https://doi.org/10.1515/cclm-2021-0744 — Clin Chem Lab Med 2021; 59(9): eA53–eA97

Congress Abstracts

60th National Congress of the Hungarian Society of Laboratory Medicine

Online Congress – August 26–28, 2021

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PL.1

The HUNGARIAN JENDRASSIK-award lecture
Fifteen years of experience in medical laboratory accreditation

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One and key of the priorities in laboratory medicine is improvement of quality management system for patient safety. Quality in the health care is tightly connected to the level of excellence of the health care provided in relation to the current level of knowledge and technical development. Accreditation is an effective way to demonstrate competence of the laboratory, a tool to recognize laboratories world-wide, is linked to periodical audits, to stimulate the maintenance and improvement of the quality, which leads to high standard of services for clients (patients, health care providers, etc.). The strategic plans of IFCC and EFLM include focusing on accreditation of labs based on ISO standards and cooperation with European Accreditation and national accreditation bodies. IFCC and EFLM recognised that *ISO 15189:2013 Medical laboratories - Requirements for quality and competence*, encompasses all the assessment criteria specified in the policy of quality. The last version is oriented to process approach with detailed division and clearly defined requirements. The accreditation of labs improves facilitation of accurate and rapid diagnostics, efficiency of treatment and reduction of errors in the laboratory process. Accreditation is not about who the best is, but who has a system of standard procedures with aim to improve the quality and patient safety. Quality system is about people, with people and for people.

The Institute of Laboratory Medicine has been accredited since 2008 and has been operating a quality management system since 2006.

In my presentation I would like to present the quality management system of the Institute, its development and implementation process, the impact of changes in the quality standard and our experiences.

PL.2

The FOREIGN JENDRASSIK-award lecture
Postgradual clinical pathology training in the United States: Observations from the past 20 years

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The Clinical Laboratory Improvement Amendments of 1988 (CLIA) set the federal regulations and standards for all U.S. facilities that test human specimens. CLIA regulates laboratory certifications, safety, and quality for all test complexity levels, as well as the training and certification requirements for clinical laboratory testing personnel and lab directors. For physicians, the most common avenue for certification is the completion of a residency program in Clinical Pathology and examination by the American Board of Pathology. Clinical pathologists then may enroll in a fellowship program followed by an exam and certification in a subspecialty. Due to the large number of medical laboratories in the U.S. and various organizational and economic factors, PhD colleagues can also qualify to become laboratory directors. They must hold a doctoral degree in a chemical, physical, biological or clinical laboratory science from an accredited educational institution, gain postdoctoral experience in a clinical lab and/or enroll in a training program. Accepted board certifications for PhDs include ABCC – American Board of Clinical Chemistry, offering certifications in Clinical Chemistry, Molecular Diagnostics, and Toxicology, and various other specialty boards. The Clinical Chemistry certification also includes training in automated hematology, coagulation, urinalysis, serology, and infectious diseases. Accreditation to these programs fosters excellence

and helps attract qualified individuals. Certain aspects of the American experience may be useful in Hungary when building of a similar clinical pathology training and certification program for qualified PhD colleagues is considered.

Suggested reading: Lorenz RG et al. Acad Pathol 2018;5:1-8

PL.3

The HONORARY MEMBERSHIP-award lecture Cardiac biomarkers in COVID-19

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Coronavirus disease 2019 (COVID-19) causes an ample series of respiratory and non-respiratory derangements, which lead to development of critical illness, multiple organ failure, up to death. A high frequency of arterial thromboses has been described in many studies. As specifically concerns myocardial injury, some degrees of heart involvement could be found in the vast majority of patients with severe illness. The consequent increase of cardiac biomarkers in general, and cardiac troponins in particular, is hence attributable to this frequent onset of cardiac injury in patients with COVID-19. Cardiac troponin increases can be found in 42% of patients with severe illness and 26% in those with milder disease. A strong association has then been found between increased values of cardiac biomarkers, especially cardiac troponins, and unfavorable outcome of COVID-19. Cumulatively, an increased value of cardiac troponins may be associated with an over 5-fold higher risk of developing severe illness and up to 25-fold higher risk of death. Another important aspect is that not only cardiac troponin levels are important predictor of worse outcome, but also their relative increase is directly related to unfavorable disease progression and mortality, with such risk increasing from 1.17 folds for values just exceeding the upper reference limit, up to over 2.4 folds when cardiac troponin levels is 50-fold increased over the upper limit of normal. The value of other cardiac biomarkers, such as natriuretic peptides and cardiac fibrosis biomarkers, is also currently addressed, with important evidence in favor of their assessment. An algorithm can also be elaborated that would make the sense of cardiac troponin testing in patients with COVID-19, with identification of patients at higher risk of developing cardiac injury, either directly triggered by SARS-CoV-2, or indirectly consequent to cytokine storm and thromboinflammation. According to this model, non-evolving cardiac troponin values, along with normal levels of other biomarkers of inflammation or cardiac dysfunction, may safely limit the necessity to perform other diagnostic investigations such as transthoracic echocardiography or stress testing in patients with suggestive signs or symptoms of cardiac involvement.

PL.4

The HONORARY MEMBERSHIP-award lecture The role of laboratory testing before and after COVID-19 vaccination

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The COVID-19 pandemic has affected all aspects of society, dramatically changing our day-to-day lives and habits. It has also changed clinical practice, including practices of clinical laboratories. This pandemic, in fact, led to an impressive increase of the visibility and value of clinical laboratories to the population at large. The key role of laboratory testing in modern medicine requires laboratory professionals to take on more, ever new responsibility with the aim of improving not only analytical performance but appropriate requesting and utilization of laboratory tests, as well as the integration of laboratory information with all other diagnostic and clinical data. Laboratory testing is needed for the right diagnosis even in asymptomatic/presymptomatic subjects using both molecular and antigen-based tests, for the appropriate monitoring and prognostication of

disease severity by using biochemical, coagulation, hematological tests and for epidemiological surveillance. In addition, laboratory testing particularly serological assays to measure SARS-CoV-2 specific antibodies play a key role in evaluating the efficacy of vaccines already developed and under development. In real life, antibody testing should be used to develop prioritization strategies, to reduce unnecessary potential side-effects in those people who have already developed the disease and to better evaluate the immune response particularly in immunosuppressed patients and patients under therapies. Finally, an increasing interest is to integration of serological data with cell-mediated immunoresponses.

SE1.1

Minimal retesting interval (MRI): how to use it in clinical laboratories?

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Introduction: The increasing number of more-and-more specific laboratory tests results in their inappropriately frequent usage that can end-up in “overtreatment” of patients and enhancement of laboratory costs. In order to circumvent this problem minimal retesting intervals (MRI) can be introduced in clinical practice by defining the minimum time before a test should be re-tested. The *National Minimum Re-testing Interval Project* initiated by the ACB and RCP defined the most important MRI values covering the areas of clinical chemistry, hematology, immunology, toxicology, microbiology and cellular pathology in 2013 and 2015.

Aims: Based on literature data the requirements, the possible implementations and the technical and financial outcomes of MRI testing will be discussed.

Results: The definition of MRIs must rely on evidence-based guide-lines and state-of-the-art clinical practice, must be established together with the ordering physicians and depends a lot on the clinical situation in which it is used. Its practical application can be realized in the clinical information systems by defining “hard”- or “soft”-stops at test requesting. The maximal decrease in the test number can be upto 50% (in the case of tumor markers or autoantibodies) but the reduction of costs can be driven by the most abundant tests (HgbA1c, lipids, sTSH, Vitamins, ferritin, folic acid).

Conclusions: Implementation of MRI testing requires thorough communication and consensus between the clinicians and laboratory specialists. Clinical information systems can provide a proper interface for physicians to handle pre-defined MRI regulations. Routine application of MRI testing can reduce the number of required tests and diminish the costs of laboratory testing.

SE1.2

Pandemic lesson on pre-analytical quality indicators

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The use of quality indicators (QIs) is essential to measure and evaluate the quality and effectiveness of laboratory testing. Quality indicators have been constantly changing since 2008. The latest update by IFCC and EFLM was made at the 2016 Padua Consensus Conference. The new Model of QI (MQI) has been classified into four priority scores (mandatory, important, proposed and evaluated) to help identify critical steps where development action is needed.

Our QI database (collected between 2016-2020) was re-checked retrospectively according to MQI (revision 1.0, January 2017). We wondered if QI data would be affected by the lack of phlebotomy education held monthly due to the 2020 pandemic. In our laboratory, we use 10 mandatory pre-analytical QIs from the IFCC list (n = 25). 5 of them were between 3-4 Sigma, 2 of them were between 4-5 Sigma, and 3 of them were better than 5 Sigma. There was no significant change, except for one (samples collected in wrong container), which deteriorated significantly (Sigma mean = 5.2 between 2016

and 2019, Sigma = 4.6 in 2020). Another was also deteriorated (clotted samples with anticoagulant), but not significantly (Sigma mean = 4.0 between 2016-2019, Sigma = 3.9 in 2020).

The study showed that total QIs did not change in 2020 compared to previous years, so the general phlebotomy education used so far is ineffective because processes cannot be improved with this tool. In the future we need to focus on those departments where the pre-analytical errors detected are common. However, for some indicators, data collection also needs to be changed so that QIs can be analyzed according to requesters, which is very difficult to achieve with manual data collection. Manual QI collection is time consuming and does not provide detailed data, so where possible it should be automated.

SE1.3.

The role of evidence-based medicine and diagnostics in future Hungarian healthcare system

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In the personalized, value-based healthcare system, laboratory medicine – due to its horizontal position – has a very high level of influence and critical role to support the clinical decisions along the entire patient journey. We are faced with a steadily increasing amount of biological (e.g., diagnostic) information about the diseases, about the physiological pathways of the patients, the potential success of the applied therapy-combinations or -sequences. This phenomenon and the broader and more targeted therapeutic spectrum create a special pressure around and in the health care system to develop faster into a more personalized direction and in an added value-centric way. The newly developing healthcare system – where the clinical outcomes of the individual patient with their diseases and different molecular responses are in the centre – needs to be patient journey-based, guideline-driven, flexible, innovation-friendly, evidence- and value-based. Synergies between clinical teams and diagnostic teams (supported by digital tools) are critical and very much needed. We need to be very personalized but also professional and quality-conscious to provide the rights and needed information with appropriate interpretations in time; to create the best clinical decisions in the proper situation. To do this, the system needs innovations in terms of healthcare organization, technology, digitalization, legislation (e.g., patient law), health economy, financing. The change in the mindset and in the system could be effectively supported by interdisciplinary cooperation between different healthcare competencies. Our interdisciplinary expert team has elaborated a “white paper” on potential applications of these ideas in future Hungarian healthcare system.

SE1.4

Study to optimise the practices of test repetition within laboratory network

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Several research groups have found that automatically measured laboratory results with modern methodologies are accurate. Accordingly, it also becomes necessary to optimise the practices of retesting. In our study we examined 1844 repeated alert results according to the recommendation of Clinical Laboratory Improvement Amendments regarding to the total allowable error limit. Of these, 929 were hematological and 915 were chemical investigations. Of the 1844 results, 1701 (92%) remained within the margin of error. Hematological measurements were performed on two different types of analyser. The highest percentage (12%) of the hematological tests, the platelet counts were out of error range on both instruments. In this case the range of the platelet count was from 2 to 53 G/L (low / critical risk range). Mostly the chemical results (triglyceride: 80%) were out of error range, but 100% of these were outside of the measuring range. For glucose, we found that 29% of the repeated

results were out from the CLIA recommendation, and only 4% of these results were outside of the measuring range. These results were from 0.4 to 2.3 mmol/L in the low range and from 25.2 to 54.9 mmol/L in the high range. All measured result on same type of analyser in the Budapest Central laboratory remained within the error range. All these results were from 1.3 to 2.6 mmol/L in the low range and from 20.0 to 32.2 mmol/L in the high range. So, within this interval the automaton measures accurately. We established the following recommendation: all laboratories should review their practices of test repetition. If the initial result is within the measuring range of the laboratory instruments, retesting is not necessary for almost all automated examinations, but it is important to assess the accuracy of each instrument before determining the repeatability limits.

SE1.5

Implementation of autoverification system at the University of Debrecen: 15 years experience

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Introduction: Autoverification (AV) is a safe and efficient post-analytical process that reduces the number of laboratory test results requiring review and manual validation and consequently reduces the turnaround time.

Aims: A laboratory information system (LIS) based test verification system was introduced to reduce turnaround time, improve detection of laboratory error and patient safety.

Results: As a result of continual upgrading of the AV algorithm over the last 15 years at our Department, an average autoverification rate of 76% has been achieved. Through the years the AV algorithm has been systematically applied to all the routine diagnostic units at our laboratory, and the rate of autoverification in the various laboratory units is as follows:

Clinical chemistry: 62%, Immunochemistry: 81%, Therapeutic Drug Monitoring: 73%, Tumor markers: 62%, Endocrinology: 70%, Chromatography: 77%, Electrophoresis: 77%, Immunology: 59%, Hematology: 68%, Hemostasis: 55%.

Furthermore, the impact of autoverification algorithms was also monitored on the turnaround time. Turnaround time analysis of routine tests showed a remarkable decrease in turnaround time with an ever broader application of autoverification.

Conclusions: Our findings strongly support the integration of AV in the LIS-based workflow. A well-designed set of autoverification rules and algorithms improves productivity, and facilitates prompt decision-making and allows focus on problematic test results and preferably improves patient care.

SE1.6.

Assessing quality of interpretative comments in haemostasis

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Interpretation of laboratory results in clinical context of the patient is an integral part of postanalytical activities of laboratories. Although works are in progress in EQA organisations to set the criteria of high-quality interpretive comments (ICs) in different fields of laboratory medicines, no harmonised standards of ICs exist. The aim of this survey was to establish quality criteria for evaluation the quality of ICs in haemostasis and validate this system in 270 laboratories of 11 European countries. Participant laboratories were asked to analyse then classify two samples from patients with bleeding symptoms. Those laboratories which on the basis of their routines would have reported results together with ICs were asked to share in the survey the ICs they would have given in real life into their native language. Scoring system of ICs established in this survey included the following aspects: 1) analytical content; 2) tentative diagnosis; 3) provided advice

to the clinician; 4) clarity of the phrasing; 5) length of ICs. Quality of the provided ICs have been analysed in consensus of 3 pairs of coagulation experts. While vast majority of laboratories provided analytically correct results on both samples, the classifications of the two samples were correct only in 47% and 83%, respectively. 90% of laboratories indicated to add ICs beside numerical results in real life and 80% shared their ICs in the survey. Shared ICs were found diverse and only small proportion of them reached highest scores. Findings of our survey confirm that standards on the content and wording of ICs are required. Those who add ICs might need support with trainings and EQA schemes in native languages of the users with internationally harmonized assessment of performance.

LS1.1

Comparison of monocyte distribution width (MDW) with different sepsis markers in emergency care

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Sepsis is still a leading cause of death. The goal of emergency care is to identify and risk-stratify patients with suspected or established sepsis, since early detection is associated with better survival. Recognition is mainly based on clinical picture but some biomarkers (presepsin, IL-6, PCT, CRP) and other parameters such as neutrophil/lymphocyte ratio (NLR), white cell count (WCC) might assist early diagnostics. We targeted to compare the feasibility of MDW with other sepsis markers. One-hundred and thirty four patients were recruited (61 male) in our comparative, prospective study. Mean age was 55 ± 20 years. Patients were involved after informed consent. Patients were dichotomized according to confirmed infection/sepsis or no infection/sepsis after diagnostic procedures. Comparison was carried out using Mann-Whitney U-test, significance was determined at $p<0.05$. In 25 (19%) cases sepsis was confirmed while in 108 (81%) no sepsis was present.

Significant difference was detected between infected/septic versus non-infected/non septic patients in case of presepsin (270 [186–543] vs. 192 [127–284] pg/mL), IL-6 (52.33 [19.36–128.68] vs. 8.05 [2.18–22.58] pg/mL), PCT (0.096 [0.041–1.013] vs. 0.033 [0.020–0.069] pg/mL, CRP (46.75 [19.88–134.58] vs. 2.85 [1.53–13.63] mg/L) and also in case of MDW (20.37 [18.81–23.03] vs. 18.42 [174.14–19.85]) WBC (12.09 [9.09–18.29] vs. 8.23 [6.7–10.16] G/L) and NLR (7.43 [4.615–18.125] vs. 2.97 [2.018–4.383]).

Based on our results MDW seems to be a promising sepsis marker compared with other such parameters. Due to the low number of cases further measurements are planned.

LS1.2

Role of Monocyte Distribution Width (MDW) in early recognition of sepsis and progression follow-up in emergency settings

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Early detection of infection could not only lead to early diagnosis, but it prevents treatment delay or worsening of the clinical condition. A new line of evidence indicates that monocyte distribution width (MDW) could be a promising new biomarker in sepsis management.

In our recent work a retrospective study was conducted to investigate the performance of MDW in clinical decision making in diagnosing infections comparing it to other biomarkers, such as C-reactive protein (CRP), procalcitonin (PCT) and white blood cell count (WBC). Overall 1075 adult patients were involved in the study and all data were obtained from the hospital's medical record system, with the approval of the University Ethical Committee. The patients were admitted to the Emergency Department (ED) during the study period (September 2019 - December 2020) with signs of suspected infection. Patient cohorts were created based on Sepsis-3 criteria (no infection N=365, infection N=309, sepsis N=322, septic shock N=79). MDW was measured as part of the blood picture by DxH900 hematology analyzer (Beckman-Coulter). CRP and PCT were determined by cobas 8000 and cobas pro analyzers, respectively (Roche).

MDW showed remarkable performance both in identifying infection and signaling the progression of the inflammation. MDW levels were significantly different in all groups (19.52-27.96, p=0.000-0.009). MDW showed almost the same performance in septic shock as PCT (AUC 0.810 (PCT) vs 0.769 (MDW)), and performed better than CRP. We recommend the use of MDW as a sepsis indicator in emergency settings due to its reliability, availability and financial affordability.

LS1.3

Serial assessment of humoral immune response to Pfizer-BioNTech vaccination in Szigetvár Hospital

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Vaccination is in focus against the corona virus pandemic. The Pfizer-BioNTech vaccine was reported 95% effective at preventing COVID-19 disease. Our aim was to explore relationships among the humoral immune response and age, BMI, post-vaccination reactions respectively. Besides, the antibody response in subjects with breakthrough infections was also examined. A total of 325 health care workers from Szigetvár Hospital, with or without previous COVID-19 disease were included after full-dose of Pfizer-BioNTech. Serial blood samples were drawn at 2 weeks, 1, 2 and 3 months after receiving the second dose of vaccine. The first samples were tested for IgM and IgG antibodies for SARS-Cov-2 spike proteins using the Beckman-Coulter Access SARS-CoV-2 IgM and IgG, but later only IgG antibodies were determined. Higher IgG levels were detected in the age group (<47.6 years as median) at 2 weeks (p=0.007), 1 month (p=0.003), 2 months (p=0.03) and 3 months (p<0.001). In the female group, a significant inverse correlation appeared between BMI and IgG antibody level at 3 months post-vaccination (-0.183; p=0.02). Among persons with subfebrility/fever as post-vaccination reaction, a higher IgG level was detected 1 month (p=0.023), while in those with joint pain, IgG levels were higher at 12 days (p=0.04) after vaccination. In those who have had COVID disease, lower IgM levels were measured 12 days (p<0.001) and higher IgG levels 1 month (p=0.018) after vaccination. After the second dose, four breakthrough infections have been developed during 3 months follow-up. In this group, lower IgM levels (at 2 weeks, p=0.04) and higher IgG levels (at 3 months, p=0.014) were measured after vaccination. These findings suggest that the humoral immune response is influenced by demographic (age, gender, BMI) and post-vaccination factors. In post-COVID subjects, a lower IgM and higher IgG anti-spike protein antibody levels were revealed.

SE2.1

Lessons learned in an external quality assessment scheme of steroid testing with respect to liquid chromatography-tandem mass spectrometry assays

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is recognized as the gold standard for assaying endogenous steroid substances in biological matrices. We participated in the international external quality assessment (EQA) scheme offering testing for the largest number of steroid analytes between 2018-2020 using a method developed, validated and routinely used in our laboratory for profiling 16 steroids in human serum. Aldosterone (ALDO), androstenedione (AD), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), 11-deoxycortisol (11DF), 17α-hydroxyprogesterone (OHP), cortisol (F), progesterone and testosterone (TT) were tested. Consistently good performance of the method employed at our laboratory was achieved for AD, DHEA, 11DF, OHP and TT. Consistently poor performance was observed for ALDO, DHEAS and F. The number of laboratories using LC-MS/MS was up to 5, and the number of peers was up to 2. In several cases it was not the difference of the result submitted from the mode mean but the standard deviation of all submitted results that eventually determined the performance of our method. 11DF was present at concentrations lower than our limit of detection (1 ng/dL) in most distributed samples, nevertheless, several laboratories submitted physiologically relevant concentrations.

Despite the increasing use of LC-MS/MS in bioanalysis, most clinical laboratories still rely on immunoassays; hence, the emergence of EQA schemes offering testing for clinically important analytes (e.g. dihydrotestosterone or 21-deoxycortisol) or conducted with the participation of a higher number of LC-MS/MS laboratories is yet to come. Switching to novel evaluation tools, e.g. the clinical concordance of methods would nevertheless be desirable in the available schemes.

SE2.2

Remnant cholesterol and its relationship with lipoprotein particle number and size determined by proton nuclear magnetic resonance spectroscopy

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According to recent international guidelines remnant cholesterol concentration (RC) should be included into the basic lipid panel as a risk factor of atherosclerotic coronary disease (ACD). The aim of this pilot study was to compare results of RC with lipoprotein particle number and size determined by proton nuclear magnetic resonance spectroscopy (PNMR) in a randomly selected group of probands without clinical manifestation of ACD. Patients and methods: Data of 50 randomly selected probands from outpatient clinics without clinically manifest ACD (age 37 – 72 years, 38 men, 12 women) were evaluated. Lipoprotein particle number and size was measured with INFAI NMR spectroscopic assay (Germany). Basic lipid parameters (total, LDL and HDL cholesterol – TC, LDLC, HDLC) were measured by routine laboratory methods. RC was calculated as TC - (HDLC + LDLC). The concentration of RC was 0.58 ± 0.20 (min. 0.10; max. 1.40) mmol•L^{-1}. It comprised 11±3 (1 – 18)% of TC and 17±2 (6 – 41)% of LDLC. The concentration of RC was associated directly with the PNMR measured VLDL and LDL particle number ($r = 0.6324$ and 0.4243; $p<0.001$ and 0.01), with the number of small LDL particles ($r=0.3845$; $p < 0.01$) and with the size of LDL particles ($r = 0.4625$; $p < 0.01$). According to our pilot study the

inclusion of RC into basic lipid panel is a rational idea because it is atherogenic and can comprise as much as 41% of LDLC. RC concentration is significantly associated with the PNMR measured lipoprotein particle number and size. To prove the validity of this hypothesis further studies on sufficient number of participants are necessary.

SE2.3

Effect of statin therapy and cholesterol supplementation in Smith-Lemli-Opitz syndrome

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Smith-Lemli-Opitz syndrome (SLOS) is an inherited disorder of cholesterol biosynthesis. Deficiency of 7-dehydrocholesterol-reductase (DHCR7) causes high 7-dehydrocholesterol (7DHC), low cholesterol, dysmorphism and somatomental retardation. Current strategies combine statin to decrease toxic level of DHC and cholesterol supplementation. Patients: Out of 12 SLOS patients (age>1 year) cholesterol supplementation and statin therapy was combined in 9 patients. In most cases statin was suspended when transaminases (AST, ALT) increased, as it may refer to liver damage. Finally, we could follow only three patients treated with statin over three years. Their lipid markers, liver function and clinical condition were monitored. Results: *Patient-1* received atorvastatin (2-3 mg/day) and cholesterol supplementation since 1y age. After 4 years cholesterol increased from 1.2 to 2.3 mmol/L, TG was normal (~1.6 mmol/L), but clinical condition did not improve. *Patient-2* received atorvastatin (2-7 mg/day) and cholesterol supplementation between age 1-10y. Cholesterol fluctuated between 1.6-3.4 mmol/L, TG was 0.7-0.9 mmol/L. He developed slowly and entered a special school. In *Patient-3* after 3 years simvastatin therapy (3 mg/day) and cholesterol supplementation cholesterol increased (2.77-3.1 mmol/L), then decreased when statin was suspended because of high CK (240 U/L). TG was 0.5 mmol/L. He walks, but can't speak. Conclusion: Cholesterol supplementation and low dosage statin is a debated approach in SLOS. It may increase cholesterol and decrease 7DHC, but may cause liver or muscle damage. Monitoring of lipids, dehydrocholesterol, CK and liver function is recommended. As 7DHC produces toxic oxysterols, antioxidant and cholesterol supplementation may be advantageous.

SE2.4

Response of the endogenous steroid homeostasis to extreme level of physical stress

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Monitoring the adrenal steroid homeostasis under extreme physical stress may support the assessment of fitness level, illicit anabolic steroid use and any adrenal disorder of athletes. Male adult handball players underwent vita maxima load by treadmill tests. Blood was taken before exercise (baseline), at peak of load (peak) and 30 min into restitution (restitution). The concentrations of 14 endogenous steroids were assessed in these three phases. Multivariate statistical analysis revealed the activation of all adrenal biosynthetic pathways. Significant changes (dominantly increase, in 88.5% of analytes tested) were detected for 6, 9, and 11 steroids at baseline vs. peak, peak vs. restitutiton and baseline vs. restitution, respectively. Moderate to strong correlation was identified between the concentrations of androstenedione (ADRN) and 11-deoxycortisol, ADRN and dehydroepiandrosterone (DHEA), DHEA and corticosterone (CCON), as well as

between CCON and cortisol (CTOL). DHEA/CTOL ratio increased continuously from baseline to restitution. Testosterone (TEST)/CTOL ratios were highest at the peak, but were lower in restitution than in baseline. Conclusions: mineralocorticoid, glucocorticoid and androgen pathways are activated simultaneously under physical exercise. Steroid levels change most remarkably between baseline and restitution. The evaluation of the concentrations of adrenal steroids and their ratios at various phases of extreme physical stress may deliver important biomarkers of the physiological traits of athletes from a very young age.

SE2.5

Reliability of free and total triiodothyronine measurements

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Introduction: In the laboratory assessment of hyperthyroidism, the determination of triiodothyronine (T3) is only a third-line marker. Its thyroidal excretion is small relative to the circulating total amount. Thus, under physiological circumstances, it can only indirectly reflect thyroid function. Nevertheless, in certain diseases, it is absolutely necessary to determine it to confirm or exclude T3 hyperthyroidism. In the Hungarian laboratory practice, which is mainly done according to the TSH-based algorithm, free T3 is only measured after low TSH and normal T4 finding. However, several guidelines recommend measuring TT3 only or TT3 and FT3 together with immunoassays. This is not a coincidence, as FT3 accounts for only a thousandth of circulating TT3 and T3 also binds to binding proteins with much lower affinity than T4. Therefore, it can be assumed that TT3 and FT3 finds should be close to each other. From an analytical point of view, however, the TT3 promises to be more reliable. With all this in mind, we aimed to examine how the FT3 and TT3 results correlate with each other on two measurement systems. Materials and methods: 167 patient sera with subnormal TSH and normal FT4 were analysed for FT3 and TT3. Among them 105 samples were measured on the Siemens Atellica system (Cohort_I) and 62 on the Roche Cobas system (Cohort_II: all clinical data from patients were known). Results: T3 hyperthyroidism based on FT3 levels was measured much more frequently in both systems than in the TT3 results (Cohort-1: 54% vs. 14%; Cohort-2: 21% vs. 4.8%). At 6–12-month follow-up, FT3 levels were 2.5 times more likely to be misleading for T3 hyperthyroidism than TT3 results, which resulted iatrogenic hypothyroidism in a few patients. Conclusions: Our results support that, measured on both systems, the TT3 method is superior to FT3, so it is recommended to supplement the currently used algorithm with TT3 measurement. This could reduce the possibility of an inappropriate therapeutic decision.

SY1.1

Postgraduate training in the field of laboratory medicine at the University of Debrecen, Hungary

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Introduction: Laboratory profession is a cornerstone in medical service and should be practiced by professionals. Ways to achieve a sufficient knowledge in the field can be heterogenous, starting from basic training (medicine, pharmacy, science) and the largely varying postgraduate training areas in various European countries.

Methods: We at the Department of Laboratory Medicine at the University Medical School in Debrecen, Hungary launched a project in 2012 when we aimed to introduce regular written exams to trainees in laboratory medicine. In the period of 2012-2020 we set out to generate complex tests to medical residents and trainees in clinical biochemistry. We created

overall 42 tests covering four large areas (i) clinical chemistry (ii) hematology (iii) heamostasis (iv) immunology and molecular testing and graded the trainees during their residency periods. The types of questions were of three major groups: multiple-choice questions, analysis of laboratory cases, short assays.

Results: Overall 26 people started their postgraduate studies in North-Eastern Hungary 13 of them with MD degrees and 13 with MSc/PhD degrees. There was a dropout rate of 15%, mostly among clinical biochemists. The four separate parts of the 'mid-term' tests resulted in variable outcomes. The range of test performance was 38%-91% with a mean of 65%. The poorest performance was observed in cytogenetics, and in areas of laboratory medicine with complex case analyses and where detailed interpretative comments were required. It could be observed that MDs performed much better in Hematology compared to MSc/PhD graduates. However PhD graduates with a research background in molecular biology usually performed better in molecular testing.

Conclusion: Clinical laboratories require well-trained experts in areas of validating laboratory results, clinical consultations, result interpretations and method development. This knowledge can not be gained by the daily diagnostic work only. We concluded that formal exams were required that also created a competitive atmosphere where trainees were stimulated for better performance.

SY1.2

European Federation of Clinical Chemistry and Laboratory Medicine: opportunities offered to laboratory professionals

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The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) was established in 2007 in Amsterdam. It is legally registered in Belgium, its office is in Milan, Italy. It represents the laboratory medicine specialists of 42 countries' national societies. The Hungarian Society of Laboratory Medicine is also a full member. The current President is Ana-Maria Simundic from Croatia. I will present some useful opportunities offered by the EFLM to the lab professionals of its member societies that may be of general interest.

Individual members of the EFLM Academy have free access to EFLM webinars, to Clinical Chemistry and Laboratory Medicine (CCLM), the official scientific journal of EFLM (as well as of the Hungarian Society), and to documents of the Clinical Laboratory Standards Institute (CLSI). Academy members also get e-mail notifications of EFLM issues, and the bimonthly newsletter, the EuroLabNews. The Hungarian Society established block enrolment of its members: this means that members who apply during the year and those who confirm their current membership at the end of the year, will be enrolled by the national society into the Academy and registered by EFLM Office for the coming year. The annual fee is 15 Euros/individual member, which is paid directly by the national society. EFLM also starts to organize postgraduate courses at reduced rates for Academy members. The (EFLM) Biological Variation Database has also been managed by the EFLM since 2019 (Westgard database previously). The EFLMLabX is a web service to enhance contact between lab specialists in a laboratory exchange programme.

SE3.1

Serum ACE2 activity for the assessment of disease prognosis in COVID-19

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Angiotensin-converting enzyme 2 (ACE2) represents the main receptor for SARS-CoV-2 to enter endothelial cells. Interestingly, controversial data have been reported for soluble ACE2 levels in COVID-19. Here we analyzed circulating ACE2 activity to correlate with laboratory parameters as well as the severity and outcome of COVID-19 disease. We determined serum ACE2 activity from 66 subjects with moderate COVID-19 and 110 critically ill patients. ACE2 levels were correlated with IL-6 and ferritin concentrations as well as clinical outcome. Baseline ACE2 activity was significantly higher than normal in patients with severe COVID-19 (54.4 [36.7-90.8] vs. 34.5 [25.2-48.7] mU/L in non-severe patients; P<0.0001). Circulating ACE2 showed a significant (P<0.0001) but moderate correlation with IL-6 (r=0.345) and ferritin (r=0.295). Substantial area under the receiver operating characteristic curve (ROC-AUC) value was determined for baseline ACE2 to indicate disease severity (0.701 [95% CI 0.621-0.781]; P<0.0001). Furthermore, significantly higher serum ACE2 was measured in non-surviving vs. surviving COVID-19 patients (54.6 [37.3-94.7] vs. 35.6 [25.3-58.5] mU/L; P<0.0001), and increased ACE2 level before treatment predicted a poor outcome with a ROC-AUC value of 0.679 [95% CI 0.600-0.759] (P<0.0001). Overall, serum ACE2 activity correlates with COVID-19 severity and acts as a new prognosis biomarker.

SE3.2

COVID-19 associated coagulopathy in acute ischemic stroke patients receiving intravenous thrombolysis

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Coronavirus disease 2019 (COVID-19) has been associated with profound hemostasis changes and a high risk of venous thrombotic events. Little is known about hemostasis alterations in COVID-19-associated acute ischemic stroke (AIS). In this prospective observational study, blood samples of 69 AIS patients, all receiving intravenous recombinant tissue plasminogen activator, were taken on admission. SARS-CoV-2 RT-PCR test was performed in all patients and acute infection was confirmed in 8 cases (COVID-19+ group). Convalescence or vaccination was proven by an anti-SARS-CoV-2 antibody test in 5 patients (post-COVID/post-vaccination group). Screening tests of coagulation, D-dimer, fibrinogen, von Willebrand factor (VWF) antigen, factor VIII (FVIII) and factor XIII (FXIII) activity, clot-lysis assay, ACE activity and ROTEM analysis were performed from the blood samples. Stroke severity was determined by NIHSS. Short- and long-term outcomes were defined at 7 and 90 days post-event by ΔNIHSS and the modified Rankin Scale. Stroke severity was significantly greater in the COVID-19+ group. VWF antigen levels were markedly elevated in the COVID-19+ group as compared to non-infected and post-COVID/post-vaccination groups (329±94 vs. 244±75 and 210±66%, respectively, p=0.027). FVIII levels were parallel to VWF levels and showed significant elevation in the COVID-19+ group. Short-term outcomes of therapy did not differ between groups. Conclusion: Elevated FVIII and VWF levels in COVID-19-associated AIS seem to be linked to endothelial cell injury and are associated with more severe stroke.

SE3.3

Experiences of COVID-19 patients' viscoelastometry testing and case reports

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COVID-19 is commonly associated with macro- and microvascular thrombosis and endotheliopathy. The hemostatic balance in COVID-19 is typically shifted toward a hypercoagulable state, sometimes associated with hypofibrinolysis. As a point-of-care, viscoelastic whole blood coagulation test, ClotPro® is used to measure simultaneously the function of

clotting factors, platelets, blood cells and the state of fibrinolysis system. ClotPro® measurements were routinely carried out in parallel with platelet function (Multiplate®) and routine hemostasis tests from anticoagulated whole blood and plasma of SARS-CoV-2 positive patients with moderate or severe disease. Standard ClotPro® tests were the EXtest (tissue factor (TF)-activated assay), INtest (ellagic acid-activated assay), FIBtest (functional fibrinogen assay), TPAtest (r-tPA within an extrinsic pathway-based assay) and the ECAtest (ecarin-based assay). For impedance platelet aggregometry, we used arachidonic acid, ADP, TRAP and ristocetin as agonists. Platelet count was also determined. COVID-19 patients were routinely treated with LMWH and antiplatelet therapy. The hypercoagulable state could be characterized by larger and firmer clot: elevated MCF (maximal clot formation) in EX-, IN-, FIB- and TPA tests; elongated CFT (clot formation time) in ECA test; elongated LT (lysis time) in TPA (test). Tests performed using Multiplate® device have shown decreased platelet function in most cases. Even in patients without antiplatelet therapy, we found reduced *ex vivo* aggregation with all four agonists compared to healthy controls and the normal reference range. Viscoelastometry in COVID-19 patients revealed an increased resistance to fibrinolysis, occasionally with, fibrinolysis shutdown".

SE3.4

Antigen specific immune response to SARS-CoV-2 vaccines

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Specific antibody and cellular response to SARS-CoV-2 vaccines has not been sufficiently mapped yet. Hungarian healthcare workers are largely exposed to the SARS-CoV-2 pandemic. Our aim was to investigate the specific immune response to mRNA vaccine (Pfizer) and its change over time in a sample of health care workers. We also aimed to examine the role of age in this process. For implementation 55 volunteer health workers were included in the study. Specific antibody tests were used to examine immunogenicity at 10 days and 2 months after the second dose of mRNA vaccine. Generic Assay IgG + test measures specific IgG against Spike1 and 2 proteins and nucleocapsid specific antibodies. The Roche Elecsys® Anti-SARS-CoV-2 S is an immunoassay for the quantitative determination of antibodies to the receptor binding domain. Ten days after the second dose all subjects showed a significant antibody production by both tests. The older age group responded with lower antibody levels (geometric means were 1915 and 1205 BAU/mL measured by Roche test in age groups below 55 years and after 55 years of age, respectively). Two months after the second dose, there was a significant decrease in antibody levels in both age groups. The decline is expected to continue, therefore the measurement will be repeated for another 3 months. Furthermore, we consider it necessary to supplement the study with cellular immunoassays. Preliminary results on these additional measurements will also be discussed in the presentation. The study was founded by the grant: Befektetés a jövőbe Alap: „Koronavírus fertőzéssel szemben természetes védettséget adó faktorok vizsgálata és alkalmazása plazmaterápiában", ID No: 2020-1.1.6-JÖVŐ-2021-00010.

SE3.5

The effect of COVID-19 pandemic on autoimmune testing in Hungary – results of a multicentric survey

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from the European Autoimmunity Standardization Initiative (EASI), Hungarian group

The first wave of COVID-19 pandemic has disrupted almost all areas of the health care services to some extent throughout the world. The present study aimed to provide reliable data on the impact of the COVID-19 pandemic on autoimmune testing in Hungary. This work was part of a European multicentric study conducted by the European Autoimmunity

Standardization Initiative (EASI). Eight Hungarian laboratories associated with an EASI member participated in the survey. Clinically relevant autoantibodies were selected as follows: anti-dsDNA, anti-MPO, anti-PR3, anti-CCP, RF, anti-CL and anti-β2 GPI (both IgG and IgM isotypes) as well as anti-tTG IgA. Monthly data on test number and the number of positive results were collected for the whole year of 2019 and 2020 for each parameter. Our results showed a mean decrease of 23% in the number of autoantibody tests in 2020 compared to 2019. The decrease was the most pronounced in April (81%) with minor differences between individual autoantibodies. With respect to the rate of positive results, a mean increase of 15.3% was observed for the same period. Again, the maximum increase was observed in April (53.7%). However, the differences in this regard were larger among the tested autoantibodies. These results support the importance of an effective strategy for the coordination of autoimmune testing in challenging situations, such as the current pandemic. In the light of the effect on delay in diagnosis and/or treatment of patients with autoimmune diseases, this strategy is highly required.

SE3.6

Determination of free amino acids in plasma and urine as predictors in COVID-19 disease

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The amino acid profiles are usually analyzed in metabolic disorders and other illnesses, such as sepsis. Certain plasma amino acids could be used as predictors of the severity and prognosis of the disease. In the present study, we measured free amino acids (AA) in plasma and urine samples, fractional excretion (FE) of plasma and urine AA, and plasma and urine creatinine levels were also determined. We analyzed 188 plasma and urine samples of COVID-19 positive patients by Shimadzu Nexera X2 UHPLC with fluorescence detector. Samples were collected by Department of Emergency Medicine from January to April, 2021. We used Mann-Whitney U-test and ROC curve for statistical analysis. We correlated plasma AA concentration and FE with mortality and the need for mechanical ventilation. In case of mortality, plasma serine, phenylalanine and threonine concentration were significantly different from the control group ($p<0.001$) and showed high sensitivity (Ser:63.6%; Tre:77.6%; Phe:96.2%) and specificity (Ser:73.1%; Tre:65.4%; Phe:52.1%). The FE of arginine was significant ($p<0.001$) showing 69.2% sensitivity and 67.3% specificity, but it was less proved to be a good predictor of COVID-19. For mechanical ventilation the following p-values were obtained: plasma serine, glycine and phenylalanine: $p<0.001$, in case of FE of glycine: $p=0.002$ and arginine: $p=0.003$. In terms of significance, we compared our results with CT scores and other laboratory parameters of patients, and the effect of certain diseases (e.g., diabetes, cardiovascular disease, kidney disease, hypertension) was examined on the outcome of COVID-19 disease.

LS2.1

Laboratory accreditation 2021 - how to use our sources for preparation?

Bekő G.
Central Laboratory of Central Hospital of Southern Pest National Institute of Hematology and Infectious Disease, Budapest, Hungary

No abstract was received.

LS2.2

How is accreditation supported by Unity?

Szakony Sz.
St. Imre Teaching Hospital, Budapest, Hungary

No abstract was received.

LS2.3

New launch of Molecular Quality Controls

Pugnale S.
Bio-Rad Laboratories

No abstract was received.

LS3.1

COVID-19: Serology, Vaccines and Variants

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has prompted the scientific community and the pharmaceutical companies to put the maximum efforts for developing vaccines able to contain the spread of SARS-CoV-2.

Serology tests were produced very soon with different sensitivities and specificities depending from the methods and technologies utilised.

Presently, many vaccines have been developed and authorized for use in human beings in different Countries. All of them are based on a version of the spike glycoprotein characterized at the beginning of the pandemic. However, they differ by their level of efficacy against COVID-19.

SARS-COV-2 as other RNA viruses mutates continuously. Genome sequencing analysis showed a nucleotide substitution rate of about $1 \times 10{-}3$ substitutions per year that lead to the emergence of variants through point mutations, insertions, deletions and recombination.

There is concern about the ability of the current vaccines to protect against the emerging viral variants. Mutations in the spike glycoprotein may affect transmission dynamics and the risk of immune escape. In this review, we address the different technological platforms in use for developing COVID-19 vaccines, the impact of emerging viral variants on virus transmission, hospitalization, and response to current vaccines as well as rare but important adverse reactions to them. Finally, different methods for measuring the antibody response to vaccine are reported including the importance of using the WHO international Standard for creating a common language for reporting data.

SE4.1

Comparing the performance of two, targeted next generation sequencing panels for myeloid malignancies

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Myeloid malignancies are clonal diseases caused by altered differentiation and proliferation of hematopoietic stem cells. For diagnosis, prognosis stratification or optimisation of treatment strategies, screening for at least six to ten gene mutations are recommended according to international guidelines (ELN and NCCN). By exploiting the benefits of massively parallel sequencing, higher precision and better cost-efficiency can be achieved. We utilized two commercially available targeted myeloid NGS gene panels. By the Illumina Trusight Myeloid kit, 54 genes on 83 samples were tested. With the Swift Accel-Amplicon Myeloid Panel, 23 genes were analysed on 30 samples. Results were confirmed by other molecular genetic methods for IDH1, IDH2, NPM1 and FLT3 genes. In comparison, the results were matched in 100% for IDH1 (11/11), IDH2 (18/18), NPM1 (28/28) and FLT3-tyrosine kinase domain (15/15) mutation positivity. However, FLT3 internal tandem duplication (ITD) results differed in 50% by fragment analysis and NGS (36/18), caused by the limitation of bioinformatic pipeline, location and length of the ITD. Based on our experience, genes that are difficult to amplify (e.g. CEBPA) should be checked by other methods (e.g. Sanger sequencing), however Swift panel proved to be superior in respect of read depths and region coverage in case of CEBPA gene. Number of genes, samples and type of sequencing cartridge must be chosen carefully to achieve optimal and high-quality output. With proper optimization and careful planning, NGS is a powerful tool for mutation screening and contributes to better understanding of mutation profile in myeloid malignancies. Supported by EMMI IV/219-4/2021/EKF, TKP2020-NKA-19.

SE4.2

Detection of measurable residual disease markers in acute myeloid leukemia by digital droplet PCR

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Monitoring measurable residual disease (MRD) in acute myeloid leukemia (AML) plays an important role in predicting relapse and outcome. The aim of this study was to investigate the role of NPM1 gene four-base pair insertions (two assays) and IDH1/2 single nucleotide variation mutations (four assays) as MRD markers. Measurements were performed by sensitive droplet digital PCR (ddPCR) on DNA and on mRNA (only for NPM1) samples. The limit of blank (LoB) and limit of detection (LoD) for the assays were determined with mutation negative samples (n=22-38). NPM1 ddPCR assays showed lower LoD than IDH1/2 assays (0-0.015% vs 0.09-0.12%). NPM1 mutant variant allele frequency (VAF) below 0.01-0.05% and IDH1/2 VAF below 0.2% was considered as MRD negative. DNA MRD negativity was proved to be a valuable MRD marker predicting overall survival in selected subgroups of marker positive patients (NPM1: p=0.029, n=116; IDH1/2: p=0.003, n=62 after induction therapy). NPM1 MRD was measured both on DNA and mRNA (n=39). Similarly to DNA, two or three log reduction was observable in mutant NPM1 mRNA expression after induction. Altogether 46% of the $NPM1^{mut}$ mRNA samples were detected as negative in the matching DNA samples. $NPM1^{mut}$ mRNA assay was proved to be more sensitive (median: 1.3-log; range: 0.0-2.78-log) compared to $NPM1^{mut}$ DNA assay (VAF) in samples with concomitant positivity both on mRNA and DNA level. In summary, NPM1 MRD can be measured from DNA, but the mRNA method is more sensitive. IDH1/2 MRD assays do not reach the sensitivity of the NPM1 methods. Supported by EMMI IV/219-4/2021/EKF and ÚNKP-20-3-II-SE-79.

SE4.3

Detection of microchimerism in allogeneic hematopoietic stem cell transplantation with droplet digital PCR

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Chimerism analysis (analysing the proportion of donor cells in peripheral blood and bone marrow samples of the recipient) is the most important diagnostic procedure to evaluate the efficiency of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The test provides information on engraftment, graft rejection or relapse. Short tandem repeat (STR) detection by fragment analysis is the current "gold standard" for chimerism assessment with 1-5% limit of detection (LoD). A more sensitive technique is the droplet digital PCR (ddPCR) capable of measuring microchimerism (below 1%).

We analysed the usefulness of ddPCR-based chimerism analysis against the STR analysis. With the application of 16 deletion/insertion polymorphisms (DIP) markers and Y chromosome-specific marker, 97% informativity was achieved in cases with a single donor (129 recipient-donor pairs). The LoD of microchimerism testing by ddPCR was improved by almost two order of magnitudes compared to STR (0.05% vs. 1-5%). According to the DIP test, microchimerism in the range of 0.05-1% could be confirmed in 171 cases (67%) among STR negative samples (n=253). The new method may also be capable of earlier relapse detection compared to the fragment analysis based STR analysis. Introduction of new indices (e.g. increment factor) that measures the increasing tendency of hematogenesis of recipient origin could further improve the determination of early relapse events.

DIP test is an accurate and sensitive measurement of hematopoietic chimerism and proved to be a highly effective tool in routine clinical diagnostics. Supported by EMMI IV/219-4/2021/EKF.

SE4.4

Identification of an acute myeloid leukemia case with variant t(8;21) by multidimensional flow cytometric analysis

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The 2016 WHO classification of acute myeloid leukemia (AML) is determined by recurrent genetic abnormalities including t(8;21) translocation. Conventional flow cytometric analysis, which is a crucial part of the diagnostic procedure of AML, shows only slight association in case of few CD markers with genetic aberrations. Multidimensional dot-plots have been proven to be more reliable for screening AML cases with specific recurrent genetic abnormalities, e.g. t(8;21). We present the case of a 6-year-old male patient who was investigated by flow cytometric and cytogenetic analysis in 2015 due to suspicion of *de novo* acute leukemia based on previous laboratory findings. In the bone marrow sample, flow cytometry confirmed the increased rate (33%) of myeloblasts and cytogenetic analysis revealed a rare t(8;16) translocation and loss of Y chromosome (karyotype: 45,X,-Y,t(8;16)). We retrospectively analysed the flow cytometric data by multidimensional dot-plots and the result suggested the presence of *RUNX1-RUNXT1* fusion gene. In order to clarify the origin of this fusion gene, fluorescence in situ hybridization (FISH) was performed using t(8;21) translocation-specific probe. Analysis of metaphase chromosomes revealed a cryptic variant translocation pattern with participation of three chromosomes – chromosome 8, 16 and 21. The fusion gene was present on the derivative chromosome 8. Reverse transcriptase-polymerase chain reaction (RT-PCR) also confirmed the presence of the *RUNX1-RUNXT1* fusion gene in the patient sample. This case illustrates the importance of integrating novel analytical methods in the routine diagnostic workflow to ensure the accurate diagnosis and prognosis even in atypical cases.

SE5.1

Genotype-phenotype relations in hereditary hemorrhagic telangiectasia

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant multisystemic vascular disease with a prevalence of 1:5,000-1:10,000. Diagnosis is based on clinical Curacao criteria. Approximately 85% of HHT cases have heterozygous family-specific mutations in the *ENG* or *ACVRL1* genes. We present our results of systematic genetic and clinical screening of Hungarian HHT families in a 10-year period. Probands were diagnosed by otorhinolaryngological examination, visceral arteriovenous malformation screening and genetic testing for *ENG/ACVRL1/SMAD4/RASA1/GDF2* genes. At-risk individuals (family members) underwent clinical examination and family-specific mutation testing. Eighteen *ENG*, 16 *ACVRL1* and 1 SMAD4 mutations were identified in 21, 26 and 1 families, respectively, with 117 individuals carrying the family-specific mutation (92 with definite, 20 with suspected and 5 with unlikely HHT by Curacao criteria). We found a novel mutation in *RASA1* gene in a patient with only 1 Curacao criterion present.

As no laboratory assay is available in HHT, genetic testing has a major role. It is important in the confirmation of HHT in young asymptomatic HHT family members. Moreover there are mutations with specific clinical consequences (eg. *ACVRL1* and *MADH4* can associate with pulmonary hypertension, *RASA1* may associate with basal cell carcinoma). Identifying founder effects simplify the genetic diagnosis in the corresponding geographic region.

SE5.2

Role of next generation sequencing in identifying hemorrhagic disorders and its use in differential diagnosis

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Diagnosis of rare bleeding disorders is challenging and there are several differential diagnostics issues. Molecular genetic studies including next-generation sequencing (NGS) are useful tools to overcome these problems. The aim of our work was to provide correct diagnosis by NGS for cases with uncertainty in laboratory diagnosis. Two large gene panels were constructed, one covered 14 genes (NGS panel 1) selected on the basis of unresolved differential diagnostic issues in von Willebrand disease (vWD), fibrinogen disorders, and in hereditary hemorrhagic telangiectasia (HHT). The second one (NGS panel 2) covered 14 candidate genes in platelet secretion disorders. Libraries were created by QIAseq targeted DNA custom panel, the MiSeq System and Sanger sequencing were used for NGS and for validation, respectively. Ninety-six patients were recruited into each NGS study. Clinical data were collected and detailed laboratory studies were performed before patient-selection.

The mutation detection rate in NGS panel 1 was almost 100%, and the causative mutations (n=28) were found in vWD (4 patients were re-classified as hemophilia A based on our results). N=10 mutations were identified in fibrinogen disorders helping to predict clinical phenotype and two rare variants of HHT were explored. The mutation detection rate in NGS panel 2 - as expected - was lower, n=37 variants were found, some of them with uncertain significance.

Genetic testing provides a higher-level evidence for diagnosing bleeding disorders, however in case of novel variants pathogenicity should be carefully determined before providing interpretative result.

SE5.3

Rare autosomal trisomies in prenatal diagnosis I.: biological background, cytogenetic laboratory work-up in spontaneous abortions and ongoing pregnancies

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Constitutional aneuploidies are often associated with poor obstetric outcome causing miscarriage, stillbirth, or multiplex congenital abnormalities. Rare autosomal trisomies (RATs) are trisomies other than those involving the chromosomes 13, 18 or 21. Although RATs are less common and usually occur in a mosaic state, laboratory diagnosis, patient management and genetic counseling can be a real challenge. Although it is well-known since the late eighties, that RATs are also a significant group of chromosomal aberrations, their importance is increasing in the era of NGS (next-generation sequencing) in noninvasive prenatal tests (NIPT) and preimplantation genetic testing (PGT-A). In the first part of the presentation, we outline the biological background of RATs, the cytogenetic laboratory processes to determine the precise type of chromosome aberration in the context of fetoplacental mosaicism from chorionic villi, amniocytes and product of conceptions (POC). Between 2007-2021, we prospectively analyzed 156 samples of POC, encompassing spontaneous abortions including blighted ova from the first trimester, also specimen from pregnancy terminations with serious ultrasound findings. Among the first trimester miscarriages we identified 32 RATS (39%) together with 9 complex aberrations (11%), while pregnancy loss was due to common trisomies in 6 cases (7%). In line with this, our prenatal database between 2014-2021 also contains RATs detected in 15 ongoing pregnancies (7%) in alignment with 203 common or structural aberrations (93%). We present the statistical analysis of our database and compare the distribution and importance of RATs with the latest data of the literature.

SE5.4

Rare autosomal trisomies in prenatal diagnosis II.: case studies, patient management, therapeutic consequences.

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Rare autosomal trisomy (RAT) is a type of chromosomal disorder, which is an important issue in connection with non-invasive prenatal testing (NIPT), preimplantation genetic testing for aneuploidy (PGT-A), and patient management with recurrent abortions. In the second part of our presentation, we demonstrate the challenges of counselling in terms of prognosis via case studies. Our statistics together with the ones in recent publications define to what extent should we expect aneuploidy as a causative factor behind early miscarriage. We attempt to outline the difficulties of interpreting RAT results of genome-wide NIPT and discuss the limitations of targeted NIPT in connection with age related aneuploidy detectable in the second trimester. Data from in vitro fertilized embryos show that in early stage of development cells can function with chaotic chromosome content, and even embryos can overcome mosaic aneuploidy resulting in an apparently healthy child. PGT-A utilizes NGS-based analysis, and can detect not even RATs, but also small segmental aneuploidy. As there is no scientific consensus yet on how PGT-A should be properly applied, case studies of viable mosaic RATs in the second trimester or those surviving to term may draw attention to the ethical concerns of embryo selection.

SY2.1

Recent progresses in diabetes therapy

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In the last a few years, therapy of type 2 diabetes mellitus (T2DM) is one of the fastest developing areas of medicine. Incretin therapies using either dipeptidyl peptidase-4 inhibitors (DPP-4-i) or glucagon-like peptide-1 receptor agonists (GLP-1-RA) are the most promising ways in some patients. On the other hand, inhibition of sodium glucose cotransporter-2 (SGLT-2-i) enzyme seems to be the best in another group of T2DM patients. GLP-1-RAs decrease the risk of atherosclerotic cardiovascular disease (ASCVD) and renal risk. SGLT-2-is reduce the risk of hospitalisation for heart failure and the risk of renal complications, as well. However, SGLT-2-is are effective in risk reduction also in ASCVD patients, and, moreover, in

non-diabetic heart failure and non-diabetic chronic kidney patients SGLT-2-is are beneficial. Both GLP-1-RAs and SGLT-2-is are neutral in the development of hypoglycaemia and they decrease the body weight of obese patients. HbA1c-decreasing effect of GLP-1-RAs is more pronounced than that of SGLT-2-is. Regarding the benefit of the combination of these two therapies we have metabolic results only, and there are no data about its cardiovascular effects. The gastro-intestinal side effects of GLP-1-Ras and the low eGFR (<45 ml/min/1.73m^2) in case of SGLT-2-is are the main limitations of the use of these excellent drugs.

SY2.2

Current trends in childhood diabetes

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WHO classifies diabetes mellitus as type 1 (T1D), type 2 (T2D), gestational diabetes and other specific forms. During the past years, we have seen a great progress in understanding several genetic defects of beta-cell function and in insulin action. Frequency of both T1D and T2D is increasing worldwide in all age groups. Genetic and environmental factors are determinants and we have measures to screen and predict diabetes in childhood. Meanwhile, population-wide screening is not recommended for T1D, risk-based screening for T2D is applicable in clinical settings. The presentation of diabetic ketoacidosis is common in childhood, especially in the youngest age-group. Aims of long-term treatment are to avoid metabolic derangements, provide normal life-style, somatic, mental and psychologic wellbeing, physiologic growth and development, but the ultimate goal is to avoid long-term complications. Principles of treatment relies on pharmacologic and life-style interventions in association with comprehensive patient education. Intensive insulin treatment for T1D is universal either by multiple daily injections or use of portable pump completed by self-monitoring of blood glucose, continuous subcutaneous glucose monitoring and HbA1c measurements. Nowadays, glycaemic monitoring and treatment are supported by data collection, analysis and communication via information technology systems and devices and the artificial pancreas concept is a fruitful promise for the near future to attain the closed-loop system in the clinical practice.

SY2.3

A comprehensive analysis of Hungarian MODY patients

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One of the monogenic forms of diabetes mellitus, maturity-onset diabetes of the young (MODY) is a heterogenous group accounting for up to 2% of all diabetes cases, and frequently underdiagnosed or misdiagnosed to type 1 or type 2 diabetes. More than a dozen MODY genes have already been identified to date. Molecular testing and classification of them is of great clinical importance to make a correct treatment decision and estimation of the prognosis. Most prevalent subtypes are HNF1A, GCK, and HNF4A. State-of-the-art genetic testing for MODY is by next-generation gene panel sequencing. Here we show the results of a 10-year period of genetic analysis of Hungarian MODY patients.

A total of 450 unrelated index patients with suspected MODY diagnosis and their 202 family members have been analyzed by NGS gene panel testing. Whenever copy number variation was suspected, multiplex ligation-dependent probe amplification method was used. All detected small-scale mutations were confirmed by Sanger sequencing. Detected variants were classified according to the ACMG standards and guidelines.

Overall 132 patients were positive for a variant classified as Pathogenic or Likely Pathogenic in one of the MODY-causing genes with a total of 89 mutations, resulting in a 30% of positivity rate. More than 70% of the mutations were found in the *GCK*, 20% of the mutations were found in the *HNF1A* and the remaining 10% in other MODY-causing genes. Out of the family members 95/202 tested positive for a MODY-causing mutation. Genetic diagnosis led to treatment changes in case of both HNF1A and GCK MODY.

SY2.4

Autoantibodies in Diabetes

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Diabetes mellitus (DM) is a complex and heterogeneous disease. In predisposing genetic backgrounds, external factors (e.g. infection) may trigger autoimmune processes that impair insulin-producing beta cells and lead to the development of diabetes mellitus. Several autoantibodies may be present at the time of diagnosis of DM: Islet cell autoantibodies (ICA), glutamic acid decarboxylase antibodies (GAD), insulinoma-associated-2 autoantibodies (IA2) and antibodies to insulin (IAA). It is known that the appearance of antibodies can precede the appearance of clinical symptoms of the disease by up to years. These antibodies are most commonly associated with the development of T1DM and play a key role in the investigation of genetically predisposed children. However, autoantibodies are also important in the diagnosis of adults. In patients with a previously uncertain or type 2 DM diagnosis, it may be important to test for antibodies to clarify the type of DM. For example, the appearance of ZnT8 antibodies may predict the so-called transmission of LADA (latent autoimmune diabetes) to the insulin-dependent DM form. Multiple antibodies can often be detected in a patient and the antibodies may disappear over time. The presentation shows in more detail the characteristics of autoantibodies associated with DM.

SY2.5

Blood glucose monitoring by telemedicine

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Telemedicine and digital technology are the newest ways of communication between patients and medical staff. The main points of it are: use of communication technologies, covering large distances and involving health care professionals who provide care to particular patients. The use of digital technology in diabetes is named as „digital diabetes". It involves methods used for glucose control, moreover transmission, processing and presentation of data.

Regarding glucose control the use of conventional glucometer is the most widely available method. Another option is to use CGMS (continuous glucose monitoring system), that measures not serum but interstitial glucose levels every 5 minutes. The advantage of CGMS is that health care providers may get to know hidden hypoglycaemic events and the tendency of glucose level curves is monitored much better. In those using CGMS not only the number of hypo- and hyperglycaemic episodes can be determined but the time spent above (TAR) or below (TBR) or in target (TIR) range, too. Calculated results, like average glucose levels, glucose management indicator (GMI – similar to HbA1c), variation coefficient referring to fluctuations in glucose levels are given. The use of real time sensor in combination with insulin pump provides the possibility to suspend basal insulin administration automatically to prevent the development of hypoglycaemic episodes. Data can be transmitted from a geographical distance and results may be discussed by phone or e-mail.

Digital technology simplifies the glucose monitoring processes for patients and is a great help in the everyday practice for health care providers to discover hypo- and hyperglycaemic episodes, the tendency of glucose level curves and therefore is a crucial point of decision-making.

SY2.6

Investigation of parameters associated with high HbA1c levels. A retrospective analysis of a large university database.

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We aimed to identify patients who are at increased risk for high HbA1c levels defined as HbA1 value above 7%.

Methods: From our database we collected 193176 HbA1c records. We assessed the rate of normal and high HbA1c in different groups defined according age and clinical chemistry parameters indicating comorbidities. We tested whether high HbA1c is normalized later. We also investigated if microalbuminria was routinely tested with HbA1c.

Results: The rate of high HbA1c peaked between 15 – 20 years of age (55%). This rate drops to 30% between 20-25 years of age and remains around 30% until 85-90 years of age. Men are at higher risk for high HbA1c than women. eGFR, GPT and cholesterol levels are comparable in individuals with normal and high HbA1c and per se are not associated with the risk of having high HbA1c. Uric acid levels in individuals with normal and high HbA1c differed significantly (288 (interquartile range, IQR 184, 369) and 310 (IQR, 239, 380) μmol/L (p-value < 0.0001). High HbA1c measured for the first time was normalized just in one-third of patients for the 2nd measurement. In patients presenting with high HbA1c (n=64833), microalbuminuria was tested just in 6321 (9.7%).

Discussion and Conclusion: This analysis revealed the susceptibility of adolescents and male patients for metabolic disturbances in diabetes. Impaired renal and liver function and hypercholesterolemia have no relevant effect on HbA1c, the efficacy of antidiabetic efforts is clearly limited. Clinicians quite often dismiss microalbuminuria testing. These data identify possible targets for intervention.

SY2.7

Thumb gangrene caused by accidental *Clostridium* infection in a patient with newly diagnosed Type 2 diabetes mellitus

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A 55-year-old male patient was referred to our hospital with a disregarded right hand thumb gangrene. Around one week before he was admitted to the ward, when he suffered an injury. He presented a swollen thumb with wet, necrotized skin; thenar and dorsum of the hand with diffuse swelling up to the wrist and extreme pressure sensitivity. Laboratory findings showed: leukocytosis, increased CRP, glucose and HbA1c level. Renal function and coagulation profile was normal. Metformin along with i.v. AM/CL was initiated. Hand radiography revealed: the distal joint of the thumb showed complete luxation. There were several smaller, sometimes confluent and transparent areas around the joint, vs gases were visible, indicating the presence of a gas-producing bacteria. The soft tissue was swollen. During the surgery the first finger could not be rescued and amputation was performed. Microbiological examination of the surgical specimen was found to be positive for *S. constellatus, Anaerococcus sp. C. sordellii* and toxin producing *C. tetani*. Histopathological findings: extensive necrotic areas could be identified under the epithelium with massive neutrophil infiltration. Negative pressure wound therapy was initiated and thereafter elimination of inflamed lesions could be observed and normal granulation tissue filled in the surgical area. His laboratory findings confirmed the regression of inflammation. A multidisciplinary

team approach and regular examination is important in conditions that increase the risk for clostridial soft-tissue infection, such as diabetes.

SY2.8

Improving the mathematical relationship between Hemoglobin A1c and calculated plasma glucose

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Hemoglobin A1c (HgbA1c) is one of our best tool to assess the patients' glycemic status, but patients and healthcare professionals adjust their diet and medication day-by-day based on plasma glucose (PG) measurements. Thus, an easy and accurate conversion between HgbA1c and glycemic status would help their therapeutic efforts.

Despite HgbA1c production is controlled by the intracellular glucose concentration and non-insulin dependent glucose uptake seems to follow the non-linear Michaelis-Menten kinetics, available conversion calculators between estimated average PG and HgbA1c are using a linear calculation method. To improve this, we present the results of our statistical analysis. We have analyzed plasma glucose and HgbA1c values from 99,815 simultaneous tests from the past 5 years. For comparison, simultaneous fructosamine plus plasma glucose (N=18,894) and fructosamine plus HgbA1c (N=22,793) levels were also processed.

We have found that average HgbA1c values assessed by incremental 0.5 mM PG ranges demonstrated a smooth, non-linear curve. Using the Michaelis-Menten equation (Vact=(Vmax*PG)/(Km+PG), we found the best fitting curve was at Km=8 mM PG (R2=0,97) and at Vmax=14% HgbA1c. The average discrepancy between calculated and observed HgbA1c was 0.23% in the 4 – 25 mM PG range. Fructosamine vs. PG also demonstrated a non-linear curve, while plotting HgbA1c against fructosamine values showed a linear curve.

While it is difficult to measure or even estimate average PG, accurate measurement of fasting PG is simple. Thus, entering fasting PG values into our improved calculation method may help patients to better adjust their diet to reach their target HgbA1c levels.

LS4.1

Associations between cardiac failure and diabetes

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No abstract was received.

LS4.2

Diabetes – heart failure – prevention/POCT in primary care: years won for patients?

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Tumors and cardiovascular diseases, constituting some of the most common causes of death of our time, would be considerably more recognizable with regular screening, which could be done with absolute certainty as a part of primary

care. Were we able to recognize tumors, diabetes, high blood pressure and consequent health problems, such as cardiac failure, the number of years lost for patients suffering of these diseases could be significantly reduced. The key point is finding those patients for whom the use of such methods would be especially beneficial. If the knowledge and experience amassed by the GPs could be combined with the use of examination with devices, patients with more serious health problems would be easier to select and they could receive the required treatment in special care units far sooner. Point-of-care devices are perfectly suitable for this task. They can be utilized efficiently and economically in primary care for treating acute problems, as well as for screening for and treating chronic diseases. Point-of-care methods can be highly effective in, among other things, measuring the level of HgbA1c for monitoring the metabolic state of patients with diabetes, the level of NT-proBNP for screening for cardiac diseases, the level of hsCRP for ascertaining the presence of residual inflammations or cardiovascular risk, as well as screening for microalbuminuria and nephropathy. Over the course of the last fifteen years, we utilized point-of-care testing methods in the case of both acute and chronic problems. Such testing methods are indeed expensive. Nevertheless, their systematic use is considerably more economical for the treatment of patients with chronic diseases than the treatment of patients with symptoms already present in special care units. This is especially true in the case of practice communities still forming, as well as those working in close cooperation with each other where primary care can be provided in the vicinity of the residences of the patients. The currently available, quality-assured point-of-care diagnostic devices and their use in primary care is internationally accepted. The Electronic Health Service Space is suitable for providing the required IT-support for recording data.

LS4.3

Replacement of the main laboratory instruments “on-the-fly”: an example of renewing analysers without affecting the daily routine work

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The renewal of almost the complete laboratory instrumentation without having significant influence on the daily routine in a fixed basic area requires a concerted action between the laboratory staff and the instruments providers. The aim of this presentation is to demonstrate a set of steps to prepare the laboratory for changing, the milestones of replacing instruments and the fine tuning of the system in order to reach the highest possible efficacy in the Department of Laboratory Medicine in Szeged. In addition, the benefits of the new automated clinical chemistry, immunochemistry, haemostasis platform connected to preanalytical and postanalytical modules will be also discussed.

PS1.1

Epigenetic regulation and protein expression of catecholamine pathway markers in glioblastoma

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GMB is the most aggressive tumor of the central nervous system. Current therapies only improve the survival by a few months. New molecular treatment targets are needed. Our recent epigenomic analyses revealed that neurotransmitters and their receptors are not only physiological mediators of interneuronal communication, but are also involved in modulating the development of GBM. Here we quantitated the expression levels of four selected catecholamine pathway markers (alpha 1D adrenergic receptor—ADRA1D; adrenergic beta receptor kinase 1—ADRBK1; dopamine receptor D2—DRD2; and synaptic vesicle monoamine transporter—SLC18A2) by

immunohistochemistry, and compared the results with the methylation levels within the promoter+genes of these markers in primary and recurrent GBMs and control brains. Promoter+gene methylation levels of these markers were also determined in an independent database cohort of sequential GBM pairs. The analyses revealed partial inverse correlations between catecholamine marker expression and promoter+gene methylation levels in tumors and control brains. We found no differences in the promoter+gene methylation levels of these markers in either our own or in the database sequential GBM pairs, in spite of their higher protein expression in primary vs. recurrent GBMs. We conclude that the regulation of catecholamine expression is only partially related to DNA CpG methylation, and additional mechanisms influence the expression of these markers in progressive GBM. This study supports the involvement of catecholamine pathway markers in GBM, and endorses their further explorations as potential treatment targets.

PS1.2

Drugs giving false positive result in the lymphocyte transformation test (LTT)

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For the *in vitro* diagnosis of drug hypersensitivity reactions, lymphocyte transformation test (LTT) is used for decades. Although this cell proliferation assay bears moderate sensitivity, its specificity is relatively high. However, we noticed that with certain drugs [ascorbic acid (AA), unfractionated heparin (UFH) and low molecular weight heparins (LMWH), drugs used for arthrosis, iron and folic acid supplements] LTT was positive in prominently high numbers. Our aim was to investigate, whether false positivity was behind the positive LTT results with these drugs. We performed LTTs on healthy controls and random persons; moreover, we assessed the rate of positivity in the diagnostic LTTs performed in 10 years at the Department of Dermatology and Allergology. Using these approaches we could prove that LTT was indeed false positive with AA and drugs containing AA presumably due to an analytical interaction, and with UFH and LMWH at higher concentrations. Based on our results we recommend to avoid performing LTT with AA and AA containing drugs, and to perform LTT with decreased concentration (0.1-1 IU/mL) with UFH and LMWH.

PS1.3

Meropenem, piperacillin and tazobactam serum concentrations in pediatric patients undergoing continuous venovenous hemodiafiltration

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Antibiotics are frequently administered to critically ill pediatric patients undergoing continuous venovenous hemodiafiltration (CVVHDF). Little is known about the pharmacokinetic characteristics of these drugs in these patients. The aim of the study was to perform the preliminary investigation of the time-dependence of the trough serum concentrations of meropenem, piperacillin and the beta-lactamase tazobactam after repeated dosing. Native blood was collected from 4 CVVHDF patients (age: 0.45-17.4 years, body weight: 3.2-30 kg) receiving 26.7-30.6 mg/kg meropenem 2 or 3 times a day, and from 3 patients (age: 0.06-1.68 years, body weight: 2.5-7.5 kg) receiving 66.7-83.3 mg piperacillin and 8.3-10.4 mg/kg tazobactam 3 times a day immediately after terminating the first infusion of the drug (i.e. first peak), and then repeatedly at troughs (i.e. just preceding the administration of the next dose). The concentrations of the drugs were determined using a commercially available reagent kit for HPLC. The trough concentrations of all three substances decreased over time. The ratios of piperacillin/tazobactam trough concentrations showed a steady increase. The results highlight the importance

of the introduction of individualized antibiotic dosage regimens in the treatment of critically ill pediatric patients receiving CVVHDF. A premise for the individualization is the frequent measurement of drug levels followed by pharmacokinetic modelling.

PS1.4

Metabolic indices as markers of insulin resistance in gravidity – a pilot study

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Introduction: Pregnancy could be described as a period with insulin resistance as well as a physiological dyslipidemia. The aim of this study was the estimation of two new metabolic indices – triacylglycerols-fasting glycemia (TyG) and triacylglycerols-C-peptide (TyCP, proposed by us) – in attempt to quantify the degree of insulin resistance during pregnancy.

Patients and methods: 92 women were included in this study. Order of the pregnancy was from 1st to 3rd, age 22 – 47 years, BMI 16.65 – 39.18. All of them had monofetal vital gravidity, 102 samples were analyzed (34, 36 and 32 in the 1st, 2nd, and 3rd trimester). Maternal serum was analysed for fasting glycemia (Glc) by certified methods in Alfa Medical labs, Slovakia. Triacylglycerols and total cholesterol were analysed with validated automatized methods on Cobas-Integra analyser. C-peptide (CP) was assessed by electrochemiluminiscence method (Elecsys C-peptid; Roche). Metabolic indices were calculated as:

$$TyG = \ln\left(\frac{TAG\left[mmol.l^{-1}\right].Glc\left[mmol.l^{-1}\right]}{2}\right)$$

And for TyCP Glc was replaced by CP ($nmol.l^{-1}$).

Results: TyG was 0.98 ± 0.48 (min. 0.03; max. 2.05) and TycP -0.74 ± 1,04 (-2.86 to 1.40). Correlation analysis showed significant correlation between both markers and weeks of gravidity (r = 0.736 and 0.694); Tyg and TyCP (r = 0.808), and between TyCP and total cholesterol (r = 0.73), all $p<0.001$.

Conclusions: These markers provide information about metabolism of saccharides and lipids in a complex way thus enabling better assessment of their changes during gravidity and perspectively also of gravidity related pathological conditions.

PS1.5

Investigation of biological therapeutic agents (Adalimumab, Infliximab) in the clinical laboratory

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Tumor necrosis factor alpha (TNFα) inhibitors are widely used to treat patients with various inflammatory processes. Adalimumab and infliximab are the two most commonly used anti-TNFα drugs for immune mediated disorders including rheumatoid arthritis, Crohn's disease, and ankylosing spondylitis. Both agents are chimeric monoclonal antibodies capable of binding and thereby blocking the function of TNFα, which is responsible for the inflammatory state. During treatment, some patients may develop antibodies against adalimumab or infliximab, which may decrease plasma levels of anti-TNFα and impair the efficacy of these medicines, reflected by the recurrence or progression of symptoms.

In line with the growing clinical demand, the test for TNFα inhibitors and their antibodies are available at the Department of Laboratory Medicine of Semmelweis University since June 2020. Measurements are performed with LISA TRACKER Duo Adalimumab and LISA TRACKER Duo Infliximab (Theradiag, Croissy Beaubourg, France) ELISA Kits.

The LISA-TRACKER Duo Adalimumab test was validated for Humira® and other biosimilar medicines such as Imraldi®, and the LISA-TRACKER Duo Infliximab test was validated for Remicade® and other biosimilar medicines such as Remsima® or Inflectra® and Flixabi® or Renflexis® for routine laboratory monitoring of the levels of drugs present in the systemic circulation and the antibodies that may be produced against them.

In this poster we present the conditions, challenges of introducing the study and, also, some results we obtained so far.

PS1.6

Avoidance of endogenous matrix interferences for an optimal signal-to-noise ratio in ELISA assays

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Background: The achievement of highly specific analyte detection is key to any serological assay development. For biomarker assays, unwanted matrix effects can be caused by endogenous molecules with similar structure to the target analyte or their natural ligands and ligand analogs. Endogenous substances, such as natural, poly-reactive antibodies, autoantibodies (heterophiles), or human anti-animal antibodies together with other unsuspected binding proteins that are unique to the individual, can interfere with the reaction.

Aim: To assay serum antibodies by indirect ELISA, we aimed to eliminate a variety of false positive and negative reactions attributed to the principle.

Methods: We treated plates of conventionally used ELISA assays with PVA-based synthetic blocking solution, followed by sample pre-treatment using IgM Reducing Assay Diluent (BioRad); a buffer enriched by mammalian proteins and recommended for matrix equalization to eliminate "sticky" or non-specific interfering proteins. We run trials using self-developed assays (anti-MMR IgG) and ready-to-use commercial kits (EUROIMMUN AG, Germany).

Results: We experienced a clearer detection; less equivocal and low positive samples, due to a significantly improved signal-to-noise ratio. A better parallelism between samples and standards was achieved, allowing for a more precise determination of analyte levels.

Conclusions: Specificity of problematic assays (e.g. tests with higher coefficient of variation regarding manufacturer's positive, negative controls and in-house control samples), may be - at least partly - restored by adding the abovementioned two simple steps to the operational protocol. The benefits of improved assay selectivity and elimination of measurement repetition clearly outweigh the cost of the initial investment. Elimination of substances that alter antibody binding decreases the chance of false-positivity, thus promoting the establishment of a correct diagnosis.

PS1.7

Repeated interference testing according to new guideline

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Introduction: Our laboratory has been using automatic hemolysis, jaundice, lipemia (HIL) detection since 2015, at the same time we introduced the rejection of HIL-sensitive parameters. Since the introduction of automatic HIL detection, an average of 71% of all hemolytic samples are 1+. For 4 chemical parameters (lactate dehydrogenase, creatinine, uric acid, iron) 1+ hemolytic samples already cause a significant change, therefore the results of these samples were not reported to physicians.

Materials and Methods: The Clinical and Laboratory Standards Institute recommendation for interference testing (CLSI EP07) was updated in 2018. Based on the new recommendation, interference tests were performed again for the 4 parameters most sensitive to hemolysis. Compared to the previous interference testing, this time we used two different concentrations of base pools. The stock spiking solution was at a concentration 20 times the concentration of the interferent. We combined stock spiking solution and base pool at a ratio of one part stock spiking solution into 19 parts base pool. Measurement concentrations were chosen at medical decision points. The observed difference was compared to a predetermined allowable difference.

Results: Within the most common 1+ hemolysis, another threshold (+) was established below which hemolytic interference did not yet cause a significant difference in the 4 most sensitive tests.

Conclusion: 45% of the 1+ hemolytic samples come from the Emergency Department. Therefore, it is particularly important that the number of results retained due to hemolysis is kept to a minimum. Based on the interference test performed according to the new CLSI EP07 guideline, we can report 62% of the 4 most sensitive parameters of the previously retained results.

PS1.8

Recognition of *in vivo* haemolysis caused by sepsis

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The treatment of haemolysed specimens and differentiating between *in vitro* or *in vivo* haemolysis is a significant challenge in clinical laboratory diagnostics. Although the incidence of *in vivo* haemolysis is reported in the literature to be less than 2%, it is important to identify such cases because the final rejection of these samples by the laboratory is considered a serious error.

The patient who arrived to the Emergency Department complained high fever, dyspnoea, and hypoxia, and had dark red urine. In his first sample, 4+ haemolysis was observed. Generally, we do not attempt to provide results from such a roughly haemolytic sample, but after obtaining the same specimen quality for the third time in repeated blood sampling that affected all tube types, the serum was placed in the chemistry analyser. The patient's chemistry parameters did not yield the expected values usually observed during massive haemolysis (K^+ = 3.59 mmol/L; CK = 156 U/L; TBIL = 80.9 μmol/L). Moreover, Gram-negative rods were discovered in the peripheral blood smear in addition to schistocytes and procalcitonin level was 40.7 ng/mL.

The suspicion of *in vivo* haemolysis was reinforced by the equal appearance of 4+ haemolysis affecting all samples, normal serum K^+ and creatine kinase, elevated total and unconjugated bilirubin concentrations, and negative urine sediment by macroscopic haematuria. *In vivo* haemolysis could have been demonstrated by an incidental decrease in haptoglobin levels, which test is not available in our laboratory.

In the case of a highly haemolytic specimen from repeated sampling *in vivo* haemolysis might be suspected. While reporting the results, it is important to be aware of the interference phenomena caused by haemolysis, which may help to detect rare *in vivo* haemolysis, especially if it occurs in a severe form such as shown above, which may have been caused by the septic condition of the patient. Among the 8,725 haemolysed samples observed in our laboratory in the past year, a total of 209 were 4+ strengths, resulting in a single massive and obvious case of *in vivo* haemolysis.

PS2.1

Development of an RNA based prostate cancer screening liquid biopsy panel for Next-Generation Sequencing

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Classical pathological diagnostic methods in cancer cases are rapidly replaced by molecular diagnostic assays e.g. next-generation sequencing. However, it is often inapplicable due to the low quality and quantity of the DNA obtained. Nevertheless, current techniques are unable to detect mutations, copy number changes, gene fusions, and gene expression changes simultaneously. Prostate cancer is the second most commonly diagnosed cancer and the sixth leading cause of cancer death among men. The use of serum tPSA level as a screening marker is widely accepted even though tPSA has a false positive rate of about 70% and a false negative rate of about 20%. Tumor sampling by needle biopsy is an uncomfortable, invasive procedure and often proves to be unnecessary. We report the development of an RNA-based diagnostic gene panel, capable of screening for prostate tumors and monitoring the progression of the disease employing non-invasive sampling from urine. The panel contains the most prominent targets for screening purposes, which are often mutated in prostate cancer, including those targets, which are important for proper personalized cancer therapy. We set up the panel using multiplex PCR and NGS on FFPE samples. The panel can detect variants in 6 genes and fusions between TMPRSS2 and ERG genes. The method could be further tested on RNA isolated from urine samples, enabling a more precise and wider diagnostic technique to map the complex background of prostate cancer.

PS2.2

Extracellular circulating miRNAs as potential biomarkers in neurological disorders: A preliminary study

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Neurological disorders, such as multiple sclerosis or epilepsy are organic brain disorders with neuropsychiatric symptoms, and represent severe social and economic burden on our society. In the last decade, numerous studies focused on the molecular background of these disorders. One important aim is to investigate the potential role of micro RNAs (miRNA) in the pathophysiology of these disorders and their potential application as peripheral biomarkers to assess disease progression and therapeutic response. Results from such studies are often rather ambiguous or hard to explain, due to the wide scale of used RNA isolation-, miRNA detection methods and different starting material. For further examination of this issue, we investigated the expression levels of circulating miRNAs in peripheral blood and brain

samples originating from humans and experimental mice. In the present study, we report on findings originating from patients with epilepsy (EP) and multiple sclerosis (MS). In addition to that, we used an animal model: C57BL/6 male mice were used for induction of temporal lobe epilepsy (TLE). 52 serum samples from MS patients, 71 serum samples from EP patients and mouse brain samples were investigated. TLE mice were terminated 1, 2, 3 and 4 weeks after intra-hippocampal injection of kainic acid. Brain tissue was removed from animals, and samples containing the prefrontal cortex, hippocampus and cerebellum were collected and immediately frozen on -80°C. After blood/brain tissue collection, total RNA was isolated, and RNA was described into cDNA. For downstream workflow droplet digital PCR reaction was prepared, using specific miRNA primers which were collected based on previous literature data. ddPCR is an extremely sensitive qPCR system, that allows absolute quantification of miRNAs in case of low yield samples as well. Our preliminary findings demonstrate an association between miRNA expression levels in human serum samples and clinical examination including MRI in MS patients.

PS2.3

Serum actin binding proteins: estimation of reference intervals and possible clinical significance in sepsis

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Serum gelsolin (GSN) and Gc-globulin are extracellular actin scavenger proteins depletion of which in systemic inflammation might contribute to the development of organ failures. We aimed to estimate reference intervals for serum GSN and Gc-globulin according to CLSI guidelines and to elucidate their clinical role in sepsis. We obtained blood from 126 apparently healthy individuals aged between 22 and 80 years. Serum GSN and Gc-globulin levels were determined by automated immune turbidimetric assays on a Cobas 8000/c502 analyzer. We also investigated sera of 41 septic patients. Among septic patients, organ failures and 7-day mortality were investigated. Reference interval (RI) for se-Gc-globulin was 291.20 – 515.40 mg/L (2.5 - 97.5 percentiles). Because of differences in sex regarding se-GSN levels, separate reference intervals were established: for males (n=63), RI was 57.72 – 110.85 mg/L, for females (n=63), RI was 55.30 – 106.71 mg/L. Intensive care patients exhibited lower serum GSN and Gc-globulin levels than controls (median GSN levels: 14.20 vs. 77.85 mg/L; median Gc-globulin levels: 263.43 vs. 394.19 mg/L; $p<0.001$). First-day serum Gc-globulin levels seemed to be able to facilitate the diagnosis of septic shock (ROC-AUC: 0.89; $p<0.05$), acute lung injury (ROC-AUC: 0.72; $p<0.05$) and acute liver failure (ROC-AUC: 0.86; $p<0.05$). Only first-day serum GSN levels predicted 7-day mortality (ROC-AUC: 0.74; $p<0.05$). Serum GSN and Gc-globulin may have promising diagnostic and predictive values in sepsis. The proposed reference intervals for GSN and Gc-globulin might be helpful in future studies investigating different inflammatory diseases.

PS2.4

Melatonin, as a novel anti-inflammatory marker in sepsis

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Timely diagnosis and effective therapy of sepsis is still one of the most challenging medical conditions in intensive care. Melatonin plays an important part in regulating the circadian rhythm, but it may also have an anti-inflammatory effect besides being a potent antioxidant as well. Selective serotonin reuptake inhibitors (SSRIs) require weeks to achieve their antidepressant effects, however, they may also acutely elevate melatonin levels in the circulation, possibly resulting in

decreased inflammatory mediator levels in sepsis. This could be an indirect effect of SSRI medications (iv. Citalopram) in patients with sepsis.

Blood and urine samples were taken from 21 septic patients without SSRI treatment and 9 septic patients with SSRI treatment at the ICU. Previous studies have shown that urine 6-sulfatoxymelatonin (6-SMT) concentrations show good correlation with the total level of melatonin in the circulation. Urine 6-SMT levels (ng/mL) were measured using an ELISA method based on the competitive principle (IBL International GmbH). Wilcoxon and Mann Whitney U tests were used in the 22th version of the SPSS program for statistical analysis. We found a significant increase in urine 6-SMT levels between the septic patient groups without and with SSRI treatment on the second (3.99 vs. 14.21 ng/mL, $p<0.05$) and third (3.55 vs. 8.39 ng/mL, $p<0.05$) night of follow up. The decreasing tendency of C-reactive protein (CRP) and Procalcitonin (PCT) was more explicit in the SSRI treated patients compared with non-treated patients, although this difference was not statistically significant. A sensitive ELISA method is already available for measuring urine 6-SMT levels, which may prove that SSRI treatment could have beneficial effects regarding the successful treatment of sepsis.

PS2.5

Laboratory diagnosis of Polycystic Ovary Syndrome – therapeutic difficulties in risk of thrombophilia
A case study

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Polycystic Ovary Syndrome (PCOS) is a hormonal disorder common among women of reproductive age (5-26%). PCOS may begin in childhood, but most symptoms occur later, in parallel with maturation and they are caused by higher-than-normal levels of androgen hormones. Main symptoms: irregular menstrual cycles, cysts in ovaries and high androgen levels which may result in physical signs, such as excess of facial and body hair, and occasionally severe acne and male-pattern baldness. Insulin resistance is also present in 70% of the cases. Later it may be accompanied by obesity, type 2 diabetes, cardiac complaints and infertility. Laboratory tests also play an important role to establish the diagnosis. Basic treatment: birth control pills and antidiabetic treatment. Unfortunately there are a lot of cases when PCOS is not detected, e.g. associated acne is considered as part of adolescence even if barely or not treatable at all with the standard dermatological agents.

In our case, a 24 year old female's main symptoms were very severe generalized acne and mild hair loss existed since her early puberty. She was no longer fully responding to retinoid treatment either. Ultrasound confirmed polycystic ovaries. Hormonal and loaded blood sugar levels were normal, while insulin resistance was detected. Steroid Hormone Binding Protein was abnormally low. Birth pill therapy could not be considered due to thrombophilia (Leiden mutation, lower Protein S and FXII). She is currently being treated with metformin that seems insufficient. Uncertain laser treatment and increase of antidiabitic treatment remain as last resort.

PS2.6

Case History of Two Patients with Factor XII Deficiency – The Diagnostic Complications and Experiences in the COVID-19 Pandemic

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Factor XII deficiency, also known as Hageman factor deficiency, named after the first patient diagnosed with the disorder in 1955. Literature estimates this deficiency to have an incidence rate of 1 to 1 million people. It is most often incidentally discovered during preoperative or routine coagulation tests causing an isolated, severally prolonged APTT.

We report the case history of two patients whose hemostatic tests revealed significant decrease of clotting factor XII activity (<3%). With the help of these cases, we discuss the practical algorithm for differential diagnosis and the influence of confounding circumstances (preanalytical processes, reagent properties, use of anticoagulant, etc.).

The first patient is a 67-year-old outpatient man, requested by the Hematology Department for investigation of suspected bleeding disorder (PT, APTT, Thrombin Time, Fibrinogen, FVIII-, FIX-, FXIII-activity, von Willebrand factor antigen level and activity, screening of platelet function with PFA and CBC). The other is a 50-year-old invasively ventilated man with acute respiratory distress syndrome (ARDS), positive for SARS-Cov-2 from the Intensive Care Unit, whose daily APTT results showed varying prolongation from 45 seconds to over 240 seconds, depending on the therapy used.

Throughout our case-studies, we aim to underline the difficulties in diagnostic procedures in the COVID-19 pandemic, including access to anamnestic details, drugs and therapies used (e.g. convalescent plasma-therapy, therapeutic dose of LMWH), possibility of lupus anticoagulant presence, ongoing infections etc., and share our practical experiences.

PS2.7

Pregnancy increases the risk of severe complications and worse outcome in COVID-19-infection (case report)

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Pregnancy is a physiologically suppressed immune state in order to protect the fetus and for this reason infection may be more severe for pregnant in comparison to non-pregnant females. V.A. 37 year old, 24-week pregnant was admitted 5 days after symptoms appeared due to progressive breathing difficulties caused by COVID pneumonia that was confirmed by PCR. Alongside mechanical ventilation, she was given convalescent FFP due to the significant increase of IL-6. According to empirical protocol ceftriaxone-azithromycin prophylaxis was used. At that time the microbiological screen was still negative. Her oxygenation continued to worsen and she was transferred to the GOKVI to receive ECMO treatment (extracorporeal membrane oxygenation). Following the insertion her IL-6 level was still low and her inflammation markers haven't improved, although her anemia required continued transfusion. That time systemic infection hasn't been shown, but multidrug resistant *Acinetobacter baumannii* was cultured from tracheal mucus. Due to next consultation, C-section was carried out. Despite anticoagulant treatment, thrombus was detected. Chest and abdominal hematoma was behind her anemia. By that time MACI was cultured from hemoculture, central cannula and urine. Severe sepsis, hemorrhagic shock and DIC developed and the patient passed away. As a result of this and similar cases, domestic regulation was created in the same way with international recommendations on March the 24th. The use of mRNA vaccines have been recommended in the case of pregnant and lactating mothers by the Infectious Disease branch of the College of Healthcare Professionals, but of course taking into consideration the individual circumstances of each case. Her life may have been saved by the vaccine as well.

PS3.1

Cytokine profile in SARS-CoV-2 infection and the effect of tocilizumab and convalescent plasma therapy

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COVID-19 is an infectious disease caused by the SARS-CoV-2 virus. It mainly affects the respiratory tract, but due to the production of a wide range of cytokines it can exert severe effects on the entire human body. Our aim was to determine the

alterations in cytokine profile among COVID-19 patients at intensive care unit. We enrolled 126 patients with COVID-19 and measured the concentration of 20 cytokines with Magpix Luminex Xmap (Merck). We compared the results between patients who recovered from COVID-19 disease to subjects who passed away. Furthermore, cytokine levels were compared among patients who received IL-6 receptor inhibitor tocilizumab or the convalescent plasma therapy vs. those who received neither intervention. We found higher IL-6, IL-8, IL-10, IL-15, MCP-1 and TNF-α levels in patients who passed away compared to those who recovered (median [quartile] pg/mL; 36.1 [3.2-90.5] vs. 120.2 [24.3-440.0], 22.9 [18.5-34.9] vs. 43.3 [24.8-74.3], 15.7 [2.6-53.8] vs. 65.6 [19.9-241.0], 13.1 [8.2-19.3] vs. 19.0 [10.5-36.3], 569.8 [367.0-903.0] vs. 918.5 [628.1-2029.0], 31.7 [20.5-49.5] vs. 44.4 [28.5-75.2], respectively, $p<0.001$). In addition, IL-8 and IL-10 levels were higher under plasma therapy compared to tocilizumab administration (44.7 [28.7-72.0] vs. 23.7 [20.0-37.7] and 75.4 [38.8-360.3] vs. 15.7 [2.6-42.9] pg/mL), respectively, while IL-10 was also higher without any of these therapies compared to tocilizumab treatment (48.4 [9.8-114.8] vs. 15.7 [2.6-42.9] pg/mL). In conclusion, high cytokine levels represent a major mortality risk in COVID-19 disease and while tocilizumab has some effect on cytokine profile, a higher level of inhibition is needed to effectively reduce the cytokine storm during intensive care unit therapy.

PS3.2

Effect of COVID-19 infection on Diamine-oxidase enzyme concentrations

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Histamine is a major mediator released by the immune system as a result of SARS-CoV-2 virus invasion, that can lead to a cytokine storm and consequently multiple organ failure. In patients who suffer from DAO (Diamine-oxidase) deficiency there are high levels of circulating histamine. Higher histamine release associated with SARS-CoV-2 virus-induced mastocytosis worsens the inflammatory process generated by the infection. Two genes can play a fundamental role: NOS2, which expresses inducible nitric oxide synthase (NOS2), and AOC1, which encodes diamine oxidase. In the first quarter of 2020, our laboratory received 1353 DAO enzyme test requests (mean value: 11.38±7.65) of which 1029 were requested by physicians and 324 were requested by private patients. In contrast, in the first quarter of 2021, 1925 were requested by physicians and 408 were performed for private patients from the total of 2333 DAO examinations (mean value: 9.58±6.83). A sandwich ELISA technique was used to determine the enzyme activity with two polyclonal antibodies against recombinant DAO. The mean ages of the two groups studied were nearly the same (40.34±15.28 and 39.72±14.52 years). In 2021, we found much more histamine intolerance patients. The relation to SARS-CoV-2 virus infection was suspected. In the first three months of 2021, 63% of all tests already showed low DAO values compared to 54% measured in the previous year. Statistical analysis showed a significance difference ($p<0.0001$). In the third wave of the COVID-19 pandemic in 2021, the DAO levels of studied patients were significantly lower compared to the previous year. The high incidence of histamine intolerance may require further investigation. We still do not know whether the SARS-CoV-2 virus infection directly contributed to the development of these symptoms of histamine intolerance or exacerbated pre-existing histamine intolerance.

PS3.3

Investigation of serum lactate dehydrogenase isoenzymes by electrophoresis in patients with COVID-19 pneumonia

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Serum total lactate dehydrogenase (LDH) was increased in severe COVID-19 and showed a positive correlation with disease severity. However, no data are available about the profile of LDH isoenzymes in COVID-19. We here studied

whether the increased activity of a particular LDH isoenzyme, such as LDH-3 typically released from the lung, could be exclusively observed. Sera of 22 consecutive severe COVID19 patients were collected showing abnormal total LDH levels measured by photometric method on a Cobas® 6000 analyzer (Roche, Mannheim, Germany) to investigate LDH isoenzymes using gel electrophoresis (Hydragel, Sebia) and densitometric evaluation. The percentage and relative activity values of these isoenzymes were correlated with laboratory parameters and the degree of lung parenchymal involvement based on chest CT. Total LDH was elevated in the range of 272-2141 U/mL that significantly correlated with LDH-3 activity (r=0.765, P=0.0001). LDH-3 levels showed a modest but statistically significant association with serum ferritin (r=0.437, P=0.042). None of LDH isoenzymes was predominately abnormal in COVID-19 and there were 2 subjects even without a higher than normal isoenzyme ratio. Larger mid-zone fractions of LDH-3 with LDH-2 or LDH-4 were detected in 8 subjects diagnosed with ≥50% pulmonary parenchymal involvement, while LDH-2 alone was augmented in moderate lung extension (n=4). In critically ill states, liver dysfunction (n=6) or hemolysis (n=2) were also presented via elevated LDH-5 or LDH-1, respectively. Overall, distinct LDH isoenzymes can contribute to the differently increased total LDH activity in severe COVID-19.

PS3.4

Laboratory follow-up of convalescent plasma treatment in B-cell depleted patients with COVID-19 pneumonia

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Convalescent plasma (CP) with anti-SARS-CoV-2 antibodies tends to be an effective therapeutic approach to aid the treatment of COVID-19 in B-cell depleted patients lacking humoral response to SARS-CoV-2. We here report a series of 20 subjects (13 males and 7 females, at the age of median [range] 56 [27-76] years) with various hematological malignancies and COVID-19 pneumonia. These patients were under anti-CD20 therapy or other chemotherapy and demonstrated a profound B-cell lymphopenia based on flow cytometry analysis of peripheral blood lymphocyte subpopulations. Consequently, they showed undetectable baseline anti-SARS-CoV-2 immunoglobulin (Ig) levels before CP treatment according to Elecsys® SARS-CoV-2 nucleocapsid- and S1-RBD-specific total Ig tests (Roche, Mannheim, Germany). Importantly, 10 patients already received the first dose of CP within 5 days of initiation of antiviral therapy, while the other half were treated with plasma only after 5 days of remdesivir treatment. Although a substantial improvement in clinical symptoms was documented in both subgroups in the presence of improving SARS-CoV-2 Ig levels and decreased inflammatory response monitored via C-reactive protein and ferritin levels as well as oxygen independence, PCR positivity was sustained longer in those subjects who were treated with CP in a delay. CP treatment was well-tolerated, and no adverse event was reported in these cases with resolving pulmonary infiltrations. In conclusion, routinely available serology tests can be utilized to monitor the effect of CP treatment in COVID-19 patients suffering from impaired humoral immunity.

PS3.5

Humoral immune response after the Pfizer vaccination

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Pfizer vaccine consists of a lipid nanoparticle shell to deliver a mRNA to the cytoplasm of host cells. Based on this technique, cells synthesize the SARS-CoV-2 spike protein, which shows the strongest immune effect. Spike protein is displayed on the host cell surface and is recognized by the immune system. Thirty-four colleagues were followed to investigate their humoral immune response. Blood samples were collected before vaccination, at 12 days after the first

dose and 14 days after the second dose. Seven different COVID-19 serology tests were performed: two types of IgG; of IgM; of IgA and anti-S1-RBD IgG neutralizing antibody levels. Recruited participants were allocated into 3 groups: 1) 26 were seronegative, 2) 5 had been previously contacted with SARS-CoV-2 infection and 3) three subjects had an ongoing, asymptomatic COVID-19 infection. Twelve days after the first dose, who previously had not been in contact with the virus, 73% had positive anti-S1/S2 IgG and 81% demonstrated anti-S IgA antibodies. In those who had been contacted with the virus showed unusually high levels of anti-S1/S2 IgG and anti-S IgA. Individuals with ongoing infection had serious adverse symptoms after vaccination. At 14 days after the second dose, 95% from the first group had high anti-S1/S2 (>100 AU/ml) and high neutralizing antibody levels. Anti-S IgA was positive among all of them, while anti-S IgM antibodies were detected in 75% of the cases. In group 2, antibody levels did not rise further, instead, in some cases the antibodies decreased drastically. In conclusion, Pfizer vaccine effectively triggers the development of the humoral immune response. According to our study, vaccination is not recommended during an ongoing infection and for patients with previous COVID-19 infection and high antibody levels, thus the second dose of vaccination should be considered at a later time than usual.

PS3.6

The prognostic importance of interleukin 6 in corona virus disease 2019

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Inflammation has a leading role to increase the severity of COVID-19 disease due to induction of a cytokine storm. Inflammatory biomarkers, such as interleukin-6 (IL-6), C-reactive protein (CRP) and procalcitonin (PCT) have been used to predict the progression and mortality of COVID-19. We performed IL-6, CRP and PCT emergency measurements from COVID-19 patients and a prognostic value was evaluated based on these results. We analysed 215 patients retrospectively and devided them into three subgroups according to severity: 1) outpatient subjects with mild symptoms, 2) patients with moderate symptoms (administered to the Infectology) and 3) those with severe symptoms (that required ICU treatment). IL-6 values were measured with chemiluminescence immunoassay (Siemens Advia Centaur XPT) and were compared to CRP and procalcitonin levels. Results were expressed as median (min.-max). The median value of IL-6 was 6.9 (<2.7-23.2) pg/mL in mild COVID-19 (n=25), 27.2 (2.7-355.4) pg/mL in the moderate group (n=110) and 98.0 (9.1->55000) pg/mL in severe cases (n=80). All patients with IL-6 level over 15,000 pg/ml died (76% of all deceased patients). We found no correlation between CRP and procalcitonin levels compared to baseline IL-6 levels, however in 90% of severe cases PCT was over 2 μg/L indicating a bacterial or fungal infection which were confirmed with positive haemoculture. In conclusion, according to our results, in COVID-19 patients IL-6 can be used as a prognostic marker, forecasting the development of cytokine storm, which can be also caused by a secondary sepsis supported by high PCT levels and positive haemoculture in our study.

PS3.7

The association of suPAR with IL-6, CRP and procalcitonin in COVID-19 patients

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SuPAR is an inflammatory marker expressed during immune activation. High suPAR level is a strong prognostic marker and has been associated with a number of immune mediated acute and chronic disease. While high suPAR levels have been confirmed in COVID-19 patients, the alterations during patient care is lacking. We aimed to follow the changes in

suPAR levels during intensive care unit treatment and to compare these data to other inflammatory markers, such as IL-6, C-reactive protein (CRP) and procalcitonin (PCT). Blood samples were collected from 12 COVID-19 patients at 6-9 different time points throughout the nursing time. Serum suPAR concentrations were measured with suPARnostic® Flex ELISA assay, IL-6 and PCT were determined with chemiluminescence immunoassay, while CRP was analyzed with immunoturbidimetry.

We found a significant correlation between suPAR and IL-6 levels in 7 patients (Spearman's correlation coefficient, [r] = 0.79; 0.94; 0.98; 0.82; 0.94; 0.94; 0.75, $p<0.05$), a strong relationship between suPAR and CRP in 3 cases (r = 0.93; 0.94; 090, $p<0.05$) and a positive association between suPAR and PCT in 6 patients (r = 0.82; 0.94; 0.79; 0.98; 0.76; 0.94, $p<0.05$). In conclusion, our data suggest that suPAR levels in patients with COVID-19 during intensive care unit treatment alter in a similar fashion than IL-6, CRP and PCT levels but with some differences. SuPAR does not change rapidly (which is very common to IL-6), and it rather changes in a more balanced way, which explains why we did not find any correlation in almost half of the patients. Therefore, suPAR could be a more suitable marker for the monitoring of patients with COVID-19 than IL-6.

PS3.8

COVID-19-associated coagulopathy in pregnancy: a prospective, case-control study

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The new coronavirus infection (COVID-19) is associated with significant changes in hemostasis parameters, however, little is known about COVID-19-associated coagulopathy in pregnancy. In this observational case-control study, 39 women with acute COVID-19 infection at 36-40 gestational weeks of their pregnancy (COVID-19+ group) and 21 healthy age- and gestational week-matched pregnant women were enrolled (COVID-19- group). All women were outpatients and acute infection was confirmed or ruled out using SARS-CoV-2 RT-PCR or antigen test. In addition to screening tests of coagulation, D-dimer, fibrinogen, von Willebrand factor antigen, chromogenic factor VIII (FVIII) activity, factor XIII (FXIII) activity, in vitro clot-lysis, angiotensin convertase enzyme (ACE) activity, and anti-SARS-CoV-2 antibody levels were measured. In the COVID-19+ group, APTT was significantly increased, while PT, TT, fibrinogen and D-dimer were not significantly different as compared to the COVID-19- group. FVIII activity was significantly lower in the COVID-19+ group (183.7±47.7%) as compared to COVID-19- group (226.7±62.5%, p=0.01). Similarly, FXIII activity was reduced in the COVID-19+ group (82.3±23.5% vs. COVID-19- group: 96.2±26.6%, p=0.04). Pregnancy-associated complications including HELLP syndrome were observed in 2 cases of COVID-19+ group, with marked alterations of coagulation screening tests, clot-lysis and D-dimer levels. Conclusion: Pregnancy associated with SARS-CoV-2 infection in the third trimester leads to reduced levels of FVIII and FXIII activity, most likely as a result of increased coagulation activation and consumption.

PS4.1

Changes in granulocyte-platelet interactions after coronavirus vaccinations

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Leukocyte-platelet interactions have been described in inflammatory and coagulation processes as well as in vascular disorders. During the interactions, platelets support the activation of polymorphonuclear cells, establishing the formation of platelet-granulocyte heteroaggregates. Platelets can also form heterotypic aggregates with monocytes and

lymphocytes, the phenomena were described as platelet satellitism. However, these interactions have not been evaluated in post-vaccination conditions. Widespread vaccinations in adults against the recently emerged novel coronavirus provide the opportunity to examine platelet satellitism related to the immune response on vaccines. We examined the proportion of granulocytes presenting platelet satellitism in peripheral blood smears of vaccinated and non-vaccinated individuals who were not presenting an ongoing inflammation at the time of analysis. Quantitative determination of anti-spike antibody has been performed in case of vaccinated participants. By analyzing the peripheral blood smears, we could observe a significantly higher proportion ($p<0.0001$) of granulocyte-platelet satellitism in the group of vaccinated individuals ($15.21 \pm 5.61\%$) compared to those without vaccinations ($6.77 \pm 2.17\%$). Granulocyte-platelet interactions showed a close relationship with the elapsed time since vaccinations and the concentration of anti-spike antibody. Our results indicate that the proportion of platelet-granulocyte heteroaggregates in peripheral blood smear suffers substantial changes during the immune processes following vaccinations, which could be related to the strength of the immune response.

PS4.2

Exclusion of pseudothrombocytopenia on Sysmex XN-1000 Hematology Analyzer

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Based on our laboratory SOP, all blood samples with a platelet (PLT) count under 90 G/L are examined by microscope to rule out the possibility of pseudothrombocytopenia. The aim of this study was to develop a simple, time-saving method that excludes PLT aggregation to avoid unnecessary blood smear testing. 443 samples with a PLT count between 30-90 G/L were examined retrospectively. PLT aggregation was confirmed in 60 cases. The association between the most common Sysmex XN-1000 PLT flags ('Giant PLT', 'PLT Abnormal Distribution', 'PLT Clumps'), PLT histogram, optical PLT count, O-PLT scattergram and PLT aggregation was determined by Chi-square test, significance level was set $p < 0.05$. Specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV) of the above parameters were determined. Based on the results of the Chi-square test, the 'PLT Abnormal Distribution' flag alone was not significantly associated with platelet aggregation ($p > 0.05$). From all the other parameters, O-PLT scatterplot had the highest correlation ($p = 6.94 \times 10^{-14}$), sensitivity (> 99%) and NPV (> 99%), however its assessment may be difficult and requires practice. The latter is also true for the PLT histogram ($p < 0.0001$) which was discarded due to its lower specificity, sensitivity, NPV and PPV. 'PLT Clumps' flag ($p < 0.0001$) had the highest specificity (89%) and PPV (38%). We preferred 'Giant PLT' flag ($p < 0.05$) over the O-PLT count ($p < 0.001$) because of its higher specificity (72% vs 55%). We developed a method with an overall specificity of 94%, sensitivity > 99% and NPV > 99% that excludes PLT aggregation in the case of negative PLT scattergram and in the absence of "PLT Clumps" and "Giant PLT" flags. Based on our calculations, 39% of unnecessary blood smear tests can be filtered out by this method.

PS4.3

Investigation of alpha2-plasmin inhibitor C-terminal heterogeneity in normal human plasma samples and its effect on in vitro clot lysis time

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Alpha2-plasmin inhibitor (A2PI) is the main inhibitor of plasmin. The secreted C-terminally intact form of A2PI (PB-A2PI) is proteolytically cleaved in the circulation. The C-terminally cleaved form lacks the plasminogen-binding site (NPB-A2PI), it remains an active plasmin inhibitor, but reacts more slowly with plasmin.

Our aim was to investigate the effect of the incorporation of PB-A2PI and NPB-A2PI into fibrin clots on the lysis of the clot.

Citrated plasma samples from 86 healthy individuals were clotted by thrombin and calcium. Total-A2PI and PB-A2PI levels were measured by sandwich ELISAs from the original plasma and the extruded serum samples, and the concentration of NPB-A2PI was calculated from these values. An *in vitro* clot lysis assay was performed using platelet poor plasma samples. Total-A2PI, PB-A2PI and NPB-A2PI levels (mean±SD) in the plasma samples were 64.3±7.5 mg/L, 43.5±4.7 mg/L and 20.8±5.5 mg/L (32.0±6.1%), respectively. 43.6±5.9% of the plasma PB-A2PI and 62.2±12.4% of the plasma NPB-A2PI was found to be incorporated into the clot. Plasma total-A2PI, NPB-A2PI levels and the amount of incorporated NPB-A2PI showed significant correlation with 50% clot lysis time (CLT50) ($r=0.313$, $p=0.003$; $r=0.387$, $p<0.001$ and $r=0.256$, $p=0.017$, respectively), while PB-A2PI levels did not correlate ($r=0.053$, $p=0.629$) with lysis parameters.

Our results suggest that non-covalent incorporation of the C-terminally truncated NPB-A2PI may have an effect on clot lysis and it should be further investigated.

Funding: GINOP-2.3.2-15-2016-00043, NKFI-K120633

PS4.4

Newly diagnosed chronic lymphocytic leukemia during symptomatic COVID-19: case reports

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Patients with malignant diseases have high risk of severe or critical forms of COVID-19. Chronic lymphocytic leukemia (CLL) can dysregulate both the adaptive and innate immune response. CLL-related immune dysfunctions may involve T- and B cells, the phagocytic cells and the complement system. SARS-CoV-2 infection also affects the function of the immune system, causing mainly the depletion of CD4+ and CD8+ T cells. When these diseases meet, the weakening of the immune system could help to avoid the fatal overreaction of the immune and inflammatory response. In our cases CLL manifested during a severe COVID-19 pneumonia. A 43-year-old man with IDDM was admitted to hospital in February with bilateral COVID pneumonia. He was transferred to hematology due to a vertebra CT showing signs of malignancy. He had 17.2 G/L WBC, 62.9% lymphocyte, 33% pathological B cells. The diagnosis of CD38+ B-CLL with two subclones was made. He had no paraproteinaemia but high levels of IgM and IgG type antibody against SARS-CoV-2 S protein. The other case is a 53-year-old man, hospitalized in March with severe COVID pneumonia. Signs of B-CLL was found (WBC 123 G/L, lymphocyte 91%, hemoglobin 107 g/L). Flow cytometric assay showed 82% pathological B cells with the diagnosis of $CD38^-$ B-CLL. The IgH gene rearrangement was positive. He had 1.2 g/L IgG kappa type monoclonal paraprotein. These two patients being in Rai 0-1 stage recovered after COVID pneumonia without shifting into a critical state in intensive care unit. The long-term follow-up of patients with CLL that manifested during symptomatic COVID-19 may further improve our knowledge about the immune system being attacked from several pathways.

PS4.5

Possibilities of fibrinogen measurements in patients with COVID-19

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Hypercoagulability is a common complication of COVID-19. Fibrinogen, similarly to other well-known acute phase reactants is increased, and hyperfibrinogenemia is considered to be one of the mechanisms for COVID-19 coagulopathy.

Derived fibrinogen assay (PT-Fg) is used in our laboratory. In an earlier study we found a correlation between the PT-Fg and the Clauss method in the linearity range (fib: 0.6 - 7.0 g/L, n=96, r^2=0.9176).

The aim of our study was to determine whether the PT-Fg assay in COVID-19 could be used by extrapolating the result (above the linearity limit), or the sample should be diluted before the measurement. We also looked at the correlation between the PT-Fg- and the Clauss fibrinogen assay.

78 PCR-confirmed Sars-CoV-2 infected patient samples were analyzed in parallel with diluted PT-Fg and Clauss method. All of the 78 patients had PT-Fg result above the linearity range (>7 g/L, 7.06 - 11.89 g/L). Our results were analyzed by Pearson's correlation analysis.

PT-Fg results from extrapolated and diluted samples correlated significantly (r^2 = 0.8374). With the Clauss method the correlation was better for the extrapolated results of the PT-Fg than for the PT-Fg measured from the diluted samples (r^2 = 0.8475 versus r^2 = 0.756).

Our results show - knowing the limitations of the procedure - that the fast and cheap PT-Fg assay is an appropriate method to measure fibrinogen from COVID-19 patients sample.

PS4.6

Haematological diseases with paraproteinemia in association with COVID-19

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The novel SARS-CoV-2 corona virus is a risk factor for worse outcome in patients with haematological tumors. As haematological diseases with paraproteinemia affect a wide range of the population, we aimed to examine how COVID-19 infection affects disease outcome. We also aimed to present a few instructive case reports. We enrolled 24 patients to the study (newly diagnosed or undergoing therapy or directly after stem cell transplantation or prepared for future stem cell transplantation). We compared their paraprotein and free light chain concentrations, IL-6, procalcitonin, CRP and D-dimer results. For patients who were not hospitalized (42% of all examined patients) the median data were: age: 60 years, paraprotein: 1.05 g/L, lambda: 8.02 mg/L, kappa: 9.7 mg/L. For hospitalized patients (the remaining 58%): median age: 66 years, paraprotein: 4.9 g/L, lambda: 18.8 mg/L, kappa: 48.85 mg/L. 42% of the latter group were administered to intensive care (paraprotein: 4.65 g/L, lambda: 16.85 mg/L, kappa: 48.85 mg/L). 16% of all patients died because of a relapse or their disease transformed to leukaemia or they were directly after transplantation.

Conclusion: The severity and outcome of COVID-19 is affected by a number of factors associated with paraproteinemia. Namely, the stage of the disease, whether the treatment had already been started or not, whether the patient is in remission or in a relapse or whether the patient needed any additional treatment or not.

PS4.7

Thrombin generation in patients with inflammatory bowel disease

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Inflammatory bowel disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC), both predominantly affecting the gastrointestinal tract. Accumulating evidence suggests that hypercoagulability may be associated with thrombotic risk and may play a role in the pathogenesis of IBD. Here we aimed to find out whether thrombin generation (TG) is increased in IBD, and whether TG parameters are associated with disease activity. Thirty-eight patients with IBD (CD/UC: 27/11) and 69 age- and sex-matched healthy controls were enrolled. TGA was performed from platelet poor plasma. Lag time, endogen thrombin potential (ETP), peak thrombin, time-to-peak and velocity index were calculated. Clinical parameters and disease activity (partial Mayo score, Crohn's disease activity index) were registered. Patients were followed and blood samples were taken in active and quiescent disease stages. ETP was significantly higher in patients vs. controls (1896±39 vs. 1468±35 nM*min, p<0.0001). Peak thrombin was also significantly increased in patients (377±98 vs. controls: 285±91 nM, p<0.001). ETP was significantly higher in UC patients with more active disease (ETP: pMayo 0-1:1837±170 vs. pMayo 2-3:2287±447 nM*min, p=0.047). Conclusions: in IBD patients, the extent of TG was significantly higher as compared to controls, which might be associated with increased thrombotic risk. Changes in disease activity resulted in parallel changes of thrombin generation, indicating that hypercoagulability might be a feature of the active disease.

PS4.8

Thrombin generation in patients with non-traumatic intracerebral hemorrhage

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Non-traumatic intracerebral hemorrhage (ICH) accounts for 10-15% of all strokes and leads to a higher rate of mortality as compared to ischemic strokes. Here we aimed to find out whether the thrombin generation assay (TGA) could predict outcomes in ICH patients. In this prospective, observational study 83 consecutive ICH patients (age: 66.71 ± 12.11, male/female: 54/29) and 162 healthy controls were included. CT, detailed clinical and laboratory investigations were performed from patients on admission. TGA was performed on stored platelet poor plasma obtained on admission. Lag time, endogen thrombin potential (ETP), peak thrombin, time-to-peak were calculated. Short- and long-term outcomes of ICH were defined at 14 days and 3 months post-event according to the NIHSS and the modified Rankin Scale (mRS), respectively. Peak thrombin was significantly higher in patients as compared to controls (392±91.4 vs. 314±85.94 nM, p<0.0001). Time to peak parameter was significantly longer in patients. A peak thrombin parameter above the upper quartile in patients conferred a risk for worse long term outcome (mRS 2-6, OR: 6.91, 95%CI: 1.77-24.29, p=0.004). Lag time and time to peak showed a modest, significant negative correlation with intracerebral bleeding volume (lag time: r=-0.2406, p=0.0448, time to peak: r=-0.3665, p=0.0018). Lag time, ETP and time to peak parameters showed significant correlation with CRP. Conclusion: in patients with ICH, TG was increased as compared to healthy controls, which might be explained by the presence of higher inflammatory parameters in patients. Peak thrombin measured on admission might be useful to predict outcomes in ICH patients.

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