasses towards smaller particles. Concentrations of cholesterol synthesis and absorption markers were diminished in CRC patients, but their HDL particles were enriched with cholesterol precursors and phytosterols when compared to healthy individuals. LCAT and PON1 activities were lower, while CETP activity increased in CRC patients. **Conclusions:** Our research demonstrated that CRC patients are characterized by profound changes not only in HDL-cholesterol levels, but also in markers of HDL structure and functions. These results reopen the possibility of using HDL as prognostic and diagnostic marker in CRC and other malignant diseases. Moreover, the involvement of HDL particles in cholesterol uptake by malignant cells lays the groundwork for an innovative pharmacological approach of using these particles as vehicles for anticancer drugs.

Non-invasive Methods for Diagnosing Hepatic Fibrosis in Chronic Hepatitis C

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Background: Chronic infection with Hepatitis C virus (HCV) is a leading cause of liver-related morbidity and mortality worldwide and predisposes to liver fibrosis and end-stage liver complications. Liver fibrosis is the excessive accumulation of extracellular matrix proteins, including collagen, and is considered as a wound healing response to chronic liver injury. Its staging is critical for the management and prognosis of chronic hepatitis C (CHC) patients. The aim of this study is to evaluate the diagnostic performance of APRI and FIB – 4 by comparing it with staging of hepatic fibrosis from transient elastography, in patients with chronic hepatitis C. **Methods:** This is a correlational observational study conducted in retrospective. The study included 88 patients. From the population of patients with chronic hepatitis C who attend the Gastro-Hepatology department at QSUT, patients who were diagnosed with hepatitis C during 2017 – 2019 were selected through a convenient sampling method. **Results:** The correlation between APRI and FIB – 4 is positive ($r=0.87$, $N=88$, $p<0.01$). The correlation between APRI and elastography is positive ($r=0.67$, $N=88$, $p<0.01$). The correlation between FIB – 4 and elastography is positive ($r=0.67$, $N=88$, $p<0.01$). Diagnostic performance for the evaluation of significant fibrosis (F2) of APRI and FIB - 4 compared to elastography resulted to be good with AUROC 0.67 for APRI and 0.73 for FIB - 4. Diagnostic performance for the evaluation of cirrhosis (F4) of APRI and FIB - 4 compared with elastography it was good with AUROC 0.77 for APRI and 0.7 for FIB -4. **Conclusion:** The study shows that both APRI and FIB - 4 have a good diagnostic performance in staging liver fibrosis. **Keywords:** hepatic fibrosis, chronic hepatitis C, elastography, APRI, FIB – 4

CLOSING LECTURE

Current and Future Advances and Challenges in Laboratory Medicine

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In vitro diagnostics (IVDs) provides objective information supporting “Evidence Based Medicine” constituting a basis for accurate and fast diagnosis which leads to appropriate and more effective therapy, targets drug treatments according to patient's response, causes reduction of morbidity, provides risk prediction and reduction, allows improved compliance, monitors recovery from disease and effects of treatment which allow for reassessment and updating of therapy, shortens length of hospital stay, lowers risk of hospital infection, and improves the quality of life of patients. Clinicians are under increasing pressure for better clinical outcomes, and IVDs contribute positively to the quality of health care through screening, diagnosis, monitoring therapy, assessment of medical interventions and therapy. IVDs are a clear and rational investment in health care. IVDs have a broad scope ranging from sophisticated technologies at the cutting edge of research and development performed in clinical laboratories to simple self-test. The overall IVD market will double over the next 10 years, driven by an aging population and an increase in non-communicable and chronic diseases in both mature and emerging markets in spite of changes and challenges, increasing pressures to prove medical value, and a more stringent regulatory environment. The next generation of POC platforms is expected to grow slightly faster than the central lab market. The new European in Vitro Diagnostic Regulation (IVDR) creates a new environment for IVD companies in terms of product development, management of product lifecycle, and commercialization approach. IVD companies need to re-register their entire IVD portfolio under the new regulation by the end of the five-year transition period. CE arcing requirements, clinical and performance requirements, post-market vigilance and surveillance, a new device identification system based on Unique Device Identifiers (UDI) as well as a European databank on medical devices (EUDAMED) are introduced as new concepts for the IVDR. Clinical evidence demonstrates scientific validity, analytical performance, clinical performance, performance evaluation and their mutual relationship. IVD Manufacturers are required to develop post-market surveillance reports to monitor specific elements of safety, clinical performance, and risk/benefit ratios which may lead to a completely new infrastructure for innovation in the field of IVDs in the European Union. Laboratory Medicine should focus on Advanced & Integrative Diagnostics in order to increase its visibility beyond providing well-functioning technical service. Every possible attempt should be performed to take part in the center of the medical dialogue and to prevent to be considered as a second healthcare provider. Innovations in digitalization and automation will provide more accurate, faster results and reduction in cost.

POSTER ABSRTACTS

Advanced analytical techniques

ICP-MS Multi-element Determination – Potential Applicability in Clinical Laboratory Practice

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Background: The interest to inductively coupled plasma mass spectrometry (ICP-MS) is steadily increasing, owing to the high analytical potential for simultaneous determination of trace and ultra-trace elements and throughput of the method. However, some drawbacks have to be carefully considered, when ICP-MS method is developed, to ensure reliable approach for correction of the possible spectral and non-spectral interferences. The aim of this study was to develop and validate ICP-MS method for multielement determination of Mg, Cu, Zn, Se and Rb in human serum, and to verify its applicability by patients with thyroid disorders. **Methods:** A microwave assisted acid digestion procedure for human serum samples preparation, as well as an optimized pseudo matrix-matched calibration approach for ICP-MS were suggested. The analytical method was validated regarding linearity, instrumental LoDs, method LoQs, selectivity, trueness and precision. ICP-MS analyzer (iCapQ, Thermo Fisher Scientific, Germany) was used to quantify Mg, Cu, Zn, Se and Rb serum levels by patients with hypothyroidism (n = 33), hyperthyroidism (n = 41) and age sex matched euthyroid controls (n = 49). **Results:** The method LoQs achieved for Cu, Zn, Se and Rb were respectively 20, 13, 9.3, 55 nmol/L and 1.9 μmol/L for Mg. The intra-assay CVs, inter-assay CVs and bias% for the different elements ranged respectively 1 ÷ 2%, 1.9 ÷ 3.3% and -3.6 ÷ +1.6, with $R2 \geq 0.999$ for all elements. Results showed that the serum Mg and Se concentrations were significantly lower in both dysthytoidism groups ($p < 0.05$), while the serum Cu levels were significantly higher only in hyperthyroid patients ($p < 0.001$) vs control group. **Conclusion:** The proposed ICP-MS method showed to be reliable for Cu, Mg, Se, Zn and Rb simultaneous determination in serum samples and demonstrates a potential feasibility as a good analytical choice in clinical laboratory practice. **Keywords:** inductively coupled plasma mass spectrometry, trace elements

Detection of Mianserin by Tandem Mass Spectrometric Method

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Background: Mianserin is a tetracyclic antidepressant with relatively few anticholinergic and cardiovascular side-effects. Its clinical efficacy is comparable to that of tricyclic antidepressants. It is a potent antagonist at 5-hydroxytryptamine (5 HT) receptors and also has antihistaminic properties. Most frequently reported side effects of mianserin are sedation and weight gain. Other side effects are the occurrence of increased appetite, headache, and postural hypotension. The aim of this study was to develop a rapid and simple determination method for the determination of mianserin. **Methods:** Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an electrospray ion source (ESI) operating in positive mode. The precursor/product ion transitions of mianserin and internal standard (carbamazepine) were m/z 265.2/91.1 and m/z 237.1/193.1, respectively. Sample preparation procedure was briefly as follows: 100 µL of the internal standard (carbamazepine) were added to 200 µL sample and vortexed for 1 min. Protein precipitation was achieved by adding 550 µL of acetonitrile. The mixture was centrifuged at 2000 g for 10 min. The supernatants were evaporated under nitrogen. The residue was dissolved in 200 µL acetonitrile:water (20:80, v:v) and 30 µL were injected for analysis. **Results:** The linearity range and correlation coefficient were 1.95–1000.0 ng/ml and 0.996 respectively. The retention time of mianserin and total run time were 1.03 and 3 min, respectively. The intra- and inter-assay imprecision ranged from 3.2% to 9.8%. The inter-day accuracy values ranged from 93.26 to 114.58%. The mean extraction recovery of the method was found to be 95.58% and matrix effect values were less than 12.7%. **Conclusion:** A simple, rapid, cost-effective and robust LC–MS/MS method has been developed for the detection of mianserin.
Keywords: drug level monitoring, LC-MS/MS, mianserin

Fast Determination of Intraconazole by Liquid Chromatography Tandem Mass Spectrometry

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Background: Itraconazole is a broad-spectrum triazole antifungal agent. It is poorly soluble in water and these properties make the oral absorption of itraconazole difficult. Therapeutic drug monitoring can be considered because itraconazole concentration differs between individuals.

The aim of this study was to develop a simple, fast and accurate tandem mass spectrometric method for determination of itraconazole. **Methods:** Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an electrospray ion source (ESI) operating in positive mode. The ions transitions used for quantification were selected as m/z 705.3>392.4 for itraconazole and m/z 475.2>58.0 for internal standard (sildenafil). Chromatographic separation was performed on a C18 column (4.6×50 mm, 5 µm) with a mobile phase consisting of 1% formic acid in water and of 1% formic acid in acetonitrile at a flow rate of 1 ml/min. The sample preparation procedure was briefly as follows: 100 µL sildenafil and 600 µL methanol were added to 250 µL sample and vortexed for 30 s. Afterward, the mixture was centrifuged at 2000 g for 10 min and 25 µL of the supernatant were injected for analysis. **Results:** The standard curves for itraconazole were linear within the range of 1.22-10.000 ng/ml with a correlation coefficient $r \geq 0.995$. The lower limit of quantification (LLOQ) was 2.44 ng/ml. Total run time was 5 minutes. The retention time of itraconazole was 3.03 minutes. The intra- and inter-day imprecision values were below 8.5% and 11.9%, respectively. Inter-day accuracies ranged between 87.8–114.8%. The recovery values varied between 95.9 and 103.8% and matrix effect values were less than 12.5%. **Conclusion:** We developed a fast, accurate and simple method for measuring itraconazole levels. This method allows the determination of nonadherent patients and toxic levels within a wide linearity range. **Keywords:** therapeutic drug monitoring, LC-MS/MS, itraconazole

Development of a Simple LC-MS/MS Method for the Quantification of Losartan Levels

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Background: Losartan is an orally used angiotensin II receptor antagonist for the treatment of hypertension. Losartan is superior to previous peptide receptor antagonists and angiotensin converting enzyme inhibitors due to its high specificity, selectivity and tolerability. Common adverse effects related with losartan are cough, diarrhea, fatigue, hyperkalemia, renal insufficiency, angioedema, hypoglycemia, anemia and urinary tract infection. The aim of this study was to develop a sensitive, rapid and simple measurement method for the quantification of losartan in biological samples. **Methods:** Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an electrospray ion source (ESI) operating in positive mode. Briefly, 100 µL internal standard (200 ng/mL carbamazepine) and 750 µL acetonitrile were added to 250 µL sample solution and vortexed for 30 seconds. The mixture was centrifuged at 2000×g for 15 minutes and 30 µL of supernatant was injected into the LC-MS/MS system. **Results:** The method was linear in the range 1.95-2000 ng/ml with a correlation coefficient (R2)

of 0.997. Total run time was 3 minutes. Intra- and inter-assay CV% values ranged between 2.8 and 9.6%. The inter-assay accuracy values ranged from 86.4 to 112.8%. The predicted concentrations of losartan deviated within ±15% of the nominal concentrations in autosampler (48 h), reinjection (24 h), repeated three freeze–thaw cycles. The matrix effect values were less than 12%, while the extraction recovery values ranged from 88.1% to 112.8%. **Conclusion:** We have developed a rapid, simple, cost-effective and reproducible LC–MS/MS method for quantitating losartan levels. **Keywords:** LC-MS / MS, losartan, side effect, drug level monitoring

The Effect of Wet Cupping Therapy on the Blood levels of Methylarginine Derivatives

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Background: Wet cupping therapy (WCT) is a simple and economical traditional treatment; however its mechanism of action still requires scientific interpretation. WCT is used as a prophylactic and/or complementary in the treatment of diabetes, hypertension and hyperlipidemia, and therefore thought to be beneficial in the prevention and control of cardiovascular diseases. Methylarginine derivatives such as asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-N-monomethyl arginine (L-NMMA) are independent risk factors for cardiovascular diseases. The aim of the study is to contribute to the elucidation of the mechanism of action of WCT in cardiovascular diseases by measuring ADMA, SDMA, L-NMMA and arginine levels in wet cupping and venous blood samples taken concurrently from subjects who underwent WCT. **Methods:** WCT was implemented to 50 volunteer women. Venous blood and wet cupping blood samples were taken concurrently. Venous blood and wet cupping blood samples arginine, ADMA, SDMA, and L-NMMA levels were measured using a validated tandem mass spectrometric method through a pretreatment procedure requiring derivatization with butanol solution including 5% (v/v) acetyl chloride. **Results:** ADMA [0.24 (0.09-0.45) μM vs 0.21 (0.12-0.35) μM, p=0.022] and L-NMMA [0.017 (0.01-0.11) μM vs 0.015 (0.01-0.25) μM, p=0.005] levels were statistically significantly higher in wet cupping samples compared to venous blood, while there was no significant difference between arginine [84.2 (44.2-186.4) μM vs 74.4 (22.0-149.4 μM, p=0.129] and SDMA [0.17 (0.07-0.31) μM vs 0.16 (0.04-0.30) μM, p=0.542] levels. The inter-assay CV% values for all analytes were less than 9.8% of the tandem mass spectrometric method used in the quantitation

of analytes. Recovery% values ranged from 94.1% to 108.9% for all analytes, while the matrix effect% ranged from 1.2% to 8.1%. **Conclusion:** WCT removes methylarginines, therefore the therapeutic effects of wet cupping in cardiovascular diseases may be due to the excretion of methylarginines from the body. **Keywords:** Wet cupping therapy, cardiovascular diseases, nitric oxide, methylarginines.

Validation of ELISA Method for Serum Erythroferrone Evaluation

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Background: Erythroferrone (ERFE) is newly discovered regulator of hepcidin synthesis, produced from erythroblasts. ERFE is involved into iron homeostasis, an important trace element with a dual role in the human organism. We aimed to validate ELISA method for quantification of serum hepcidin in Bulgarian population. **Methods:** Validation of immunosorbent quantification method for ERFE in serum in Bulgarian population went through several evaluations like analytical scope through calibration curve, limit of detection (LD), low (LLOQ) and upper (ULOQ) limit of quantification, middle point (MPQ) of quantification, intra- and inter-assay precision. **Results:** For calibration curve were used recombinant human ERFE with level 10 ng/ml, from which after proper dilutions the required clinically relevant values were established. Each and every standard was measured twice, and corrected against blank reagent sample. The calibration curve was four parametric; logarithmic by axis X, linear by the axis Y. LD was evaluated by ten times measured blank reagent sample. We established 0.056 ng/ml, which ensure very high diagnostic sensitivity. Evaluation of LLOQ, MPQ and ULOQ showed CV < 8% and bias < 10%. Trueness of method was evaluated using recovery procedure, which showed area from 96.7% up to 97.9%. Intra-assay precision showed CV < 5%; inter-assay repeatability – CV < 6%. **Conclusions:** The immunological ELISA method we choose for serum erythroferrone quantification showed high specificity and sensitivity during validation process. It is a new parameter in Bulgarian clinical laboratory practice. **Acknowledgements:** This project is sponsored by MU-Sofia, as part of Grant D-57/2019.

Molecular diagnostics/molecular biology

Association of Vitamin D Receptor Single Nucleotide Polymorphisms with Alzheimer's Disease in the Greek Population

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Background: Study primary objective was to investigate potential association of specific VDR gene single nucleotide variations with the development of Late onset Alzheimer's disease (LOAD) in a Southeastern Caucasians cohort. Secondary- to evaluate the relationship of those variations to APOE e4 carriers, Mini–Mental State Examination (MMSE) and Frontal Assessment Battery (FAB) scores. **Methods:** The cohort included 90 confirmed LOAD patients (median age 74 years, male 48.9% - female 51.1%, median MMSE score 21, median FAB score 10) and 103 unmatched healthy controls (median age 57 years, male 49.5% - female 50.5%). Blood samples were analyzed to determine the genotypes of TaqI (rs731236), BsmI (rs1544410) and FokI (rs2228570). After DNA extraction and Polymerase Chain Reaction (PCR) BsmI and FokI were genotyped using the RFLP method. Genotypes of TaqI were determined using LightSNiP (SimpleProbe®) real-time qPCR assay. **Results:** The frequencies (%) of TaqI TT, TC and CC genotypes in controls/patients was 34.0/48.9, 47.6/41.1 and 18.4/10.0 respectively. A statistically significant difference was observed for TaqI C allele (TT vs CT+CC, OR 0.54, 95% CI 0.30-0.96, p=0.035), for TaqI TT genotype (CC+TC vs TT, OR 1.86, 95% CI 1.04-3.32, p=0.035) and for TaqI CC genotype in relation to MMSE score <21 in the patient's group (CC vs TC+TT, OR 0.128, 95% CI 0.015-10.72, p=0.036). **Conclusion:** TaqI TT increases the risk of developing LOAD by 1.86 times. TaqI C allele might act protectively with 46% lower risk of developing the disease. TaqI CC patients have 87% less likelihood of severe cognitive impairment based on MMSE score. No association of the investigated polymorphisms was

observed with FAB score or the presence of APOE e4 allele. **Keywords:** Vitamin D Receptor SNIPS, Alzheimer disease

Plasma Progranulin Levels in Frontotemporal Dementia (FTD) Patients

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Background: Frontotemporal Dementia (FTD) is as common as Alzheimer's disease in patients <65y. Progranulin gene (PGRN) is one of many FTD-associated genes. It encodes for a 593-amino acid secreted protein that can lead to neurodegeneration in case of reduced concentrations. It can be easily detected in CSF or plasma and could assist in a referral for PGRN genetic analysis. **Methods:** In 36 well-ascertained FTD patients, we collected EDTA peripheral blood after obtaining their informed consent. We performed both plasma progranulin measurements with an ELISA method (Adipogen) and DNA genetic analysis in 21 FTD genes (among them the PGRN gene as well). **Results:** Three patients were detected with deleterious frameshift mutations, one patient with a rare missense variant of unknown clinical significance (p.T310P) and 32 were negative for any alteration in the PGRN gene. Plasma progranulin measurement of these 32 negative patients showed a normal distribution with an average 232.78 ng/ml (SD±48.57) while the 3 positive patients showed a range between 59-92 ng/ml (25-75o interquartile). **Conclusions:** Based on the used method and statistical analysis, FTD patients with plasma progranulin levels above 136 ng/ml can be safely considered that they do not bear PGRN mutations with 95% confidence while patients with <92 ng/ml should be offered PGRN genetic analysis so that they could benefit from novel, emerging Precision Medicine therapeutic approaches such as intracisternal PGRN gene therapy or intrathecal anti-sortilin antibodies. Furthermore, evaluation of PGRN gene expression through plasma progranulin protein levels could shed light in assessment of the pathogenicity of some rare PGRN gene alterations.

Determination of HLA B27 Positivity- Comparison of Two Methods

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Background: HLA-B27 is an MHC class I molecule that is associated with ankylosing spondylitis (frequency 90%), with some other rheumatic diseases but can be found also in healthy individuals (frequency 8%). The B27 antigen expression can be detected quickly by flow cytometry but the used anti-HLA-B27 antibody may cross-react, most often with HLA-B7. The producer’s recommendation is to confirm potential low positive and some negative results in the so-called grey zone with another referent method. We confirm the potentially “false” results with high sensitive molecular method - real-time PCR. **Methods:** For six mounts 441 people were tested in our lab for HLA B27 positivity. The peripheral blood with EDTA was used for both methods. All samples were processed with flow cytometry BD HLA -B27 kit (Becton Dickenson) for direct immunofluorescence staining with the two-color combination - anti-HLA-B27 FITC/CD3 PE. After erythrocyte lysis and washing, the probes were acquired and analyzed using BD Calibur and HLA B27 clinical software. Ten channels zone from both sides of referent marker was determined as a grey zone. The probes in this area were confirmed with the HLA-B27 REAR TIME PCR Genotyping kit (DNA TECHNOLOGY). **Results:** From 1 January to 25 June 2021, we determined that 4.5% (20/441) of the results fall in the grey zone. The referent marker was at channel 150. Sixteen samples were low positive - from 150 to 160 channels. Four probes were in the negative grey zone - from 150 to 140 channels. All these “suspicious” samples were confirmed with real-time PCR. The results demonstrate complete coincidence between two different methods. **Conclusions:** The flowcytometry method for HLA B27 determination is fast and very specific but due to the cross-reactivity with other HLA B antigens, some results were inconclusive. This requires confirmation by alternate methods - another flow cytometry kit or molecular methods.

Effects of CYP2C9 and VKORC1 Polymorphisms on Acenocoumarol Sensitivity and Responsiveness during the Postoperative Period after Cardiac Surgery

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Background: Cardiac surgery is associated with an inflammatory response promoting a hypercoagulable state, which requires successful postoperative anticoagulation. Our aim was to investigate the influence of genotype variants at two loci responsible on acenocoumarol metabolism. **Methods:** 200 post-op patients given acenocoumarol thromboprophylaxis were reviewed for INR values on the first five days of acenocoumarol treatment. Genotyping for VKORC1 1639G>A and CYP2C9*2&*3 polymorphisms were done by RT-PCR. Significant

differences among genotypes in INR were assessed. **Results:** Median age at surgery was 63.9 ± 10.8 years; 66.5% of patients were male. Median BMI was 28.6 ± 5.4 kg/m2. Genotype distribution for VKORC1 and CYP2C9 was G/G*1/*1–24.5%, G/G*1/*2–9.5%, G/G*1/*3– 7%, A/G*1/*1–27%, A/G*1/*2–10%, A/A*1/*1– 9.5% and A/A*1/*2–5%. Comparison of INR revealed no significant differences among genotypic groups on days one (p = 0.35) and five (p = 0. 51). Differences in INR were detectable by day three (p = 0.02). Normal responders were more likely to be subtherapeutic with INR<1.5 (p<0.05) compared to those with mutant genotype. Genotypes A/G*1/*1, A/G*1/*2, A/A*1/*1 and A/A*1/*2 are associated with a higher incidence of "hyperreactive" results (INR> 3.5) and a higher risk of bleeding. The comparison of normal responders (n = 82) to the collective cohort of hyperresponders (n = 103) revealed that the former group had both a significantly lower INR (p= 0 .006) and carried higher relative risk being subtherapeutic (61% vs. 16.2%, P < 0 .001) on the third day of acenocoumatol treatment. **Conclusions:** Presence of a mutant allele of VKORC1 1639G>A and CYP2C9 *2 and *3 increased the odds of requiring a lower mean dosage of acenocoumarol. Wild-type patients at both loci had yet to reach therapeutic INR or being subtheraputic by day three more frequently than those with mutation variants. **Keywords:** Acenocoumarol, VKORC1, CYP2C9.
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Molecular Laboratory Techniques Used in Research and Medical Practice: Applied Training Course Pre-test/Post-test Evaluation Results

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Background: Constructing and meaning of knowledge occurs through individual's own experiences. Laboratory studies, especially research, practice and inquiry-based laboratory practices are very important in this respect. Here in, we aimed to determine the possible benefit of these educations and also we planned to define this study as a template for describing the timing, duration and structure of these types of educations. **Methods:** This study was carried out during the Molecular Laboratory Techniques used in Research and Medical Practice Course. Before the course, 25 test questions were prepared according to the difficulty of the subjects by the lecturers of the course. Prepared test questions were divided into four main groups for later evaluation: DNA/RNA isolation, PCR-RTPCR, Gene Cloning and Western Blot techniques. Attendees answered the same questions before the course and after the education of 5 days then data analysis were done for the results. Also a survey was completed by attendees. **Results:** The pretest mean score was determined as 7.73±2.49 (mean ± sd) for multiple choice questions of five- answers option test consisting of 25 questions. The overall results for the posttest were determined as 14.24 ± 2.31. When the scores of the participants according to their education

period were evaluated, it was observed that the overall scores were altered depending on experience. **Conclusion:** Molecular Laboratory Techniques Used in Research and Medical Practice: Applied Training Course was found to be beneficial for the participants. In addition, we observed that the pre-test and post-test application and evaluation of the results would be useful in order to determine the missing points during the course. Educators could also evaluate themselves before going on new courses.

Endocrinology and metabolism

Sex Differences in Leptin and Its Correlation with C-reactive protein in Patients with Long-standing Type 1 Diabetes Mellitus

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Background: Leptin is a lipostatic adipokine and its biological functions are mediated by membrane-coupled receptors (LEPR or ObR), encoded by obese (ob) gene. Their expression in immune cells is reported as predominant in diabetes mellitus type 1 (T1DM). The involvement of the hormone in innate and adaptive immunity by modulating neutrophil activation as well as monocytes and macrophages capacity to produce cytokines, explains to some extent the T1DM-related chronic low-grade inflammation. Our aim was to analyze the gender influence on serum leptin concentrations and its correlation with C-reactive protein (CRP) in patients with long-standing T1DM. **Methods:** The study enrolled 159 patients, 108 of whom are diagnosed with T1DM (subgroups of male/female=56/52; mean age=43.06±10.38 years; duration of diabetes 26.23 ± 8.2 years; with a similar body mass index (BMI) and a control group of 51 age-, gender- and BMI- matched healthy people (subgroups of male/female=27/24; mean age=43.90 ± 9.12 years). Serum leptin levels were determined using an ELISA kit. CRP was measured by immunoturbidimetric method (Advia chemistry 1800). **Results:** In both groups, the sex difference in serum leptin was significant (7.85±6.33 ng/ml for diabetic women vs 3.15±3.09 ng/ml for diabetic men; $p<0.001$ and 5.86±4.23 ng/ml for healthy women vs 2.96±2.13 ng/ml for

healthy men; p<0.01). Serum leptin was significantly higher in men and women with T1DM, compared to the control subgroups (p<0.05). A significant positive correlation between leptin and CRP was also found, regardless of gender in patients with T1DM (r = 0.65; p <0.001) but not in the nondiabetic group (r=0.22; p=0.14). **Conclusion:** Our findings indicate that gender is an important determinant of leptin concentration in patients with T1DM. The correlation we observed between leptin and CRP in the clinical group supports the theory of the pro-inflammatory and pro-atherogenic effect of the hormone, respectively.

Rhythm of Melatonin, Leptin and Ghrelin in Women with Metabolic Syndrome with and without Impaired Fasting Glucose

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Background: The objective of this study was to compare serum levels of melatonin, leptin and ghrelin at 3.00 AM and 8.00 AM in patients with metabolic syndrome (MS) with and without impaired fasting glucose (IFG). **Methods:** The study included 60 women with MS, divided into two groups: with normoglycemia (n=30) and with increased fasting glucose (between 5.6 mmol/l and 6.9 mmol/l) (n=30). The diagnosis of MS was made according to the IDF criteria. The women in the study were assigned anthropometric (waist, BMI, HOMA-IR) and metabolic characteristics (serum glucose, total cholesterol, triglycerides, HDL cholesterol, insulin). Blood samples for serum melatonin, leptin and ghrelin determinations were taken at 3:00 AM and 8:00 AM. Serum melatonin, ghrelin and leptin levels were measured using ELISA kits with Sirio S microplate reader (SEAC, Italy). Statistical analysis was performed with SPSS v.19. Significance was defined as P < 0.05. Before recruitment, aim, benefit and procedure of the study was explained, and informed written consent was taken from all women. **Results:** There were no statistically significant differences in the mean age of the women with and without IFG (34.63 ± 2.39 years vs 28.62 ± 2.46 years, P > 0.05). The two groups did not differ in anthropometric and metabolic parameters except for uric acid, which is statistically higher in the group with IFG (263.39 ±31.11μmol/l vs 189.23 ±36.91 μmol/l, P = 0.048). The comparative analysis shows statistically higher mean leptin levels at 8:00 AM in women with IFG (21.32 ± 5.70 ng/ml vs 8.69 ±3.37 ng/ml, P = 0.046). There is a tendency to lower nocturnal melatonin in women with IFG compared to normoglycemic women, but the difference did not reach statistical significance (119.60±11.82 pg/ml vs 176.63±22.28 pg/ml, P = 0.063). **Conclusion:** Our findings may help to explain the incidence of metabolic syndrome in women with low night melatonin levels.

Keywords: Metabolic syndrome, impaired fasting glucose, melatonin, ghrelin, leptin.
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Myeloperoxidase (MPO) Activity Study in Obese and Subjects with Metabolic Syndrome

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Background: Obesity is one of the most common modern health problems worldwide. Numerous studies indicate the association of low-grade chronic inflammation and obesity. Myeloperoxidase (MPO) and its reactive oxidants contribute to tissue damage during inflammatory processes in the human body. The aim of the study was to examine the activity of the enzyme myeloperoxidase in the serum of obese and subjects with metabolic syndrome and to test the relationship between MPO activity and other indicators of inflammation in the blood of subjects, as well as the relationship between MPO activity and blood lipid concentration. **Methods:** The study included 175 first and second year students of the Faculty of Medicine in Foča who were divided into three groups according to the criteria of the International Diabetes Federation: normally fed (N = 106), subjects with abdominal obesity (N = 37) and the third group consisted of subjects with metabolic syndrome. **Results:** During the study, significant differences in the activity of MPO were found in all three groups of subjects, and the highest activity was measured in subjects with metabolic syndrome. We established a significant positive correlation between the indicators of inflammation such as erythrocyte sedimentation rate ($r=0.147$, $p<0.001$), fibrinogen ($r=0.204$, $p<0.001$) and and hsCRP ($r=0.563$, $p<0.001$) and the MPO activity, which indicates that with increasing amounts of adipose tissue and the accumulation of macrophages in it, there is an increased production of acute phase proteins in obese subjects. Also, we revealed a significant positive correlation between MPO activity and uric acid levels in our subjects ($r=0.312$, $p<0.001$). A positive correlation was found between MPO activity and atherogenic index, as well as between MPO and LDL-cholesterol concentration, while a negative correlation was found between MPO and HDL-cholesterol concentration. The study showed that MPO activity progressively increases with obesity and metabolic syndrome. **Conclusions:** Based on the obtained results, it can be concluded that the activity of MPO in the serum of the subjects progressively increases with obesity and metabolic syndrome, so the obtained results may be important in the pathophysiological mechanisms and complications of obesity and metabolic syndrome. **Keywords:** Myeloperoxidase, metabolic syndrome, obesity, inflammation, dyslipidemia.

Laboratory Findings of Insulin Resistance in Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a frequent endocrine disorder characterized by chronic anovulation, hyperandrogenism, and/or polycystic ovary. 50% -70% of women with PCOS have insulin resistance which contributes to the hyperandrogenism responsible for the signs and symptoms of PCOS. Many patients with PCOS present signs of metabolic syndrome concomitant with insulin resistance. The aim was to analyze the laboratory findings of insulin resistance, metabolic syndrome, and to study their correlations in patients diagnosed with PCOS. **Methods:** This is a correlational study conducted in prospective during six months, including 50 female patients, with an average age of 24.9 ± 5, diagnosed with PCOS .We used the following data: age, BMI, value of fasting glycemia, insulinemia, cholesterol, triglyceride, LDL, HDL. **Results:** 43.33% of the PCOS patients are overweight and 3.33% are obese. BMI can predict changes in insulin levels in 27% of the patients, changes in HOMA IR and QUICKI in 29 % of the patients and changes in cholesterol levels in 25% of the patients. 54.84% of the patients with PCOS present decreased insulin sensitivity and increased insulin resistance. In 67% of the patients insulin resistance can predict changes in LDL-cholesterol levels. In 53% of the patients changes in insulinemia are predicted by HOMA IR and in 54% of patients by QUICKI. **Conclusions:** Insulin resistance is present in almost 60% of patients diagnosed with PCOS. Overweight and dyslipidemia is found in 1 out of 4 patients with PCOS. **Key words:** polycystic ovary syndrome, insulin-resistance, insulin sensitivity, obesity, dyslipidemia

Comparison of Parathyroid Hormone (PTH) Immunoassays on VITROS 3600® and COBAS-E411® Analyzers

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Background: The unstable structure of parathyroid hormone (PTH) makes it difficult to determine it is concentration. Previous studies have shown that PTH is more stable in plasma. The main objective of this study was to compare two different immunochemical methods for PTH on VITROS 3600® and Cobas e-411® analyzers. The research was conducted in the Department of Laboratory Diagnostics (DLD) of the University Clinical Hospital Mostar (UCH

Mostar) in Bosnia and Herzegovina. The specific objectives of the paper were to investigate the comparability of analytical methods, and to determine the constant and proportional deviations between individual methods. **Methods:** Subjects included in the study were patients older than 18 years who were treated in UCH Mostar or were referred to DLD from the Health Centers, with the signed informed consent. The study included 132 respondents (106 women and 26 men). The methods used to analyze PTH concentration were commercially available assays; VITROS Intact PTH, chemiluminescent sandwich method (CLIA), antigen-antibody complex, and Elecsys PTH of the Cobas e-411® analyzer. **Results:** The reference range for PtH; on the Vitros analyzer is 10-65 pg/mL, and on Cobas e-411 is 14.9-56.9 pg/mL. Bland-Altman analysis and Passing Bablok regression in MedCalc software were used for statistical data processing. For Bland-Altman analysis mean difference (MD) between two measurements was 24.5853 (18.8965-30.2741) with limits of agreement D ±1.96. The results according to Bland-Altman analysis showed statistically significant constant deviation. Passing Bablok regression equation -16.856185 (-19.0484 to -13.7618) +1.676110 (1.6130 to 1.7260)x and showed statistically significant constant and proportional deviation. **Conclusion:** According to the results, it is concluded that the methods are not comparable. Bland-Altman analysis showed that the limits of the confidence interval are different from zero, which confirmed the null hypothesis about the difference and incomparability of the methods. Regression equations, obtained by Passing Bablok analysis, also confirmed that there is a statistically significant difference between these methods and analyzers and that the same method. Also, the differences in measurement by the above methods are greater than the usual analytical variability, and thus may affect the clinical outcome. **Keywords:** Parathyroid hormone (PTH), VITROS 3600®, Cobas e-411®, comparison method.

Gastrointestinal/Cardiovascular/CSF biomarkers/ Reference ranges and decision limits

Salivary sIgA and Nitrite Levels in Patients with Helicobacter Pylori Chronic Gastritis

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Background: Helicobacter pylori (HP) infection causes chronic inflammation leading to gastric ulcer or cancer. The secretory IgA (slgA) and nitrites in saliva represent some specific and nonspecific defence mechanisms against oral pathogens. As a non-invasive diagnostic biological fluid, saliva could be a suitable tool for monitoring HP infection. The aim of the present study was to evaluate the salivary levels of sIgA and nitrites in patients with HP chronic gastritis. **Methods:** A total of 140 subjects (patients and age matched controls) were enrolled in the study. The patient group consisted of 44 HP(+) and 16 HP(-) individuals with chronic gastritis hospitalized for exacerbation of their dyspeptic symptoms. The salivary sIgA and serum IgA

levels were measured using DiaMetra IgA Saliva ELISA and Beckman Coulter kits. The Mackerey-Nagel Nanocolor Nitrite kit was used for measurement of salivary nitrites. **Results:** The HP(+) patient group had significantly higher sIgA levels in comparison with HP(-) patient group (139.9±33.2 vs 98.3±18.4mg/L respectively, $p<0.0001$) and with controls (108.3±47.7mg/L, $p<0.0001$). Difference between serum IgA levels in HP(+) and HP(-) patient groups was not found, however the mean value of the whole patient group was significantly higher than this of the controls (2.4±1.0 vs 1.9±0.8g/L respectively, $p<0.01$). The mean value of salivary nitrite levels in HP(+) patients was 131.5±32.6μmol/L which differed significantly from those of HP(-) patients and controls (80.2±37.9 and 85.3±75.5μmol/L respectively, $p<0.0001$). Correlation between salivary and serum IgA levels was not established. **Conclusion:** Salivary sIgA and nitrite levels in HP(-) patients with chronic gastritis are similar to those of the control group and differ significantly from those in HP(+) patients. HP infection involves host oral specific and nonspecific immune mechanisms to counteract the pathogenic microorganism. Examination of salivary levels of sIgA and nitrites could provide additional options in monitoring patients with HP chronic gastritis. **Keywords:** saliva, sIgA, nitrite, HP infection

Fecal Calprotectin - a Useful Laboratory Marker in Gastroenterological Practice

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Background: Among the inflammatory bowel diseases (IBD) the most common are Crohn's disease and ulcerative colitis. Unfortunately, very often the symptoms of these diseases resemble complications of functional origin, which makes the diagnosis difficult. Calprotectin is a protein that is released by the neutrophil cells, which are directed to the site of a present inflammation of the intestinal wall. Calprotectin enters the feces, where it is not destroyed, but its levels rise. This test is suitable for both children and adults. In addition, it is extremely fast, accessible and painless for patients. **Methods:** We measured fecal calprotectin in 113 patients aged 18 to 80 years. 43 of them are women and 70 - men. The studied patients were divided into four groups: First group - 43 patients with IBD; second group - 17 patients with irritable bowel syndrome (IBS); third group of 33 patients with other non-inflammatory gastrointestinal diseases and fourth group - 20 healthy people. Results: We found that calprotectin levels were the highest in the first group - $x = 101.2$ μg/g; in the second group calprotectin is $x = 42.4$ μg/g; in the third group - $x = 77.2$ μg/g. In healthy controls we found an average value of calprotectin - 31.8 μg/g. **Conclusions:** Fecal calprotectin is a sensitive marker for the identification of patients with IBD. In most cases, negative calprotectin rules out inflammatory bowel disease, thus invasive tests, such as colonoscopy, are not compulsory for people with irritable bowel syndrome. **Key words:** calprotectin, IBD, IBS, Crohn's disease, ulcerative colitis

Analytical Characterization of the High-sensitive Cardiac Troponin I Assay on the Siemens Dimension EXL System

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Background: To define the analytical characteristics of the LOCI® method for high-sensitivity cardiac troponin I (hs-TnI) on the Dimension EXL system. Cardiac troponin I is prefer and recommended marker for the diagnosis and treatment of acute coronary syndrome and myocardial infarction. **Methods:** Three different levels of controls BIO RAD Laboratories (Liquicheck - Cardiac Troponins Control), mean concentrations 55.40, 3617.00 and 10975.00 ng/l) were used for the imprecision, quantifying separately within-run 20 times during a day and between-run for 20 days. Determination of the limits of the blank (LoB) and detection (LoD), we measure in a sample that contains no analyte - mean and standard deviation (SD) of sample diluent (n=20). We test 3 pools with mean values 4.8, 45.3 and 98 ng/l for 20 days to determinate limits of quantification (LoQ). The imprecision (CV) of 10% was found by interpolation for LoQ. We use a non-parametric method for 86 healthy people to calculate upper reference limit (URL) in lithium heparin samples. **Results:** The within-run imprecision (coefficient of variation, CV%) was 2.2%, 1.8% and 2.9%. The between-run imprecision was 4.5%, 3.7% and 4.8% for each control level. Total imprecision was 5.02%, 4.02% and 5.78 %. LoB=5.2 ng/l, LoD=8.98 ng/l, LoQ=11.82 ng/l (10% CV). URL was 59.7 ng/l and no differences between men and women. **Conclusions:** We found that the analytical performance characteristics LOCI® method for hs-TnI on the Dimension EXL system are acceptable and meets all guidelines recommended criteria. These results demonstrate that the hs-TnI assay is a precise and highly sensitive method for measuring Cardiac Troponin I, especially for identifies patients with risk at very low levels of hs-TnI.

CSF Biomarker Method Comparison in Dementia Patients

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Background: CSF proteins amyloid beta1-42 (Ab), tau and p-tau are now considered reliable biomarkers of amyloid plaque formation, neurodegeneration and tau aggregation resulting in neurofibrillar tangles according to the recent AT(N) 2018 classification of neurodegenerative dementias. The automation of these immunochemical measurements will increase both accuracy and widespread application. **Methods:** 35 CSF samples from patients from the neurodegenerative spectrum (Alzheimer's disease, Frontotemporal Dementia etc.) were analyzed for all CSF biomarkers with the gold-standard Innotest ELISA methods from Fujirebio and in parallel, with the new CE-IVD Roche reagents that employ Elecsys/Cobas automated platforms. An external quality assessment sample was also included (Instand, Oct 20). **Results:** The new Roche reagents are easy to implement, rapid (results within 19 min compared to 20 hours for the ELISA manual procedures) and showed excellent precision results (CV% of internal low and high quality controls<5% compared to 8-12% for ELISA). Regression, correlation and concordance statistical analysis showed the following results: a) AbRoche= 1.18xAbFujirebio - 104 (r=0.91, concordance 72%), b) tauRoche= 0.65xtauFujirebio+7 (r=0.97, concordance 97%) c) p-tauRoche=0.55xp-tauFujirebio-9 (r=0.97, concordance 80%). In order for a fair method comparison to be performed, normal/abnormal concordance was evaluated according to literature universal cut-offs for Fujirebio (550, 375, 52 pg/ml for Ab, tau, p-tau although the manufacturer provides age-specific cut-offs) and for Roche (800, 300, 27 pg/ml for Ab, tau, p-tau correspondingly). The Roche suggested Ab cut-off of 1000 pg/ml was changed arbitrarily to 800 in order to improve concordance. **Conclusions:** Our results showed high correlation for the tau and p-tau methods; the Roche tau cut-off seems appropriate for correct classification of the cases and the Roche p-tau cut-off needs slight adjustment in order for the concordance to improve. Ab values seem to converge with acceptable correlation but the cut-off value of the two methods need to be re-examined in light of the new certified Ab reference material. Final and correct adjudication of the cases should be examined during patient monitoring. Efforts for standardization and harmonization of these CSF parameters should continue worldwide.

Haematology and Haemostasis

Reference Ranges for Serum Erythroferrone in Bulgarian Population

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Background: Erythroferrone was discovered in year 2014 as regulator of hepcidin synthesis and is synthesized by erythroblasts. Erythroferrone main function is to suppress hepcidin synthesis during inflammation, thereby regulates iron homeostasis. We aimed to establish erythroferrone reference interval for Bulgarian population using validated ELISA method. Materials and **Methods:** 151 healthy volunteers were included. In all included participants intima-media thickness (IMT), ankle-brachium index (ABI), and blood pressure were measured, body-mass index (BMI) were calculated. Biological specimen (venous blood) was taken in order to evaluate total red blood cells count, erythrocyte indices – MCV, MCH and MCHC, hemoglobin, hematocrit, high sensitive CRP, serum iron and TIBC, serum transferrin, ferritin, haptoglobin, CRP, LDH, CPK, ASAT, ALAT, creatinine, glucose, lipid profile (total and HDL, LDL-cholesterol, triglycerides), hepcidin, TNF-α, IL-6. Included volunteers were divided into two age borders – a) from 18 to 50 years old and b) above age of 50. The distribution was as follows: males – total number 103, below age of 50 - 59 (57.3%), females – total number 114, below age of 50 - 69 (60.5%). **Results:** For calibration curve we use recombinant human ERFE with level 10 ng/mL, from which after proper dilutions the required clinically relevant values were established. Diagnostic sensitivity was 0.056 ng/ml. After applying specific statistical analyses and parametric distribution we found 6.3 – 15.7 ng/mL as reference range for Bulgarian population. **Conclusions:** The immunological ELISA method we choose for serum erythroferrone quantification showed high specificity and sensitivity during validation process. It is new parameter for Bulgarian clinical laboratory practice and hematology specialty.
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Myelodysplastic Syndromes, Refractory Anemia with Ring Sideroblasts as an Important Entity of Myelodysplastic Syndromes

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Background: Myelodysplastic syndromes are clinically heterogeneous disorders characterized by clonal hematopoiesis, impaired differentiation, peripheral blood cytopenia, and a risk of progression to acute myeloid leukemia. The two myeloid neoplasms defined by the presence of ring sideroblasts (RS), include a refractory anemia with ring sideroblasts (RARS), now classified under myelodysplastic syndromes with RS (MDS-RS), and RARS with thrombocytosis (RARS-T), now called myelodysplastic/myeloproliferative neoplasm with RS and thrombocytosis

(MDS/MPN-RS-T). Ring sideroblasts are erythroid precursors with an abnormal perinuclear mitochondrial iron accumulation. Our aim was to highlight the importance of Perls staining and morphology as an important step towards the diagnosis, classification and prognosis of MDS. **Methods:** This study included 44 patients diagnosed with MDS at Tirana University Hospital Center "Mother Teresa" during a period from January 2018 to October 2019. Myelograms of these patients were examined after Perls staining. **Results:** The mean age was 68 years, with a female sex predominance. Examination of 44 cases revealed the presence of ring sideroblasts in 3 patients, the presence of normoblast with siderotic granules: >5% perinuclear granules covering in ring form over 1/3 of the perimeter of the nucleus, accounting for >15% of the erythroid lineage. Erythroid dysplasia was prevalent. **Conclusion:** Morphology is an important step in the diagnosis of myelodysplasia and a first step in the MDS diagnostic algorithm. Perls staining is an important method in the diagnosis, classification and prognosis of RARS.

Assessment of Platelet Indices in Patients with Long-standing Type 1 Diabetes Mellitus

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Background: Platelet hyperreactivity is a factor which contributes towards increased risk of cardiovascular events in diabetis mellitus (DM). We analyzed platelet indices in long-term DM type 1(T1DM) patients with the aim to investigate whether platelets' morphology is altered, potentially predisposing them to cardiovascular events in the future. **Methods:** The study group consists of 83 patients with T1DM (male/female=45/38; mean age=41.23±11.06 years; duration of diabetes 29.9 ± 9.7 years) and a control group of 32 age- and gender-matched healthy people (male/female=13/19; mean age=40.29±9.65 years) during the 2018-2020 period. Platelet parameters were derived from the results for complete blood count (Sysmex XN1000). Morphological analysis of the Romanowsky-stained blood smears was performed for all patients.

Results: We observed elevated platelet indices in the clinical group, comparing them with the control group: PLT- 290.7×10^9/l ± 80 vs 256.18×10^9/l ± 44; MPV- 10.41fl ± 1.14 vs 9.98fl ± 1.34; P-LCR- 29.903% ± 7.82 vs 29.086% ± 8.38; PCT- 0.307% ± 0.074 vs 0.239% ± 0.05; PDW- 12.697% ± 11.84 vs 1.58% ± 1.71. A significant positive correlation was found between MPV and PDW($r = 0.94$; $p< 0.0001$) and a negative correlation between MPV and PLT ($r= -0.39$; $p=0.0003$), PDW and PLT($r=-0.56$; $p=0,001$); PLT and age ($r=-0.30$; $p=0.03$) in the clinical group. For 54 (65%) of diabetic patients, the evidence for macroanisothrombocytosis was positive. These patients had a value for MPV> 10 fl (mean = 11.08 fl; CV = 0.47; SD=0.68). **Conclusion:** Platelets of patients with long-standing T1DM show morphological evidence of hyperreactivity, potentially contributing to increased cardiovascular risk .Macrothrombocytes have larger biological activity. With advancing age, their proportion increases in contrast to the accelerated platelet consumption in order to maintain the constant functional platelet activity. Unlike other markers for assessment of platelet function, platelet indices do not require specialized hemostasiological equipment, which determines their rentability in clinical and diagnostic terms.

Comparison of Two Different Hematological Modules for Analysis of the Number of Platelets

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Background: Platelets (PLT), also called thrombocytes, are tiny fragments of cells that are essential for normal blood clotting. Counting of PLT is an important part of a routine complete blood count (CBC). The aim of this study is to compare PLT values obtained by measurements performed on two hematology analyzers considering assessment of their parallel use in laboratory work. **Methods:** 50 whole blood samples were included in the study. For the counting of PLT (103/μL), we used Sysmex XT-1800i reference module (fluorescent flow cytometry) and module Siemens Advia 2120 (flow cytometry). The accuracy and precision of the analyzers were checked. The results were statistically analyzed by MedCalc software. **Results:** A shortened analytical evaluation has determined the satisfactory accuracy and precision of the analyzers. The lowest number of PLT measured on the Sysmex XT-1800i was 19, and the highest 462. The lowest number of PLT measured on the Siemens Advia 2120 was 37, and the highest 391. The Scatter diagram indicates the diversity of data distribution from low, through normal to high. Bland Altman graph shows that four PLT values were distributed outside of ± 1.96 SD. In Passing-Bablok regression analysis, when comparing the Sysmex XT-1800i with the Siemens Advia 2120, the following results were obtained for PLT: $y = 8.003597 + 0.913669 x$. Intercept a = 8.0036 (95% CI 2.6096 to 19.2517). Slopes b = 0.9137 (95% CI 0.8601 to 0.9561). Result for

the slope indicates that there is statistically significant constant error. Cusum's linearity test shows that there is no deviation in linearity (P = 0.99). **Conclusions:** These two methods are not comparable and both hematological modules cannot be used simultaneously in a routine laboratory practice.

Introduction of Heparin-induced Multielectrode Aggregometry Method for Heparin-induced Thrombocytopenia Testing

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Background: Heparin-induced thrombocytopenia (HIT) is complex clinical-biological syndrome after exposure to heparin associated with high morbidity and mortality. The diagnosis of HIT needs sensitive laboratory assays and remains challeng. Immunoassays detect the presence of anti-PF4/heparin antibodies; a negative result can be used to exclude HIT; positive results are often false-positive. Serotonin release assay is a current gold standard functional assays that detect platelet-activating heparin-dependent antibodies, but is technically demanding with limited use. Objective: to implement in clinical practice heparin-induced multi-electrode aggregometry method (HIMEA) standard protocol from the platelet immunology subcommittee of the ISTH as a simple and rapid functional HIT assay. **Methods:** HIMEA method assesses the ability of serum/plasma from suspected HIT patients to activate fresh platelets from healthy donors in the presence of several concentrations of heparin. Whole blood is collected using hirudin as an anticoagulant and is incubated in the analyzer under stirring at 37 ◦C for 1 min with the patient's plasma or serum and a saline solution. Saline buffer, therapeutic (1 IU/mL) and supratherapeutic (200 IU/mL) concentrations of heparin are added into the reaction mixture and impedance changes are recorded for 15 min. **Results** are considered positive if they fulfill all the fourth following criteria: a typical sigmoidal curve reflecting actual platelet aggregation; AUC (1 IU/mL UFH) > 30 U; AUC (200 IU/mL UFH) < 50% of the AUC obtained with 1 IU/mL heparin; AUC < 30 U without heparin. **Conclusions:** HIMEA functional assay have a positive impact on patient management by reducing the time taken to confirm a diagnosis of HIT.

Antithrombin Deficiency in Pediatric Patient with Multisystem Inflammatory Syndrome in Children (MIS-C)

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Background: MIS-C is a hyperinflammatory condition arising secondary to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Antithrombotic prophylaxis is recommended in children hospitalized with severe disease who have at least one risk factor for thrombosis or a high plasma concentration of D-dimers. **Methods:** We report a critically ill 15-year-old boy, presenting with features of MIS-C who requires admission to the intensive care unit. He was found to have fever, abdominal pain, cardiac dysfunction and neurological symptoms. Laboratory values were remarkable for high levels of C-reactive protein, B-type natriuretic peptide, ferritin, procalcitonin and troponin. The patient was noted to have neutrophilia, lymphopenia and hypoalbuminemia. Antibody testing for SARS-CoV-2 IgG was positive. Because of markedly elevated levels of D-dimers he was treated with therapeutic dose low molecular weight heparin (LMWH). **Results:** D-dimers at the admission were 35.2 mg/L FEU (reference range 0.00 – 0.55). Four hours after administration of Clexane 60 mg twice daily the measured anti-Xa level was very low 0.1 IU/mL. Antithrombin (AT) deficiency was suspected and functional assay was performed. AT activity was 28% (Innovance kit, reference range 82.9 – 118.2). Anticoagulant therapy was switched to acenocoumarol. 5 days after initiation of treatment with acenocoumarol D-dimers concentration decreased to 5.6 mg/L. There was neither thromboembolism nor bleeding events during 4 months of follow-up. **Conclusion:** Standard LMWH doses do not provide sufficient anticoagulant effect in antithrombin-deficient individuals. Laboratory monitoring of LMWH in critically ill children with MIS-C may be useful to ensure adequate antithrombotic therapy. **Keywords:** Antithrombin deficiency; multisystem inflammatory syndrome in children, low molecular weight heparin, anti-Xa level.

Hypochromic microcytic anemia in outpatients

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Background: To evaluate laboratory data of hypochromic microcytic anemia on outpatients, frequencies, gender and age distribution and causes. **Methods:** We performed an epidemiological study including 384 outpatients, 85 men and 299 women, mean age 34.27 year, for a year period, with hemoglobin levels under the reference range. The blood of these patients was then tested for hemoglobin electrophoresis with Sebia HYDRASYS. **Results:** We have got 90 cases with hemoglobinopathies from which 87 or 22.66% were β-thalassemia minor and 3 or 0.78% were Sickle cell trait anemia. The iron deficiency anemia was found in 129 outpatients with a percentage of 33.59%. The rest of our patients hadn't a given cause. Hypochromic microcytic anemia was more often present in females with 77.86% to 22.14% males.

Conclusions: The presence of hemoglobinopathies and its percentage is a very good reason of continuing screening test in pregnant women. Regarding to iron deficiency anemia, the most important thing is establishing the diagnosis and reason for iron deficiency and correct it.
Keywords: Anemia, hemoglobinopathies, iron deficiency, thalassemia, sickle cell anemia.

Inflammation, inflammatory and infectious diseases

Immunoassay SARS Cov-2 Ag Quantitative Test in Saliva vs. PCR test in Nasopharyngeal Swab for Diagnosis of Covid-19

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Background: The new SARS Cov-2 emerged in Bulgaria in march 2020, reached several pandemic peaks to this day. Our goal is clinical assessment of CLEIA Lumipulse G600II SARS Cov-2 Ag quantitative test in saliva sample and reverse transcriptase polymerase chain reaction (RT-PCR) performed on a nasopharyngeal swab sample for diagnosis of infection with SARS Cov-2. **Method:** At the same time, we collected saliva sample and nasopharyngeal swab sample from 29 patients referred to lab for screening or diagnosis of SARS Cov-2 infection. Each patient was tested twice with the two different methods and respective samples. **Results:** We found no differences in the end result with the two methods. RT-PCR results are reported like positive or negative. Saliva results are reported like quantitative concentrations with cut-off value and higher or lower. Quantitative concentrations are correlated with the amount of virus infection. Thus, the severity of the disease can be predicted. **Conclusion:** We recommend this method in the routine diagnosis of SARS Cov-2 infection like auxiliary because: it can be implemented in any laboratory; more easily accessible sample; faster result; assessment of viral load; ability to track infection. **Keywords:** SARS Cov-2 Ag quantitative test; saliva sample.

SARS CoV-2 Patients at Hospital Admission and Plasma Level of KL-6 for Predicting Lung Damage

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Background: Coronavirus disease 2019 (COVID-19) is currently spreading worldwide. Patients at hospital admission are assessed by several blood laboratory tests and chest CT. This study

examined whether serum Krebs von den Lungen-6 (KL-6) level is a useful biomarker for evaluating the severity of COVID-19 patiens. KL-6 is a mucin-like high molecular weight glycoprotein produced and secreted in the serum by pulmonary type II pneumocytes. **Method:** We examined patients diagnosed with COVID-19 at St. Ekaterina Hospital between February 1, 2021, and May 1, 2021. Test method for KL-6 is CLEIA LUMIPULSE G600II KL-6 (FujiRebio). A total of 68 patients were tested, including 43 in the non-severe group and 25 in the severe group, of which four died. Compared with those in the non-severe group, more patients in the severe group were significantly older and had comorbidities. **Results:** Serum KL-6 levels were significantly higher in the severe group than in the non-severe group both at diagnosis (median, 347 U/mL) and at peak levels within one week after diagnosis (median, 797 U/mL) (both $p < 0.001$). Serum KL-6 value at peak level (369 U/mL) was used as the optimal cut-off to evaluate disease severity (sensitivity, 85.7%; specificity, 96.6%). **Conclusion:** In conclusion, serum KL-6 levels were significantly elevated in severe COVID-19 and are useful for evaluating its severity. **Keywords:** SARS Cov-2 infection, KL-6 testing, Lung damage

Hematological Inflammatory Indices Role in Predicting the Need for Intensive Care during Covid-19 Hospitalization

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Background: Inflammatory responses play a critical role in the progression of Covid-19 and disease severity. Platelets-to-Lymphocytes Ratio (PLR), Neutrophils-to-Lymphocytes Ratio (NLR), derived-NLR, Systemic Immune-inflammation Index (SII), Lymphocytes-to-Monocytes Ratio (LMR), Platelet-to-Neutrophils Ratio (PNR), have been associated with systemic inflammation. This study aims to evaluate Hematological inflammatory Indices at admission and their role in predicting the need for Intensive Care during hospitalization. **Methods:** In this study are enrolled 150 adult patients admitted in Infective Emergency and hospitalized for COVID-19 from 15 November 2020 to 15 February 2021 in University Hospital Center (UHC) 'Mother Theresa', Tirana, Albania. Blood was drawn at admission and laboratory tests were performed in Laboratory Network, UHC 'Mother Theresa'. WBC, Neutrophils, Lymphocytes, Monocytes, Platelets, MPV, PDW, RDW were performed on Abbott, Alinity H-series. PLR, NLR, derived-NLR, SII, LMR, PNR were calculated. The parameters were compared between the group of patients who needed Intensive Care and those who didn't. Statistical analysis was performed using IBM SPSS Statistics 26. P-value<0.05 was considered statistically significant. **Results:** 33% of the patients were females and 67% males. The average age was 61.94 ± 12.26 years. 23% of patients enrolled in this study needed intensive care during hospitalization. Mean WBC

and neutrophils count were elevated at admission. 68% of the patients had lymphocytopenia at admission. SII (p 0.002), NLR (p 0.009), WBC (p 0.001), Neutrophils count (p 0.001) and Monocytes count (p 0.047) at admission were significantly higher among patient that needed intensive care. Lymphocyte's count (p 0.048), LMR (p 0.032) and PNR (p 0.001) at admission were significantly lower among patients that didn't need intensive care treatment. **Conclusions:** Hematological inflammatory indices are useful and cost-effective in predicting the need for intensive care during the hospitalization for Covid-19, potentially impacting clinical outcomes and management.

The Contribution of COVID 19 Seroprevalence to COVID 19 Convalescent Plasma Collections

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Background: Many individuals for various reasons either could miss viral examinations for COVID 19 or could obtain negative results due to delayed testing. The aim of this study was to determine the seroprevalence of COVID 19 in correlation with RT-PCR test, between men and women, to collect appropriate donors for convalescent plasma (CP), and to serve as register of individuals that were infected with COVID -19 and recovered. **Methods:** This study was conducted in Tirana during 21 to 28 December 2020 and included 817 individuals. Anti – S1 protein of SARS – COV – 2 IgG antibodies (anti - IgG) were measured with EUROIMMUN, ELISA method. **Results:** Subjects' age ranged from 20 to 70 years and 63.8% (521) of them were females. Of all 393 anti - IgG positive patients, 11.8 % (89) were RT-PCR positive (p= 0.00001). The prevalence rate of anti - IgG was 48.1%. (95% CI: 44.8% - 51.7%). Anti – IgG ratio < 3.5 was 39.4 % (155) and ratio > 3.5 was 60.6% (238). In negative RT – PCR (304), the anti – IgG ratio <3.5 was 43.4% (132) compared to ratio > 3.5 that was 56.6% (172). In positive RT – PCR the anti – IgG > 3.5 was 74.2 % (66) compared to 25.8 % (23) referred to ratio < 3.5 (p =0.003). Of all 393 positive patients, 61.3% (241) were females. 62.1 % (82/132) of males and 61.0 % (159/261) of females resulted with anti – IgG ratio > 3.5. 61 from all individuals were real donors for CP, based on criteria from Central Blood Bank of Tirana (Donors 20 – 60 years old, donors without a history of blood transfusion, female donors who have never been pregnant, the anti – IgG ratio > 3.5). **Conclusions:** The seroprevalence was the main indicator test for natural infection of COVID 19. The percentage of anti – IgG ratio > 3.5 was higher than ratio < 3.5. Females were infected more often than males. Males and females with anti – IgG ratio > 3.5 were approximately the same in %. This means they could confirm almost the same moderate and severe illness. Our data served us to collected 61 eligible donors for CP.
Keywords: Anti – S1 protein of SARS – COV – 2 IgG antibodies, convalescent plasma.

Seroprevalence of Antibodies against Coxiella Burnetii among Young Children and Adolescent with Fever of Unknown Origin (FUO): a Prospective Study

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Background: Zoonoses are constantly growing group of infections, which have emerged and reemerged as the global security challenge throughout the world, included Q fever, caused by intracellular bacterium Coxiella burnetii. In humans, the disease has a broad spectrum of clinical manifestations, ranging from a limited febrile illness, pneumonia and hepatitis to life-threatening forms such as endocarditis and meningoencephalitis. Fever of unknown origin (FUO) is usually described on the period of primary infection with C. burnetii and without an early diagnosis can require prolonged treatment. The goal of the present study is to estimate the prevalence of antibodies against C. burnetii (Q fever) among children and adolescent with fever of unknown origin (FUO) in Bulgaria from 01 January 2019 to 30 May 2021. **Methods:** Blood samples from 19 children (0 – 12 years old) and 56 adolescents with FUO were collected in healthcare facilities of Bulgaria and examined with the commercial indirect enzyme-linked immunosorbent assay (ELISA) by detecting anti-C. burnetii phase II IgM and IgG antibodies. **Results:** Seven percent (n = 5) of tested children were seropositive for Q-fever. The study population included mainly adolescents (70%, 13 – 18 years of age). An additional, 4% (n = 3) of all patients included in our study were seropositive for anti-C. burnetii IgM only (suspected acute Q-fever). The most frequently reported symptoms (n≥15% of respondents) mentioned by seropositive children were: FUO (69%), headache (52%), epigastric pain (37%), constipation (26%) and fatigue (24%). Milk consumption and residence of area with differing intensities of Q-fever transmission are considered risk factors for Q-fever seropositivity. **Conclusions:** The findings of this study should be investigated further. The results are of importance both for public health and the animal health/livestock production sector. Only the determination of the rate of Q-fever among seropositive patients can give an answer to the question, of which role Q-fever plays in the complex of FUO. **Keywords:** Q-fever, seroprevalence, fever of unknown origin (FUO), young children and adolescents, ELISA assay. Acknowledgments: This work was supported by the Bulgarian National Science Fund under Grant KP-06-N33/3.

Differences in Biochemical & Hematological Parameters of Non-survive and Survive Patients Hospitalized for Covid 19

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Background: COVID 19 is defined as illness caused by a novel coronavirus now called severe acute respiratory syndrome coronavirus-2 (SARS CoV-2). On March 11, 2020, the WHO declared COVID-19 a global pandemic. This virus take 2 539 427 human lives till this moment worldwide. The aim of this study is to reveal the importance of biochemical & hematological parameters in predicting death. **Methods:** This study was carried at the Department of Medical Biochemistry and Department of infective diseases of Public Health Organization Clinical Hospital "D-r Trifun Panovski" in Bitola. Our study describes the laboratory characteristics of 66 Covid 19 infected patients hospitalized in Department of infective diseases, age of 26 to 93. The patients were divided into 2 groups as: 30 non-survive patients and 30 survivors Covid 19 positive patients. Complete blood count was determined in ethylenediaminetetraacetic acid (K-EDTA) blood samples -using Sysmex XP 300/ Sysmex XS 1000 (Sysmex Co, Kobe, Japan) according to the manufacturer's instructions. Biochemical analyses were performed on Abbot Architect CI 4100 according to the manufacturer's instructions. **Results:** 60 patients (females and males) with ages between 26 -93 (median=65) were studied. It was found significant differences between 2 groups in terms – age, urea, creatinine, CRP, LDH, D dimer, AST, CK, CK-MB, and we can concluded that this parameters predict deaths of patients. **Conclusions:** Laboratory parameters at the time of hospital admission allow us to know which patient may have a more serious SARS-CoV-2 infection. Urea, creatinine, CRP, LDH, D dimer, AST, CK, CK-MB are higher in severely ill patients. Monitoring of these markers in COVID 19 patients would therefore help in better risk stratification and management of these patients. Laboratory parameters can help to physician to predict the course of SARS-CoV-2 infection in the early stages, allowing adapting healthcare to the estimate prognosis of the disease.

Pregnancy and laboratory

The Serum Selenium Status in Bulgarian Pregnant Women and Its Relationship with Glucose Metabolic Indices

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Background: Our aim was to evaluate the status of selenium (Se) and its link to glucose metabolic indices in gestational diabetes mellitus (GDM) and healthy pregnant women in 24-28

gw of the pregnancy. **Methods:** A total of 80 pregnant women aged between 18 and 40 yr are divided in two groups: with GDM diagnosis and normal glucose tolerance (NGT) by the 2 h 75-g oral glucose tolerance test (OGTT). Only singleton pregnancies are included. The exclusion criteria are based on patient hormonal substitutional treatment, oral hypoglycemic agents and insulin therapy. Cases with hypo-or hyper thyroiditis and smokers are excluded. GDM diagnosis is done according to the criteria of American Diabetes Association. The glucose and insulin levels are measured in venous blood at fasting state. Blood glucose is measured by amperometric method (Biosen C-line), serum insulin by ECLIA (Cobas Integra). HOMA-IR index is calculated by formula. Serum Se is determined by ETAAS using matrix modification and Zeeman spectral background correction. **Results:** The serum Se in GDM and healthy pregnant groups are 694.52± 119.5 nmol/L vs. 730. 3±192.6 nmol/L with no significant statistical difference (p=0.35). Blood glucose and insulin at fast, and HOMA–IR are respectively 4.4±0.53 vs 5.3±0.37 mmol/L, p<0.005; 12.8±7.4 vs 7.2±3.9 U/ml (p=0.02); 2.4±1.8 vs 0.96±0.8, p<0.05. Very slight positive correlation between glucose and Se is established (r=0.01 GDM; 0.23 NGT group) with negative correlations between the trace element and insulin and HOMA-IR (r=-0.30, -0.38 GDM; r=-0.25, -0.19 NGT) respectively. **Conclusions:** Se point very slight tendency for decreasing in GDM pregnant women. The observations in the present study imply a very complicated link between the essential trace element and glucose metabolism indices as overall effect of many combined factors: oxidative stress in the same pregnancy and GDM, an insulin-like action property of Se, nutritional status and very complex molecular mechanisms of regulations and disorders during pregnancy and especially in GDM. **Key words:** selenium status, gestational diabetes, glucose
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First Trimester Combined Screening Test: Concentration of Serum β-hCG i PAPP-A in Women with Insulin-dependent Diabetes Mellitus

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Background: First trimester combined screening test gives early information about risk of chromosome anomalies, especially the risk of Down syndrome, as well as other, less frequent trisomies such as trisomy 18 or 13 (1,2). The aim of the research was to determine if there was

any statistic significant difference in serum concentration of β-hCG and PAPP-A in pregnant women with insulin dependent diabetes mellitus (IDDM). **Methods:** Study involved 104 pregnant women who were sent to perform the combined screening test by their gynecologist in the period from February 2020 to August 2020 to Department of Laboratory Diagnostics, University Clinical Hospital Mostar. Signed written informed consent was obtained from all patients. The subjects were divided into two groups, healthy pregnant women and pregnant women with IDDM diagnose. Serum parameters of combined screening β-hCG and PAPP-A were measured on COBAS E 411 analyzer (Roche Diagnostic, Germany) by electrochemiluminiscence immunoassay (ECLIA) method. Data was statistically analysed in MedCalc Software's VAT Version 19.0.7. **Results:** The study included 92 healthy pregnant women (88.5%), and 12 with insulin dependent diabetes mellitus (11.5%). Median concentration of free βhCG was 28.23 IU/L and PAPP-A was 2.31 IU/L in pregnant women with IDDM diagnose. In group of healthy pregnant women median concentration of free βhCG was 35.49 IU/L and PAPP-A was 3.330 IU/L. No statistically significant difference in concentration of free βhCG hormones with regards to insulin dependent diabetes (p=0.065). Statistically significant difference in concentration of PAPP-A with regards to insulin dependent diabetes (p=0.188) was not found as well. **Conclusion:** The present results showed that there was no statistically significant difference in concentration of combined screening markers β-hCG and PAPP-A found in pregnant women with IDDM compared to healthy subjects involved in the study.

Cardiovascular Biomarkers and Their Changes in Hypertensive Disorders of Pregnancy

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Background: Hypertensive disorders of pregnancy are one of the leading causes for fetal and maternal mortality worldwide. Aside from the immediate risk they pose for the pregnant woman, there is significant evidence that women after such a pregnancy have a long-term risk for the development of cardiovascular diseases. Our aim was to determine the levels of certain biomarkers indicating cardio-vascular involvement - Galectin-3, high-sensitive CRP, Interleukin-6 in patients with gestational hypertension, preeclampsia and in healthy pregnant women. **Methods:** A prospective single-center clinical epidemiological study was carried out and data

were analyzed for 123 pregnant women – 36 with gestational hypertension, 37 with preeclampsia and 50 healthy controls. ELISA method was used to determine the levels of Galectin-3, high-sensitive CRP, and Interleukin-6 and Placental growth factor in each of the groups. **Results:** The presence of the designated hypertensive pathologies was further proven by the significantly lower mean levels of placental growth factor in those women compared to controls (215.89 pg/ml for controls vs. 81.34 pg/ml for preeclampsia and 88.60pg/ml for gestational hypertension). Mean levels of Galectin-3 and Interleukin-6 were significantly lower in the control group compared to both of the pathological groups ($p<0.05$). The gestational hypertension and the preeclampsia group were not statistically different from each other for those two biomarkers. Mean Galectin-3 level was 6.53 ng/ml in the control group, 7.31 ng/ml in the gestational hypertension group and 7.59 ng/ml in the preeclampsia group, while Interleukin-6 levels were 2.77 pg/ml, 5.08 pg/ml and 8.06 pg/ml respectively. High-sensitive CRP mean levels were significantly higher ($p<0.05$) only when comparing the gestational hypertension group (6441.12 ng/ml) with the controls (5095.61 ng/ml), but there was no significant difference between the preeclampsia group (5581.02 ng/ml) and controls in our study. **Conclusion:** Hypertensive disorders of pregnancy are associated with significant changes in certain biomarkers, indicative of cardiovascular changes.

Trace elements/ vitamins/ electrolytes

Vitamin D Levels in Adult Outpatients for a Period of 8 Months

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Background: Vitamin D is critical for bone and mineral metabolism and plays an important role in immune response. It has anti-inflammatory, immunoregulatory properties and can modulate the innate and adaptive immune responses. Low levels of vitamin D have been associated with increased susceptibility to infections, immune-related disorders and other diseases. The aim of this study was to assess serum 25-hydroxy-vitamin D (25OHD) levels in adult outpatients from September 2020 through April 2021, a period with a high incidence of viral infections. **Methods:** The current study involved 367 participants (67 men and 300 women) mean age 46.31±3.5 years. Fasting blood sample was taken to measure serum 25OHD levels by the chemiluminescent immunoassay method (CLIA, Access 2-Beckman Coulter; ECLIA, Cobas e411-Roche). A descriptive statistical analysis was applied. **Results:** The mean 25OHD levels were 26.91ng/ml (67.29 nmol/l), lower than the recommended optimal value of 30 ng/ml (75nmol/l). Vitamin D deficiency (<20 ng/ml) was detected in 29.97% of the tested, insufficiency (20.0-29.9 ng/ml) in 38.96 %, and sufficiency (>30 ng/ml) in 31.07%. **Conclusion:** Low vitamin D status is being associated with many diseases, so optimizing serum vitamin D levels by improving lifestyle and

dietary supplementation would be a cost-effective measure to improve the general health of the population. **Keywords:** vitamin D status, population study

Sleep Apnea Involves Iron Homeostasis Regulation

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Background: Obstructive sleep apnea syndrome (OSA) is defined as a combination of symptoms as a result of intermittent, recurrent constraint and / or complete airway overhead airway overflow (sleep disturbance). OSA is associated with the development of insulin resistance, arterial hypertension, metabolic syndrome, systemic atherosclerosis and increased cardiovascular risk. **Methods:** 49 patients with OSA were included. Their results were compared to sex and age matched healthy control. All participants were evaluated for CBC, serum iron, ferritin, hsCRP, hepcidin, homocysteine and vitamin B12; atherosclerotic evidences were diagnosed through IMT and FMT changes. **Results:** Increased serum hepcidin concentrations were found in OSA patients with atherosclerotic evidences based on IMT and FMD changes (108.7 ± 9.9 μg/L) compared to healthy controls (19.1 ± 2.9 μg/L); $P<0.05$. A positive correlation was found in OSA patients with atherosclerotic changes between IMT and FMD to serum hepcidin ($r=0.817$, $r=0.845$, resp.; $P<0.01$). Serum hepcidin correlated positively to homocysteine and vitamin B12 in OSA patients ($r=0.874$, $r=0.839$, resp.; $P<0.01$). **Conclusions:** Brain-vascular disease risk factors are connected to obstructive sleep apnea syndrome. Dysregulation of iron homeostasis is one of the main atherogenetic factors. Early hepcidin quantification might predict an atherosclerosis occurrence in OSA patients, which might be very important for better clinical diagnosis and practice. **Acknowledgements:** This project is sponsored by MU-Sofia, as part of Grant D-52/2018.

Serum Calcium Difference in Geriatric Patients with Alzheimer and Vascular Dementia

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Background: Beside amyloid hypothesis which was accepted as a most relevant for Alzheimer disease, experience showed that current drugs didn't give improvement in cognitive functions. Cellular calcium signals regulation has high importance in neuronal physiology. The objective of this study is to investigate possible higher incidence of hypocalcemia in Alzheimer disease. **Methods:** We tested 60 serum samples collected from geriatric patients for serum calcium level on instrument Roche Cobas Integra 400, 36 with Alzheimer disease and 24 patients with vascular dementia respectively. **Results:** 32 of 36 patients or 88% have lower levels of serum calcium in Alzheimer disease patients and 16 of 24 or 66% of vascular dementia patients respectively. Alzheimer patients have median 1.95 (IQR 1.6 - 2.2 mmol/l) and vascular dementia patients have median 2.45 (IQR 1.9 - 2.6 mmol/l) in 2 by 2 table calculation we found value of $p<0.05$. **Conclusion:** Hypocalcemia is more common in Alzheimer disease then in vascular dementia. It can be proposed that calcium regulation can open up new approach to Alzheimer disease prevention and treatment. **Keywords:** Alzheimer disease, vascular dementia, serum calcium

Varia topics

Plasma KL-6 as a Potential Biomarker for BPD in Neonates

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Background: Bronchopulmonary dysplasia (BPD) is a chronic inflammatory lung disease of very-low-birth-weight (VLBW) preterm infants, associated with arrested lung development and a need for supplemental oxygen. This study has an aim to establish plasma levels of KL-6 in VLBW infants below 28 gestational weeks, depending on the presence and severity of BPD. **Methods:** Two groups patients was analyzed: A (15 infants) – without or with mild BPD; B (20 infants) – with moderate or severe BPD. The patients in both groups have no differences in gestational age, weight, Apgar scores, the severity of Respiratory distress syndrome and duration of mechanical ventilation and oxygen supply. KL-6 are analysed on day 7 and day 14 after birth. Use method is CLEIA FujiRebio LUMIPULSE G600II KL-6. **Results:** The results show that the mean plasma level of KL-6 on day 7 in group A is 315 U/mL, while in group B is 337 U/mL. In group A we find a reduction of the KL-6 on day 14 – mean level 199 U/mL, whereas in group B the mean levels of KL-6 increase – 498 U/mL, and this difference is statistically significant ($p\leq0.001$). Two infants from group B with severe BPD have plasma KL-6 above 1000 U/mL at two weeks of life. **Conclusion:** We conclude that plasma KL-6 could be an early screening marker for the detection of infants at higher risk for developing BPD. Increasing levels of KL-6 during the first two weeks of life as well as very high plasma levels of KL-6 are typical for

infants that develop severe BPD. **Keywords:** Bronchopulmonary dysplasia (BPD); very-low-birth-weight (VLBW); KL-6.

Klippel-Trenaunay-Weber Syndrome: a Case Presentation

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Background: Klippel Trenaunay Webber Syndrome (KTWS) is a rare congenital disorder characterized by asymmetric limb hypertrophy, usually of the lower limbs, as well as vascular anomalies and capillary malformations. The aim of our study is to present the laboratory findings of a KTWS case. **Methods:** A 20 year old female patient was presented at our laboratory for routine examination. We performed biochemical, haematological and haemostasis tests, capillary serum protein electrophoresis and immunofixation, as well as tests for the detection of autoantibodies. **Results:** Her complete metabolic panel and complete blood count were normal. Serum protein electrophoresis and immunofixation were normal too. CRP and ferritin levels were 92.2mg/L and 214.0 ng/mL, respectively. Coagulation test results were PT 14.4 sec, aPTT 33.8 sec, INR 1.27, D-Dimer 31.87mg/L and Fibrinogen 96mg/dL. **Conclusions:** KTWS is rare, genetic disorder, associated with the translocation at t(8;14)(q22.3;q13). Its incidence and prevalence are not known. There is no apparent ethnic or gender predilection. Diagnosis of KTWS is mainly clinical. There are no specific laboratory tests. Tests should focus on evaluation of the type, extent and severity of the malformations. In this case report, CRP and Ferritin levels are elevated due to chronic inflammation. Moreover, D-Dimer levels were elevated due to deep vein thrombosis in the patient‘s legs. **Keywords:** Klippel Trenaunay Webber Syndrome (KTWS), congenital disorder, thrombosis.

Laboratory Findings of a Decompression Sickness Case

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Background: Decompression sickness (DCS), also known as the divers' disease, is a condition arising from dissolved gases coming out of solution into bubbles inside the body, during depressurization. The aim of this study is to present the laboratory results of a DCS case. **Methods:** Male, of Greek origin, aged 36, was admitted to our Emergency Department with shortness of breath, chest pain, vertigo and nausea, following an uneventful four-hour scuba dive at 30 meters. Hematological, biochemical and haemostasis tests were performed immediately. Moreover, these tests were repeated every day for a 10 days interval. **Results:** A complete blood count revealed WBC of 15.3 cells/μL, hemoglobin of 17.2 g/dL, and hematocrit of 49%. The basic metabolic panel was normal. The hepatic function panel showed mild elevations in AST and ALT at 130.9 U/L and 102.7 U/L respectively. Significant elevations in LDH, CPK and CRP at 3562.0 U/L, 1725.1 U/L and 389.9 mg/L respectively, were observed. Serum myoglobin level was at 411.0 ng/mL and his urine color was dark red. Serum sodium and potassium levels were 140.5 and 4.53 mmol/L. Coagulation tests (PT, aPTT, INR and Fibrinogen) were within normal limits. D-Dimer levels were at 31.6 mg/L. Arterial blood gas demonstrated pH 7.42, pCO2 35.7 mmHg, pO2 65.1 mmHg, and bicarbonate 22.5 mmol/L. **Conclusions:** In a confirmed case of DCS some laboratory abnormalities were observed, related to mild elevations in AST and ALT and significant increase of the LDH, CPK and CRP concentration. In addition, high levels of D-Dimer and serum myoglobin concertation were detected. Finally, the urine colour was dark red.
Keywords: Decompensation sickness, myoglobin, D-Dimer, laboratory test results

Evaluation of TAT for Biochemistry ED Samples

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Background: Prolonged Turnaround Time (TAT) of laboratory results affects patient care as well as patient satisfaction adversely. The objectives of our study were to measure the TATs of biochemistry results to the Emergency Department (ED) and to identify the causes of prolonged TAT. **Methods:** The TATs for the biochemistry ED profile measurements during one month were used (428 samples). Data were collected from the HIS database. TAT start time was defined as the time of specimen arrival at the laboratory; end time was defined as the time of completion of the last test ordered, including the 20-minute clotting step. Outliers were defined as more than 60 minutes for biochemistry measurements. The time taken to transport blood samples to the laboratory was excluded. Incomplete tests due to haemolysis or lost specimen, as well as those for which part or all of the data was missing were excluded. Full day work cycle was divided in 2 hour intervals. Analysis was performed by means of Pareto chart, which is a bar graph. The lengths of the bars represent frequency and are arranged with longest bars on the left

and the shortest to the right. In this way the chart visually depicts which situations are more significant. **Results:** The total TAT results confirmed that the 60-minute goal for the processing of stat specimens was not being met for 10.5% of the samples. 60.3% of the prolonged TAT was recorded from 06:00 to 10:00 every day, which coincides with healthcare personnel shift changes at 07:00, as well as high volume of testing, work load and internal quality control process during this time interval. **Conclusions:** TAT is an important quality indicator of laboratory performance. Appropriate triage and discharge of patients is impacted by the timely return of laboratory test results, so TAT of laboratory tests is a key contributor to ED workflow. A Pareto chart is a basic quality tool that helps you identify the most frequent defects, complaints, or factors you can count and categorize. It is based on the "80/20 rule," which postulates that 80% of the problems come from 20% of the causes. In our study we measured prolonged TAT in 10% of stat specimens. We studied the lab processes in more detail and successfully identified human factors in process control.