

Abstracts*)

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Poster Presentation

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PL.1

The HONORARY MEMBERSHIP-award lecture

Should performance specifications for POCT be similar to performance specifications in the central laboratory?

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POCT is the most rapidly growing field in laboratory medicine. With increasing technological and analytical possibilities, an increasing number of analyses can now be carried out on POC instruments. Although the costs of POC instruments are less than hospital instruments, the number of users of POC instruments are much larger, ranging from wards in the hospitals, GP offices, nursing homes, pharmacies and last but not least tests for self-measurements. With the increasing emphasis on patient empowerment, this is a wanted development.

The ultimate goal of using POC testing is that patient outcomes should be improved and/or that it should be more cost/effective than the use of conventional laboratory testing. To achieve this, the role of POCT in the different clinical settings as well as the responsibility for introducing and manage the instruments and use of the instruments should be clearly defined. The main reason for using a POC instrument is that a rapid result is more useful than waiting for a result from a central laboratory. An essential question is therefore: Should performance specifications for POC instruments be different from that of instruments in a central laboratory. Many will say “yes”, but taking into account the different use of such instruments, performance specifications could probably be modified. Many POC instruments are used for specific clinical settings and one should therefore try to develop performance specifications for that setting. It is also probable that time and location is an important quality factor and that performance specifications can be less strict if a result is provided rapidly – especially in cases where you would like to know if the result is “very high” or “very low”; e.g. hypo- and hyperglycemia. However, if performance specifications for some POC measurement procedures should be less stringent compared to the central laboratory, it is important that this is communicated to the users of tests.

PL.2

The International JENDRASSIK-award lecture

The future of molecular biology in the diagnostic laboratories

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The recent advances in the genomic field and the development of new technologies for DNA testing started the revolution of the diagnostic laboratory. For the diagnosis DNA-based diagnostics provide a sensitive alternative to protein-based diagnostics and the mutation detection is one of the most important areas of molecular diagnostics today and can be divided into two categories: a diagnostic mode, where specific tests are designed to detect known mutations and a scanning mode, where a stretch of DNA is searched for unknown mutations.

Advances in DNA analysis to develop methods, which are increasingly specific, sensitive, fast, simple, automatable, and cost-effective, are considered paramount. These demands are currently driving the rapid evolution of a diverse range of newer technologies.

Researchers have discovered hundreds of genes that harbour variations contributing to human illness, identified genetic variability in patients' responses to dozens of treatments, and begun to target the molecular causes of some diseases. In addition, scientists are developing and using diagnostic tests based on genetics or other molecular mechanisms to better predict patients' responses to targeted therapy.

For the future of genomics is demanding the rapid evolution of miniaturization (nanotechnology) and high-throughput genotyping technologies (next generation sequencing) toward increased speed and reduced cost. The speed, accuracy, efficiency, and cost-effectiveness of DNA sequencing have been improving continuously since the initial derivation of the technique in the mid-1970s. With the advent of massively parallel sequencing technologies, DNA sequencing costs have been dramatically reduced. The recent introduction of instruments capable of producing millions of DNA sequence reads in a single run is rapidly changing the landscape of genetics, providing the ability to answer questions with heretofore unimaginable speed.

PL.3

The Hungarian Silver JENDRASSIK-award lecture

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PL.4

The Silver PÁNDY-award lecture From basic research through laboratory service to clinical research

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It is an extraordinary honor to receive the “Silver Pándy Medal” of the Hungarian Society of Laboratory Medicine and I am very thankful for that. I graduated from the University of Pécs where I specialized in laboratory medicine and in neuroendocrinology. I became university professor in 1997, and member of the Hungarian Academy of Sciences in 2004. My professional carrier can be clearly divided into three periods. Between 1972-1987, I was involved in medical teaching and was doing basic research in Pécs, Szeged and Utrecht investigating the impact of neuropeptides on brain functions and the correlations of endocrine and immune disturbances in ethanol and narcotic addiction. Between 1987-2004, I was head of laboratory at the Markusovszky Teaching Hospital and in the period between 2004 and 2013 I was chair of the Institute of Laboratory Medicine and senior vice rector of the University of Pécs, responsible for science (2010-2013), thus my main responsibility was serving patients. Currently, I am the president of the newly established Szentágothai Research Centre of the University. My current scientific interest lies in the early diagnostic biomarker research of hormonal and metabolic diseases, especially in biochemical and genetic correlates of successful in vitro fertilization. In the past 44 years, I have had a chance to serve the Hungarian Society of Laboratory Medicine, IFCC and FESCC, but I must confess that my main dedication, interest and hobby has remained research.

SE1.1

Aspirin resistance: does it exist at all?

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Aspirin is widely used in the prevention of acute atherothrombotic complications. It acetylates Ser529 residue in cyclooxygenase-1 (COX-1) and prevents thromboxane A₂ (TXA₂) formation from arachidonic acid (AA) in platelets. A variety of platelet function tests have been used for the detection of aspirin effect, but none of them was specific for COX-1 acetylation and provided inconsistent results. We developed two new reference methods for the direct and indirect detection of COX-1 acetylation by aspirin. In the first method monoclonal antibodies were raised against acetylated and non-acetylated nonapeptides corresponding to the amino acid sequence of human COX-1 525-533 residues. Using Western blotting technique the antibodies clearly distinguished between acetylated and non-acetylated COX-1 in platelet lysate. The second method measures AA-induced TXB₂ production of platelets in diluted platelet rich plasma. Using these methods no aspirin resistance was detected among healthy volunteers taking 100 mg aspirin protect for 7 days and among patients with coronary artery disease being on long-term aspirin prophylaxis. Methods routinely used to detect the effect of aspirin were evaluated against the reference methods. Only arachidonic acid induced platelet aggregation and secretion and Verify Now Assay detected reliably the effect of aspirin, while aggregation by other agonists (ADP, epinephrine, collagen) and PFA-100 closure times were proven non-specific. In conclusion, true aspirin resistance, if it exists at all, must be a rarity. In general, there is no need to look for aspirin resistance; the drug is effective. The only indications to test effect of aspirin are to detect non-compliance and interference by other drugs. For this purpose only specific methods are to be used.

SE1.2

New data in the therapy of diabetes

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Review of health insurance full set data proves that in Hungary 772.000 diabetic patients are registered. The rate of female/male is 54/46%. The majority, 94% of diabetic population has type 2 diabetes. Prevalence of type 2 diabetes increased by 71% between years 2001

and 2014. The prevalence of diabetes with age >70 is 20%. The costs of type 2 diabetes for the health insurance increased in Hungarian forints from 188 billion in 2001, to 199 in 2010 and to 216 in 2014, which is expressed in euro 711, 723 and 698 million, respectively. Regarding diabetic complications, it is noteworthy that the number of leg amputations were 3475 cases in 2005, and 3658 cases in 2014. During this period the prevalence of type 2 diabetes increased, that is why the relative prevalence of amputation decreased from 0.58% to 0.50%. Prevalence of myocardial infarction decreased from 3.9% of the first 5 years of the observational period (2001-2004) to 2.7% of the last 5 years period of 2010-2014, which reflects on a 31% relative risk reduction. During the same periods relative risk reduction of stroke was 26%. Standardized mortality achieved 4.0% in 2001, which dropped to 3.7% in 2014. Summarizing, at an increasing prevalence of type 2 diabetes, using new drugs for the therapy of these patients the costs of their management decreased and the risk of complications and mortality dropped.

SE1.3

Soluble urokinase plasminogen activator receptor (suPAR) levels and autoimmune disorders

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The assessment of the general inflammatory condition of patients with autoimmune connective tissue disorders (ACTD) is a major challenge. The use of traditional inflammatory markers including CRP-levels and erythrocyte sedimentation rate (ESR) is limited by several preanalytical factors and their low specificities. Soluble urokinase plasminogen activator receptor (suPAR) is one of the novel candidate markers that is increasingly used in immune mediated disorders. In our studies we compared suPAR levels of patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and ankylosing spondylitis with those of healthy controls. suPAR provided valuable clinical information on disease activity in RA, SLE and SSc. We identified a subgroup of remitted RA patients, who presented still clinical symptoms of inflammatory activity, which correlated to high plasma suPAR (while ESR and CRP were normal). In SLE we established specific suPAR cut-off values that support the discrimination between patients with high and those with moderate SLE activity. In patients with SSc suPAR correlated with objective measures of lung and other complications.

In the majority of ACTDs including SLE, SSc or RA, suPAR is seemingly a good biomarker that would provide valuable clinical information. However, before the introduction of this novel parameter in laboratory repertoire important issues should be elucidated. These include the establishment of appropriate and disease specific cutoff values, clarification of interfering preanalytical values and underlying conditions and declaration of age- and gender-specific reference ranges.

SE1.4

Luminescence in medical research

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Fluorescence spectroscopy including polarization technique is suitable for direct monitoring of molecular interactions and in cellular experiments as well. We worked out a polarization method for assessment of amniotic fluid microviscosity, related to fetal lung maturity. Membrane fluidity of cancerous and normal lymphocytes was also studied. A model of fluorescence polarization immunoassay was established long before its introduction to laboratory practice. The measurement of human procalcitonin (PCT) as an early sepsis marker was introduced using chemiluminescence technique. We evaluated the role of PCT in clinical practice and proved that neutrophil granulocytes and liver cells were partially a source of PCT elevation in sepsis. A perchloric acid precipitation method was developed for isolation of acid soluble serum proteins of patients. Quantitative and electrophoretic analyses showed a dramatic increase of acid soluble proteins in catabolic states (sepsis, tumors, etc.) with a marked elevation of alpha-1-acid glycoprotein. Balkan endemic nephropathy is related to exposure with the mycotoxin ochratoxin A (OTA). OTA strongly binds to albumin therefore, the toxin's binding characteristics and some desorbing ability of drugs and flavonoids were studied. A three-dimensional model of OTA-albumin complex was suggested and potential attenuation of OTA toxicity was also discussed. A simple, one-step procedure with ATP-dependent bioluminescence technique was developed for measuring RBC's ATP content. Using cellular models, it was proven that in living cells ATP strongly binds to intracellular macromolecular structures and it is considered to be a viability marker. Therefore, a multiparametric luminescent cellular viability assay was worked out measuring ATP, DNA content, esterase activity and total protein of tissue cultures for assessing dose-response curves in toxicity assays.

SE1.5

Serum levels of lectin complement pathway molecules do not determine the risk of bacterial infections in patients with cirrhosis

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Bacterial infections are a significant cause of morbidity and mortality in cirrhosis. Lectin pathway molecules of the complement system are synthesized in the liver and have a pivotal role in the innate host defense against infectious organisms. Mannose-binding lectin (MBL) and ficolins (FCNs) act as soluble pattern recognition molecules, while mannan-binding lectin serine proteases (MASPs) are effector molecules in elimination of the pathogens. Low levels of the functional proteins increase the risk of various infectious diseases but their significance has scarcely been investigated in cirrhosis related bacterial infections.

Sera of 266 patients with cirrhosis and 160 healthy subjects were assayed for the concentrations of FCN-2, FCN-3 and MASP-2 by ELISAs. In cirrhosis, a 5-year follow-up observational study was conducted to assess a possible association between lectin levels and development of clinically significant bacterial infections (CSI) and mortality.

The FCN-2, FCN-3 and MASP-2 levels were significantly lower in cirrhosis compared to healthy controls (505 vs. 769 ng/ml, 7,301 vs. 10,797 ng/ml and 212 vs. 412 ng/ml, respectively, $p < 0.001$ for all) and decreased according to disease severity as rated by Child-Pugh stage. In Kaplan-Meier analysis time to development of CSI was associated with low level of FCN-3 ($< 4,857$ ng/ml, $p = 0.028$) but not FCN-2 (< 427 ng/ml, $p = 0.068$) or MASP-2 deficiency ($p = 0.368$). Combined FCN deficiency even more than individual molecules were able to predict the development of these episodes. Patients with low level of both FCNs had a cumulative risk of an infection of 52% as compared to 31% with normal level of FCNs ($p = 0.021$). In multivariate Cox-regression analysis, clinical factors but not the serum lectin profile remained an independent predictor of CSI. Prior episode of CSI and in a stepwise manner, the disease severity as rated by Child-Pugh stage conferred higher risk for development of CSI (HR: 2.64, 95% CI: 1.74–3.99, $p < 0.001$ and 2.11, 95%CI: 1.52–2.93, $p < 0.001$, respectively).

In the present prospective study, diseases severity and prior episode of CSI but not the serum lectin profile were major determinants of the risk of CSI in cirrhosis.

SE1.6

Cellular factor XIII in corneal stromal cells

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Transglutaminases (TGs) are a family of enzymes that cross-link proteins by ϵ (γ -glutamyl)lysyl bonds and most of them have been implicated in the modulation of extracellular matrix. Here we investigated the presence of keratinocyte TG (TG-1), tissue transglutaminase (TG-2) and the cellular form of blood coagulation factor XIII (cFXIII) in the corneal tissue.

Frozen sections of normal human cornea obtained from enucleated bulbus were stained for cFXIII, TG-1 and TG-2 using poly-, or monoclonal antibodies. Detection of cFXIII was also combined with labeling for CD11b, CD34, CD45, CD68 and CD163 using double immunofluorescent staining. FITC-labeled or biotinylated secondary antibodies with Texas red-labeled streptavidin were used for the visualization of immunoreactions. Corneal stromal cells were isolated, incubated with antibodies against FXIII and CD34, and subjected to flow cytometry analysis. Western blot analysis was also performed on corneal stroma, and the expression of cFXIII mRNA was analyzed by real-time qPCR. A significant part of keratocytes showed intensive staining for cFXIII, but not for TG-1 and TG-2. Neither epithelial nor endothelial cells were labeled by anti-cFXIII antibody. cFXIII positive keratocytes were distributed unevenly in the corneal stroma; they were abundant in the sub-epithelial tertile of stroma (120 ± 10 /visual field), while they were sparse (38 ± 6 /visual field) in the subendothelial tertile. cFXIII+ cells showed co-staining for CD34, however, a significant number of CD34+ cells were negative for cFXIII. CD34+ cells were evenly distributed in the stroma. Only a few cells were stained for CD11b and CD45, they were also labeled by anti-cFXIII antibody. No cell showed positivity for CD68 and CD163. Corneal stromal cells expressed FXIII-A (68%) and CD34 (84%) by flow cytometry. cFXIII expression was confirmed by Western blotting, and real-time qPCR showed that keratocytes are capable of synthesizing cFXIII. This is the first report demonstrating the presence of cFXIII in the cornea. A significant part of CD34+ keratocytes contained cFXIII, their transglutaminase activity might be important in building up the lamellar structure of corneal stroma.

SE2.1

Problems and solutions in the laboratory diagnosis of endocrine disorders

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The laboratory diagnosis of endocrine diseases is aggravated by numerous factors: i. lack of the appropriate marker; ii. changing hormone levels as a characteristic of the disorders; iii. rarity of a disease influencing basically the diagnostic value even of a specific test, iv. the normal range is revised by new studies. Copeptin released together with vasopressin from the precursor protein, serves as a reassuring new marker and diagnostic tool in evaluation of patients with polyuria-polydipsia and hyponatremia. Apelin, an antagonist of arginine vasopressin is a new player on this field. The diagnosis of primary aldosteronism is bothered by an inherent uncertainty due to the variability of aldosterone secretion and lack of well-defined cut-off values and reliable confirmative tests making the diagnosis of primary aldosteronism rather an estimation of probability than a definite opinion. First-line screening tests for Cushing's syndrome have various diagnostic performances in special clinical settings. Liquid chromatography-tandem mass spectrometry is probably the most accurate method for assessing cortisol levels but it is not routinely used in clinical practice. The diagnosis of Cushing's syndrome is based on complicated algorithms. Recently, the normal range of 25-OH vitamin D became even more uncertain and the US Preventive Services Task Force concluded that there is insufficient evidence to screen for vitamin-D deficiency because of lack of consensus about the cut-point and limited accuracy of commercially available assays.

SE2.2

Laboratory evaluation of neuroendocrine tumours

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The prevalence of neuroendocrine tumours increases worldwide. The signs and symptoms are mainly related to the overproduction of specific hormones secreted by tumour tissues. However, a vast majority of these tumours are hormonally inactive, silent tumours and are discovered incidentally during an imaging study. On the other hand, some neuroendocrine tumour types are taking part of hereditary tumour syndromes, which are inherited autosomal dominantly and the causative role of certain gene mutations have been already well documented. The laboratory diagnosis of neuroendocrine tumours is challenging. This presentation summarizes those laboratory measurements, which are used in evaluation of neuroendocrine tumours. The specific roles of these measurements in the diagnosis, follow-up and evaluation of therapeutical response will be discussed. A brief overview about the genetic background of neuroendocrine tumours focusing on the role of mutation status in patient management will also be covered.

SE2.3

False results by presence of non-pathogenic autoantibodies in endocrine laboratory diagnostics

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The widely used immunometric assays have critically high sensitive detection technology, but their weakness is the susceptibility to antibodies interferences. These can lead to erroneous test results, which can be clinically significant and lead to misdiagnosis. The interfering substances can be characterized by their high molecular weight, their heterogeneous molecular form and their limited biological activity. The polyethylene glycol (PEG) precipitation remained the commonly available technique to investigate these dominant macromolecules. In endocrine laboratory diagnostics the disturbing endogen autoantibodies against thyroglobulin (Tg) and prolactin are frequent problems. Tg is a highly specific tumor marker for differentiated thyroid cancer (DTC), but TgAb is present in more than 25% of DTC's serum. The routine screening for macroprolactin could eliminate unnecessary diagnostic testing and treatment. MacroTSH is considered to be a rare entity. However, according to the newest studies, screening for macro TSH should be performed before hormone replacement therapy is initiated for subclinical hypothyroidism (1).

The authors' experience on these three interfering substances, supported the above-mentioned statements.

In conclusion, we are not able to control these factors in the measurements for frequently requested hormones mostly because of financial reasons. The only way to solve the problem is a close dialogue between the physician and the laboratory.

(1) Hattori N., Ishihara T., Shimatsu A. European Journal of Endocrinology 2016 174: 9-15.

SE2.4

The endocrinology of human pregnancy

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In the course of a human pregnancy a well concerted secretion and interaction of hormones between the maternal, fetal and placental compartment take place. During the follicular phase of the menstrual cycle estrogens trigger structural and functional changes within the endometrium (proliferation). In the luteal phase progesterone induces transition of proliferative endometrium into the secretory phase thereby preparing it for embryo implantation and the establishment of pregnancy. The optimal environment for implantation is provided by chemokines, growth factors, and special cell adhesion molecules produced by the endometrium. The end result of this process is the attachment of the blastocyst and the induction of local changes in the endometrial stroma resulting in decidualization. During the first trimester of pregnancy HCG plays a key role in maintaining corpus luteum function until placental development. The placenta works in a hypothalamic-pituitary-target organ-like fashion, with its own stimulatory and inhibitory feedback mechanisms. Placental hormones regulate growth and differentiation of placental cytotrophoblast and syncytiotrophoblast, growth and maturation of the placental vascular system. The fetus and the placenta produce and secrete steroids into the maternal circulation. These processes are characterized by complementary enzymatic deficiencies within the placental and fetal compartments. Until recently, estriol produced at the third trimester was widely used for the evaluation of the maternal-feto-placental unit. Nowadays in the age of high resolution ultrasonography the clinical significance of sequential estriol determination is dramatically reduced. Parturition represents the interplay between placental, fetal, and maternal compartments, characterized by increased estrogen bioavailability, functional progesterone withdrawal and increased CRH synthesis. The net result of these processes is the increased responsiveness of the myometrium to prostaglandins and oxytocin, a prerequisite for delivery.

SE2.5

Simultaneous detection of salivary cortisol and cortisone levels with a newly developed LC-MS/MS method and their diagnostic power in laboratory diagnosis of hypercortisolism

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Cortisol circulates in blood bounded to carrier proteins and only less than 10% of total cortisol is the biologically active free fraction. Laboratory diagnosis of hypercortisolism requires measurement of cortisol from serum, saliva and urine samples collected in the morning and at midnight. The aim of our work was to develop a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of cortisol and cortisone level in various biological fluids. The diagnostic capacity of our method in the laboratory diagnosis of endogenous hypercortisolism was also evaluated.

142 patients referred to the 2nd Department of Medicine, Semmelweis University with a suspected diagnosis of hypercortisolism were studied. Cortisol levels from patients' midnight saliva samples were measured first using an ECLIA test (Cobas, Roche), then the simultaneously cortisol and cortisone determination was performed by a validated in-house LC-MS/MS method.

A strong correlation between the LC-MS/MS and immunoassay measured salivary cortisol was revealed ($R^2=0,988$). Receiver Operating Characteristics (ROC) analysis showed good diagnostic performance of LC-MS/MS-cortisol and LC-MS/MS-cortisone and this diagnostic accuracy was similar to those obtained for salivary midnight cortisol measured by immunoassay. However the best cut-off value was significantly lower for LC-MS/MS-cortisol than the immunoassay-cortisol (0,232 $\mu\text{g}/\text{dl}$ vs 0,522 $\mu\text{g}/\text{dl}$).

SE2.6

Urinary Steroid Profiles of a woman with recurrent granulosa cell tumour of ovary

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Granulosa cell tumour of the ovary (GCTs) is the most frequent sex cord stromal tumour and represents 5% of all primary ovarian cancers. Ovarian granulosa cell tumour is a malignant tumour with slow progression and in some case this tumour is hormonally active. The recurrence

of granulosa cell tumour often happens after 5 years. We present the case of a 54-year-old woman with recurrent granulosa cell tumour of ovary. Urinary steroid profiles of this woman were studied in the presence of ovarian granulosa cell tumour during a 5 years period. Eight samples were obtained before and after the operation, and also during and after the chemotherapy. 24-h urinary samples were examined and the urinary concentration of 23 androgen, progesterone and corticoid metabolites was quantitatively determined by gas chromatography-mass spectrometry with selected ion-monitoring. We could observed a correlation between the levels of the urinary steroids and the progression of the ovarian granulosa cell tumour.

The work was supported by ÁOK-KA-2015-16.

SE3.1

Abnormal tyrosine hydroxylation: possible diagnostic uses

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Hydroxyl radical converts Phe to para-, meta-, and ortho-Tyr (p-Tyr, m-Tyr, o-Tyr), while Phe is converted enzymatically to p-Tyr. Theoretically, the hydroxyl radical action may cause mishydroxylation in either the unincorporated Phe, or in the protein incorporated Phe molecules. Earlier, Wittmann and coworkers demonstrated that elevated m-Tyr and o-Tyr can contribute to erythropoietin resistance, decreased arterial wall relaxation, or may influence insulin resistance. In addition, altered m-Tyr and o-Tyr levels are potential markers upon oxidative stress conditions like septic shock.

As of now, we show that various organs contain significantly different m-Tyr and o-Tyr levels in rats, an observation which may lead to the development of a working animal model. Further interesting observation is that the m-Tyr and o-Tyr ratios are also different among various organs. The model, when consolidated may serve as a useful tool to test different conditions, which may affect Phe hydroxylation in an animal model.

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SE3.2

Laboratory possibility of molecular (component-based) allergy diagnostic

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In case of positive result of allergen specific IgE, further differentiation is required for diet definition, lifestyle change and therapy planning. A good and new opportunity for the differentiation is to divide the positive allergens into their molecular components, which helps to separate the original allergens from the cross-reactive allergens, the allergens that cause systemic or mild symptoms can be distinguished and the dietary requirements can be specified. In case of children the molecular test often provides the information whether the allergy can be outgrown or not. Since November 2015 in our laboratory the measurements of molecular components of the following allergens have been available: milk, egg, soy, peanut, nut, walnut, fruits (apple and peach), crab, birch pollen, late summer weed pollen (ragweed and mugwort), timothy grass, dust mites, bee and wasp. For these tests we use recombinant and highly purified native allergens. We have created a special request form for these tests, which helps to give a personal evaluation and it is attached to the laboratory results. This presentation makes detailed analyses of the connections and contradictions of the allergen specific IgE and component-based

measurements, as well as the diagnostic algorithm. The molecular-based allergen specific IgE test must always be the second level of the laboratory testing.

SE3.3

Importance of component-resolved diagnosis (CRD) in case of pollen and food allergy - Case reports

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Background: Regarding pollen and food allergy the traditional allergological diagnostics (skin prick test and/or specific IgE) is not capable of separating the primary or secondary components that are actually causing the allergy. The latter may also occur in other pollen plant types as cross-reactive components. Common cross-components (e.g. profilins, polcalcins, other molecules) occur in ragweed, mugwort and grasses, but they are also present in the birch pollen and in many other fruits, which can even trigger oral allergy syndrome in sensitive subjects. Peanut is the strongest food allergen, the allergic reactions range from oral allergy syndrome to life-threatening anaphylaxis. The main components (Ara h1, Ara h2, Ara h9) that are risk factors regarding anaphylaxis have to be distinguished from the side/secondary components (h5 Ara, Ara h8) that can only cause mild oral symptoms. The latter occur as cross-reactive components in plant pollens such as birch pollen or in fruits, e.g. in peach (Bet v1, Bet v2).

Aims: Using the advanced component-resolved diagnosis, which also can be carried out in our country, the real allergen can be identified from the possible CRD components and the children with a higher risk of future anaphylaxis can be found.

Patients and methods: Measurements were performed by the ImmunoCap-based Phadia 250 instrument in the Svábhegyi Medical Institute. Molecular diagnostics measures the IgE responses specific to the allergen component responses. We present two pollen allergic patients waiting for immunotherapy and two peanut allergic children.

Results: The clinical cases also showed the already existing results that the CRD can reliably isolate if the immune system of the patient waiting for immunotherapy shows allergic reaction to non-specific (side)-components occurring in the allergy-causing pollen or to the given pollen-specific major allergen. Some of the children showing high values of the Ara h2 allergen components were investigated due to mild localized allergic reaction. However due to their age their immune system has met only very small amounts of peanut.

Conclusion: The specifically planned component-based diagnostics based on a thorough medical history and detailed analysis of the symptoms is now an essential step in the preparation of decisions relating to immunotherapy recommended by many European professional guidelines. The component-based allergy test shall be performed for all children sensitized to peanuts, regardless of the severity of allergic symptoms seen so far, since this method provides a considerable aid in the suitable medical attendance (epinephrine auto-injector) of the affected patients.

SE3.4

Statistical analysis of HbA1c assay distribution in a large population: preliminary studies

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In 2015, a subcommittee including authors (1-3) was set up by the Hungarian Society of Laboratory Medicine to explore the extent of use of Hemoglobin A1c (HbA1c) assays in Hungary. In this study data from Pest county, Hungary were descriptively analyzed. Fully anonymized patient data of all HbA1c assays types and anti-diabetic drug treatment (ATCC a10*) counts (A10), with or without confirmed diabetes diagnosis (ICD10 E10-14) (Db), between April 2004-2014 were extracted from the National Health Care Database. Patient age, sex, birth and death dates were also collected. Results: 79.05% of the Db+ population (n=142,610) underwent one or more Hb assays during the total study period. The ratio of HbA1c assays/year/person was in a range of 1-20 (in 2004) that decreased gradually to 4 assays/year/person by 2012. While improving over the study period of 10 years, the percentage of diabetic patients diagnosed in 2004 and still alive in 2014, undergone at least the recommended 4 HbA1c assays/year is only 5.55% (n=22,636). The average number of HbA1c assays (HbA1c rate) was 1,25/year/person still alive in 2014 (SD: 0.847, n=20,753). Age group analysis revealed that HbA1c rate was the same in 82% of the patients aged 40-79 years. Younger patients showed higher while very old ones (age >80years) a decreased HbA1c assay rate. At non-diabetic patients (Db- A10-) a single HbA1c assay over the whole study period was performed in 63,7% of the cases (out of a total 165.077, 100%) as contrasted to 8,33% (out of 86,078, 100%) in the diagnosed (Db+) and/or treated (A10+) group. Our initial results on a large population clearly demonstrate that the level of glycaemic control in Db patients is suboptimal in the studied population. Epidemiological studies to assess comprehensive relationships among the different variables are in progress.

SE3.5

Acute alcohol poisonings in the clinical laboratory routine

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The measurement of ethyl alcohol is very important in every emergency clinical laboratory because it is the most frequent toxic agent in emergency medicine. The most precise method of its quantifying is gas-chromatography, however, in clinical laboratories the enzymatic (ADH) method is much more convenient. It is easy to adapt on any clinical chemistry equipment. We use the Roche ETOH test on Cobas Integra.

From year to year we do more and more alcohol measurements in our laboratory. The number has exceeded 2000 in 2015, with 1400 from providing positive results. The age of alcohol poisoned patients ranges 10 to 80 years. Most of them are male. Alcohol poisoning itself can be dangerous, but we have often found benzodiazepine, carbamazepine and other psychoactive legal or illegal drugs in addition to alcohol in the patient's fluids. These drugs, even in moderate concentration together with alcohol may endanger the patient's life. Ethyl alcohol can be used as antidote to ethylene glycol. In these cases, alcohol measurements can serve as therapeutic drug monitoring.

In our toxicology experience we often measured only high level of alcohol instead of medical or illegal drugs in patients. We must not forget that this agent is toxic, may cause respiratory failure, acts as a central nervous system depressant, impairs sensory and motor function, slows cognition, and causes amnesia, stupefaction, unconsciousness, and possible death.

SE3.6

UriSed mini – a new category in UriSed Technology

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Urinalysis is one of the most common and important tests for screening urinary tract and kidney diseases. The manual method of analysis, which is the gold standard, is poorly standardized, labor intensive, time-consuming and operator dependent. The patented UriSed Technology was developed to reduce the shortcomings of manual microscopy through automation. The UriSed Technology is the optimized automation of traditional manual microscopy using a special cuvette as the only consumable. Sample is filled into the disposable cuvette, a monolayer of particles is created at its bottom that is analyzed by a bright-field microscope. Multiple digital images are taken, that are evaluated and the results are calculated by a neural network based high-quality image processing software, the so called Automatic Image Evaluation Module (AIEM). Since 2007 thousands of UriSed Technology based automated urine microscopy analyzers have been installed in hospitals and large laboratories all over the world. As a result of continuous development, in 2016 a new instrument category was introduced: the semi-automated UriSed mini which is also based on UriSed Technology. The UriSed mini operates with the same measurement sequence and detects 16 sediment particles similarly to the automated UriSed devices. UriSed mini provides quantitative RBC and WBC results and semi-quantitative results for all other particle types (EPI, NEC, HYA, PAT, CRY, CaOxm, CaOxd, URI, TRI, BACr, BACc, WBCc, MUC, SPRM). An additional feature for experimental and educational purposes is the manual microscopy mode that gives the user the option to have a live microscopy view on the screen to see moving microorganisms. The UriSed mini is a highly effective tool for a wide range of medical and clinical practices. Due to its reduced size compared to an automated analyzer it is also suitable for experimental labs and for smaller laboratories with lower daily test rate.

SE4.1

Errors in the extra-analytical phase influence the interpretation of flow cytometry results of minimal residual disease assessment

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Minimal residual disease (MRD) is a powerful predictor of response to treatment and clinical outcome in different hematological malignancies. Flow cytometry is a time- and cost-effective method for studying MRD in those cases where leukemia associated immunophenotypes (LAIP) can be detected on de novo blasts. However, the interpretation of some MRD results may be challenging. We aimed to explore the sources of

preanalytical and postanalytical errors or variables that influence the interpretation. Altogether 323 bone marrow or peripheral blood samples were analysed in 2015 for MRD assessment with the acquisition of 300 000 events at different time-points of treatment. 54% of samples were obtained from patients with acute leukemia. While in childhood B- and T-cell acute lymphoblastic leukemia (ALL) well-defined time-points, standardized marker combinations and gating strategies help in the evaluation of MRD, it is extremely difficult to interpret MRD results in acute myeloid leukemia (AML). In ALL 80% of reports gave unequivocal MRD result, while rest of the samples were either assessed as 'non-adequate' because of peripheral blood contamination (5%) or showed high regenerating activity of the bone marrow (15%) without LAIP of the blasts making it difficult to distinguish them from normal hematogones. In 59% of AML samples unequivocal results were obtained; 5% were considered as 'non adequate' and in 36% myeloblast or monoblast ratio was interpreted with or without shifted immunophenotype of the blasts. In case of chronic lymphoid leukemia (CLL) MRD results were always definite. In myeloma multiplex (MM) ratio of myeloma cells in the total plasma cell (PC) pool was interpreted according to international recommendations. In 83% of the reports the interpretation of MRD status was straightforward. However, in 17% of samples CD19 negative atypical PC were detected in <0.1% without intracellular light chain restriction – this PC subpopulation can be detected in normal marrows as well. Demonstration of plasma cell clonality is important for diagnosis, but the relevance of clonality assessment is less clear in follow-up samples.

In conclusion, preanalytical error causing 'non adequate' sample was identified in 5% of acute leukemia cases resulting in no MRD interpretation. Difficulties in the postanalytical phase resulted in uncertain MRD results in 36%, 15%, 16% and 0% of AML, ALL, MM and CLL samples, respectively.

SE4.2

Harmonization of deep molecular monitoring of chronic myeloid leukemia in Hungary

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Detection of *BCR-ABL1* mRNA transcripts by reverse transcription quantitative polymerase chain reaction (RT-qPCR) has been proven to be a highly sensitive diagnostic method to measure leukemic-cell burden in chronic myeloid leukemia (CML). As a result of the international standardization project, laboratories express results on the international scale (IS). This standardization covered the range of 1-4 log reduction (10%-0.01% with detectable *BCR-ABL1*) compared to an "average" expression level detectable at diagnosis (IRIS trial standardized baseline). Recommendations for defining deep molecular responses (MR) with low or undetectable *BCR-ABL1* expression (0.01-0.0032%: MR⁴; 0.0032-0.001%: MR^{4.5}; ≤0.001%: MR⁵) were initiated in 2012. The Molecular Diagnostic Laboratory of HNBTS participated in six sample exchange programs with the European reference laboratory (Mannheim), in two external quality controls to harmonize MR^{4.5} assessment (Salisbury), in two trials for external reference materials and serves as the Hungarian MR^{4.5} reference laboratory in the EUREKA CML registry (Jena). The HNBTS performed standardization with the Hungarian laboratories twice, and a sample exchange process involving deep MR is ongoing. In 2015, the HNBTS laboratory performed routine *BCR-ABL1* RT-qPCR on 687 CML samples. Among 358 CML patients followed routinely in our laboratory, 256 (72%) reached MR³, and 160 MR⁴ (45%). Similar ratios were observed in all 5 laboratories in Hungary in 2014 (MR³: 76-85%, MR⁴: 44-61%). The frequency of deep MR observed in patients with at least 24 month tyrosine kinase treatment (enrolled in the EUREKA registry in Hungary) seems to be slightly higher (preliminary data). In summary, the harmonization of *BCR-ABL1* monitoring improves inter-laboratory comparability of results. The available external reference materials may replace sample exchanges. The large number of patients reaching deep MR prompts the application of deep MR monitoring guidelines. This work was undertaken in the frame of the European Treatment and Outcome Study (EUTOS) for CML international collaboration.

SE4.3

Diagnostic issues in Antithrombin, Protein C and Protein S deficiency in the Hungarian population; experience of a large thrombosis laboratory

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The molecular genetic background of antithrombin (AT), protein C (PC) and protein S (PS) deficiency is heterogeneous. The different laboratory tests have different sensitivity to these deficiencies and may suffer from interferences making laboratory diagnosis difficult.

Our aims were to describe the mutation spectrum of AT, PC and PS deficiencies, to determine the mutation detection rates and to evaluate the functional laboratory assays in a cross-sectional single center study.

Patients with AT, PC and PS deficiencies diagnosed by routine laboratory methods (Innovance AT, Protein C reagent coagulometric and Protein S Ac, Siemens) between 2007 and 2015 were registered. Sanger sequencing of *SERPINC1*, *PROC* and *PROS1* was executed and MLPA analysis was performed in sequencing negative cases. Factor V Leiden mutation (FVL) was also detected.

Out of the AT deficient (n=124) 92 carried the AT Budapest 3 (ATBp3). In addition 17 different causative mutations were registered. The anti-FXa based AT activity assay that we used, could detect all type II heparin binding site deficiency, like ATBp3 with high sensitivity. Among the 122 and 132 patients with decreased PC and PS activity in the clotting assays, a high number of FVL carriers (FVL+) were registered. The mutation detection rate for *PROC* was 66% in FVL- and 14% in FVL+ cases, while in the case of *PROS1* it was 41% in FVL- and 19% in FVL+ cases. Nineteen and 14 novel mutations were registered in *PROC* and *PROS1*, respectively and no founder mutation was detected. Type IIb PC deficiency with normal chromogenic PC activity was detected in 8.5%.

It can be concluded that the mutation detection rate is practically 100% in AT deficiency, where the majority of patients are carriers of the founder mutation, ATBp3. The mutation detection rate is lower in PC and PS deficiencies, especially in FVL positive cases. The high rate of mutation negative cases in FVL- suggests the presence of larger chromosome alterations or epistasis. Laboratory assays for diagnosis should be chosen carefully, taking population-specific considerations into account.

SE4.4

Rare Von Willebrand Disease subtypes

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Von Willebrand disease (VWD) is the most frequent inherited bleeding disorder caused by a dysfunction of primary hemostasis. Type 2 VWD includes a wide range of qualitative abnormalities of von Willebrand factor (VWF) structure and function resulting in different subtypes. Type 2B includes VWF variants with increased affinity for platelet glycoprotein Ib. Type 2N characterized by markedly decreased affinity for coagulation factor VIII (FVIII). The authors present two cases with these VWD subtypes. Patient1 is a 13-year old girl who had prolonged menstrual bleeding. The coagulation screening tests were normal, while the PFA-100 closure times were prolonged. Von Willebrand (VW) tests, platelet aggregation and secretion studies were performed. The only abnormality was enhanced ristocetin-induced platelet aggregation, which suggests to type 2B VWD. This diagnosis was confirmed with molecular genetic test, p.P1266L mutation was found in exon 28 of the VWF gene. Patient2 is 9 year old girl without bleeding symptoms, in her case hemostasis screening tests were carried out before tonsillectomy and prolonged APTT was detected due to low FVIII activity (22%). The VWF level was in the reference range, the ristocetin cofactor activity was borderline normal. The immeasurably low VWF-FVIII binding ELISA assay result was due to the presence of p.R854Q mutation in exon 20 of the VWF gene resulting in type 2N VWD. These cases draw attention to the phenomenon that despite of the normal first line VW diagnostic tests (immunological and functional assays of VWF) results the patient could suffer from rare VWD subtypes. Provident laboratory investigation is very important for the exact diagnosis and for appropriate treatment.

SE4.5

The effects of kisspeptin on the platelet function

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The role of platelets in diverse inflammatory and immunological processes including atherosclerosis is established. Diabetes and smoking are important etiological factors in the development of atherosclerosis. Kisspeptin (KP) plays an important role in the regulation of the neuroendocrine and cardiovascular systems, and, also, that of hemostasis. KP levels are increased in atherosclerotic vessels. Kisspeptin is able to prolong bleeding time and to decrease the number of platelets. The aim of our study was to examine if KP is able to influence platelet function.

We investigated the eicosanoid synthesis of Wistar-Kyoto rats and the aggregability of platelets in healthy and diabetic animals under the effects of various inducers (ADP, collagen, arachidonic acid and thrombin-receptor activator protein 6 [TRAP-6]) in the presence of KP-13 (1.25; 2.5; 5; 10x10⁻⁸ mol/L). Furthermore, we studied the platelet aggregation of healthy smoking and non-smoking male volunteers.

The amount of thromboxane B₂ derived from arachidonic acid marked by resting platelets was significantly increased by 35.6% in the presence of 2.5x10⁻⁸ mol/L KP-13. The amount of COX₁ as determined by ELISA was increased by 33%. 5x10⁻⁸ mol/L KP-13 enhanced the collagen-induced platelet aggregability from 56.9±3.27 to 69.73±3.3 U in healthy rats, and from 57.88±3.83 to 60.2±3.43 U in diabetic rats. The most significant aggregability of human platelets was observed in the presence of inducer TRAP-6 both in case of smoking (111.1±3.81 U) and in case of non-smoking men (107.1±3.69 U). 5x10⁻⁸ mol/L KP-13 decreased the platelet aggregability of non-smoking men by 14.1%, at the same time, 2.5x10⁻⁸ mol/L KP-13 increased the platelet aggregability of smoking men by 15%.

Our data demonstrates that KP is able to alter the functioning of both resting and activated platelets. In view of our results it can be stated that KP probably plays a role in physiological and pathological processes implicating platelets.

Ethical license No: 143/2015.

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SE4.6

New Oral Anticoagulants from the Clinical Laboratory Perspective

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New Oral Anticoagulants (NOACs) are changing the anticoagulation paradigm. In contrast to Vitamin K Antagonists (VKA) and heparin, NOACs target a single coagulation factor: Factor IIa (or thrombin) for dabigatran (PRADAXA®), or Factor Xa for rivaroxaban (XARELTO®), apixaban (ELIQUIS®), and edoxaban (LIXIANA® or SAVAYSA®).

Because of their pharmacological profile, they allow a simple dose regimen and do not require routine monitoring nor dose adjustment.

However, NOACs can interfere with routine and specialized coagulation assays used in the clinical laboratory. Physicians and laboratory staff must be aware of these possible interactions in order to avoid any misdiagnosis.

On the other hand, measurement of drug plasma concentration can be helpful in emergency situations such as bleeding, overdose or urgent surgery or invasive procedure.

This presentation will review the basics of NOACs pharmacological profile, how NOACs can interfere in coagulation assays and what are the existing solutions for measuring NOAC plasma concentration when needed.

SE5.1

Patient-centered laboratory medicine in everyday practice

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Laboratory medicine is used to support patient care. We studied the poster of patient-centered laboratory medicine published by IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) and tried to apply it for our everyday practice.

Five major ways have been identified as dominant causes diagnostic error: 1. Ordering the wrong test. 2. Not ordering the right test. 3. Misapplying the test result. 4. Missing the test result. 5. Test result inaccurate. We made efforts to improve these areas.

The best way to avoid incorrect ordering is to develop local or standard guidelines for the use of tests: our laboratory participated in the development of local clinical protocols (e.g. high-sensitive troponin I, D-dimer).

The misapplication of test results can stem from cognitive failures by the clinician or from failing to understand the limitations of the test methodology. In our lab performance limitations or interfering substances (e.g. hemolysis, lipemia) are measured and their effects are indicated on our laboratory report.

Delays in the total testing process may occur at the pre-analytical, analytical or post-analytical stage. To minimize pre-analytical errors there is regular phlebotomy training in our hospital. We also monitor 38 quality indicators to test the quality of the pre-analytical phase.

The result of an appropriately ordered test can be inaccurate due to analytical or non-analytical issues. To achieve accurate analysis we use third party controls for statistical quality control (SQC) and participate in international external quality assessment program (EQAS). SQC and EQAS results are analyzed and corrective action is done if required.

Further decrease in test-related errors can be achieved only by collaborating with clinicians.

SE5.2

How to organize workflow in a large regional laboratory?

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A considerable proportion of medical decisions is based on laboratory results, thus clinical laboratories should meet the increasing demand of clinicians and patients. Huge central laboratories may process over 10 million tests annually. These act as 'result production factories' as

millions of emergency and routine tests are measured with a required speed and accuracy. At the same time, these laboratories also serve as specialized diagnostic centers where well-trained experts construct esoteric tests and interpret special test results. It is essential to improve and constantly monitor this complex laboratory service by several methods.

Sample transport by pneumatic tube system, use of an advanced laboratory information system and point-of-care testing would improve turnaround times. The optimization of test ordering may result in a faster and more cost-effective laboratory service. We present our experience on how autovalidation that was introduced 10 years ago in our Department saved time for the analysis of more complex results that require significantly more attention. We created small teams of experts (3-5 members each) responsible for special diagnostic work, and their interpretative reporting according to predetermined principles, that helps to minimize subjectivity of special reports in flow cytometry, autoimmunity, endocrine studies, cytogenetics, molecular testing and special hemostasis tests.

Although laboratory investigations have become diversely developed in the past decades, it is essential that the laboratory should be able to provide accurate results relatively quickly, and that laboratory specialists can support the diagnosis and monitoring of patients by adequate and thoughtful interpretation of special laboratory results.

SE5.3

Patient-risk based guideline on management of critical-risk results.

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Management of critical-risk results (CRR) -laboratory results that require immediate medical attention and action because they indicate a high risk of imminent death or major patient harm- is considered as a significant factor affecting patient safety. The Task and Finish Group on Critical Results collected information on the practice of European -including Hungarian- laboratories in CRR management and analysed their alert lists (ALs) some years ago. This presentation will discuss the observed CRR management practices in the light of the recommendations of the recently published standard CLSI-GP47.

Wide variations were seen between laboratories in those aspects how they developed their ALs as well as which tests were selected for inclusion in ALs. The most important unconformities in designation of ALs were that often thresholds were not set on broad consensus in institutions and that targeted timeframes of reporting were indicated rarely. Wide variations were also observed in the reporting process of CRRs among laboratories. Laboratory policies were not always patient-risk focused. Only a third of the responding laboratories established practices for proper documentation and the same proportion of laboratories monitored the performance of their CRR notifications.

There is a definite need for adaptation of CLSI-GP47 guideline in European laboratories. The Hungarian Society of Laboratory Medicine is actively working on adaptation then implementation of CLSI-GP47 standard in Hungarian healthcare setting. Hopefully, these efforts may convert the existing heterogeneous, often ineffective CRR practice into a patient-risk based CRR management that can contribute to better patient safety.

SE5.4

Difficulties of establishing cut off values and reference intervals for specific IgG levels induced by vaccination.

Special case – general problems.

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Investigation of specific IgG levels has an emerging importance in the care of immunocompromised patients. Measurement of specific IgG values before and after immunization is an accepted technique to evaluate humoral immune function and a useful diagnostic tool for the investigation of patients with immunodeficiency. Furthermore it is important to know the protective titres (cut off values) that ensure safe immunity of the patients. There is no international consensus about cut off levels and reference ranges of Diphtheria toxoid (Diph), Tetanus toxoid (Tet), Pneumococcus (PCP)-, and Haemophilus influenzae type b (Hib)-specific IgG levels. Our aims were to establish reference ranges specific for Hungarian population and refine cut off values. For this purpose the levels of IgG specific for a different pathogens were determined from sera of 144 healthy individuals (55 adults, 99 children) by ELISA method (The Binding Site).

Statistical analysis strongly supported by wide range of clinical experience was essential to interpret the data and to establish age-related characteristic reference range for Hungarian population. Our work contributed to refine cut off values for Diph-, Tet-, PCP-, Hib-IgG levels.

Our suggestion is to use „decision-making intervals” instead of single cut off value for the judgement of protection and to decide whether booster doses are necessary. As general principle this approach could guide the establishment of target ranges for other laboratory parameters. Collaboration with clinical experts is essential in this work.

SE5.5

True or false? - Spurious counts and results from the daily hematology practice

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Modern hematology analyzers are designed to provide quick and accurate blood count results in most of specimens. However, in certain cases spurious results are observed. These may be related to several situations which led to the alteration of one or more cell blood count parameter. For the identification of these erroneous results hematology analyzers generate flags or peculiar scattergrams/histograms in most of such cases. By presenting clinical cases from our daily practice using Sysmex XE-2100 hematology analyzer we would like to highlight the importance of correct interpretation of blood count results. In the first case an abnormal white blood cell (WBC) differential scattergram associated with WBC flags, no automated cell differential and a significant difference between WBC-baso and WBC-diff counts indicated the microscopic evaluation of peripheral blood smear led to the final diagnosis: Plasmodium vivax parasitemia. In the second case the evaluation of the blood collected from a patient suffering from B-cell chronic lymphocytic leukemia resulted in an extremely high WBC count with a characteristic lymphocyte morphology together caused the appearance of erroneous red blood cell (RBC) count and red cell indices associated with an abnormal RBC histogram and RBC flags. These data clearly present that beside numerical data review, the correct interpretation of blood count results must include a careful study of the scattergrams/histograms, flags and if it is necessary a peripheral blood smear evaluation.

SE5.6

Hemolysis index for chemistry analytes on Architect analyzer: determination of different interference limits

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Background: Hemolysis is the most frequent pre-analytical error in our diagnostic laboratory. In spite of the regular training of phlebotomists the incidence of hemolytic samples increased dramatically during last year. To detect hemolysis visual inspection and automated hemolysis index (HI) were applied simultaneously for one year. Methods: Visually reported hemolysis was recorded by laboratory staff. Automated monitoring of HI on every tested specimen was performed on Abbott Architect C8000 analyzer. Hemolysis interference was evaluated for 21 analytes. For each parameter, the HI corresponding to the $\pm 10\%$ change of concentrations from baseline ($\pm 10\% \Delta$) was determined, as well as to the analytical change limit (ACL) and to the reference change value (RCV). Results: The HI estimation was twice as high as the visual scrutiny (10% vs 5%). HI was expressed in ordinal values and the following distribution was received: 74% of the hemolysed samples was +1, 17% was +2, 8% was +3, 1% was +4. Considering the ACL, 17 analytes concentrations were affected by hemolysis. Considering the $\pm 10\% \Delta$, 14 parameters were affected whereas only 10 analytes remained sensitive to hemolysis when considering RCV. Conclusions: By automated estimation of HI we can detect systematically and quantify reliably hemolysis in every sample. The ACL based HI is used as flag cut-off for comment to clinicians such as "Result changed by hemolysis interference" along with the reported result. RCV based HI is retained as decision cut-off. The result affected seriously by hemolysis is not reported; instead, the comment "Hemolyzed specimen" is presented on the report.

SE6.1

Concomitant use of conventional and molecular cytogenetic methods for detection of chromosome aberrations in rare congenital diseases

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Major congenital anomalies are detectable in 2-3 % of the newborn population. Their genetic causes are attributable to numerical or structural chromosome aberrations, submicroscopic copy number variations (CNV). The classical chromosome analysis together with molecular cytogenetic methods are widely used as diagnostic tests for fast and accurate detection of chromosomal abnormalities in patients with developmental delay/intellectual disability and multiple congenital anomalies (MCA). We present our experience with concomitant application of conventional cytogenetic method, fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (CGH) for postnatal diagnosis in rare congenital disorders. G-banded chromosome analysis and/or FISH was performed on cultured peripheral blood

lymphocytes, fibroblasts and buccal cells. Array CGH was carried out on genomic DNA using CytoScan 750K array platform (Affymetrix). Genetic diagnostic workup of four cases demonstrates the efficient use of different cytogenetic methods in revealing the genetic background of the underlying syndromes. One patient had CNV associated with 3q29 microduplication syndrome. One case presented rare genomic aberrations: unbalanced subtelomeric translocation t(8;18)(p23.3;q22.3) causing partial deletion of 18q22.3 and duplication of 8p23.3. Another case showed a rare variant of Cat eye syndrome with ring chromosome 5. The fourth patient had Pallister-Killian syndrome caused by tissue specific 12p tetrasomy in mosaic form. The completion of the diagnostic procedure in medical genetics by different kind of molecular cytogenetic techniques promotes the clarification of the diagnosis for patients with rare disorders and provide information about potential reproductive or future health risks.

SE6.2

New aspects of alkaptonuria

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Alkaptonuria (AKU) is an inborn error of tyrosine metabolism. Deficiency of homogentisate 1,2-dioxygenase results in high level of homogentisic acid (HGA), which results in pigment deposits in connective tissues (ochronosis). In adults it may lead to osteoarthritis, renal stone and rigid heart vessels. Multieffective redox property of HGA with human organs has been studied recently and the results raised new aspects of AKU. In these days HGA is not considered as a harmless metabolite any more. Until the last decade for the treatment of AKU dietary protein restriction and high-dose vitamin-C treatment were recommended. As the effect of long-term medication is uncertain and dietary compliance decreases with age, we monitored urinary HGA. Urinary HGA was determined with Nylander-reagent. The colorimetric assay (5 min, 575 nm, 2,5-dihydroxyphenylacetic acid calibrator, Sigma) correlates well with GC method. To compensate for diurnal variation, urinary HGA was related to creatinine. Reference range of HGA/creatinine was also determined in 84 healthy children; it is decreasing with age. Patient-1: we introduced determination of HGA 18 years ago for a baby girl who was born with AKU. Her daily HGA excretion has been checked once a year. Urinary HGA gradually increased from 350 to 840 mg/mmol in the 3rd year. Since she was treated with vitamin-C (1g/day), the urinary HGA stayed at the initial 300-400 mg/mmol (reference range <5 mg/mmol). Her clinical condition was satisfactory, adequate to the early period of AKU. Patient-2: a 44 years old AKU patient, recently checked with heavy rheumatic complain in his hip. After 3 months of nitisinone therapy urinary HGA decreased from 408→40→15 mg/mmol, without any adverse effect. Originally nitisinone was applied in tyrosinemia, to inhibit the formation of HGA from 4-hydroxyphenylpyruvic acid. Though the drug reduces formation of benzoquinon polymer, recent studies proved ~10x accumulation of tyrosine after a 3 months treatment. Therefore restriction of protein/tyrosine intake and checking of liver function (or tyrosine) should be considered at long run treatment. Monitoring of HGA helps to evaluate metabolic condition and the effect of treatment. Nitisinone seems to be a promising new orphan drug.

SE6.3

Serendipity: exponential increase of carcinoembryonic antigen in an asymptomatic patient

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A 51-year-old man without any complain went to prostate cancer screening. The prostate specific antigen (PSA) was supplemented with carcinoembryonic antigen (CEA) without any further reason. Laboratory testing revealed a CEA concentration of 10,7 µg/L [reference interval (RI): <5 µg/L]. The parameter was repeated two consecutive years with the following results: CEA 14,4 µg/L and 21,4 µg/L. The other laboratory parameters (containing a series of other tumor markers) were in RI. At this time the patient had a clinical investigation of tumor searching, but it ended unsuccessfully. In the next two years the CEA measurement resulted in 56,2 µg/L and 105,4 µg/L. The patient had no complaints and was asymptomatic during the four year period. Prior to a new series of imaging studies for tumor searching we tried to analyse the observed results. There was an isolated high CEA level. Pre-analytical and analytical error could have been excluded because of the exponential increase of CEA. The elevation of CEA was clinically significant according to reference change value (RCV). Benign diseases could be ruled out because of the high value. The most common tumors causing CEA elevation were excluded during previous tumor searching. The main question was what type of tumor can cause CEA elevation which was not examined earlier. According to the literature medullar thyroid carcinoma (MTC) might cause CEA elevation that was not investigated before. A calcitonin (Ctn) test was ordered and its level was 2030 pg/mL (RI:<10 pg/mL). The suspected diagnosis was confirmed by preoperative examinations. The patient was treated by total thyroidectomy. The MTC proved to be sporadic: RET mutation was not found. Ctn and CEA levels still not have doubled 4 years after surgery.

SE6.4

Diagnostic dilemmas in routine prenatal cytogenetics

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Classical laboratory protocols in prenatal cytogenetic diagnosis were established during the 80s. The utilization of amniotic fluid, chorionic villi, or occasionally chord blood to gain chromosome preparations with long term culture, and the evaluation of spontaneous mitoses of the placenta revolutionized the routine work. In the 90s, the next crucial milestone was the introduction of PCR- and in situ hybridization-based techniques, together with digital imaging software for chromosome analysis. Due to the progression of molecular genetic and biochemical methods in the 2000s, a new strategy in prenatal care emerged by bringing non-invasive screening into prominence. One of the aim of our presentation is to discuss questionable patient management based on some of our problematic cases. On the basis of these examinations we emphasise the advantage of invasive diagnosis over non-invasive screening, as it yields more reliable and detailed information on the genetic constellation of the fetus. We also show cases when the precise cytogenetic diagnosis could not be determined without the inevitable molecular cytogenetic techniques.

SE7.1

Twentieth birthday of the “Society of the Hungarian Medical Laboratory Professional Staff”-From establishment to present days

P. Gáborné Vörös
President of the Society

SE7.2

The effect of use of HeartMate II left ventricular assist device and pump thrombosis on laboratory parameters

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HeartMate II is a second generation left ventricular assist device designed for long term use, providing continuous blood flow for patients. Clinically significant hemolysis may occur in such patients as a consequence of turbulent blood flow in case of device (pump) thrombosis. Therefore, following implantation regular laboratory control is required including routine clinical chemistry, hematology and hemostasis. Any increase of free hemoglobin indicates intravascular hemolysis i.e. pump thrombosis. The aim of this study was to follow these parameters in patients living with left ventricular assist device. Between 2012 and 2014, 11 implantation of HeartMate II occurred in our Department. The patients were monitored by the following parameters: plasma D-dimer, LDH, free hemoglobin and haptoglobin. D-dimer was measured by Stago Compact, LDH and haptoglobin were determined by Cobas Integra 400 plus analyzer whereas plasma hemoglobin was measured by direct spectrophotometry. Among 11 patients device thrombosis occurred in 3 cases. In these patients elevations in LDH activity (2000-7400 U/L) were detected. In addition, a decrease in plasma haptoglobin and an increase in plasma hemoglobin and D-dimer concentration were noted. By comparison, in patients without device thrombosis the LDH activity ranged between 430-1071 U/L and the haptoglobin and hemoglobin levels were within the reference range. However D-dimer was also elevated in these patients. On the basis of our results it can be concluded that regular determination of LDH activity, plasma haptoglobin and hemoglobin concentrations could be used to detect device (pump) induced thrombosis in patients living with left ventricular assist device.

SE7.3

The role of calprotectin and other biomarkers in differential diagnosis of inflammatory bowel disease

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The biomarkers of gastrointestinal diseases help to establish the diagnosis with high specificity and sensitivity or serve as additional tests in specifying certain pathological conditions. Fecal calprotectin and matrix metalloproteinase-9 (MMP-9) are novel markers in inflammatory

bowel diseases (IBD), while other tests such as serum ANCA (anti-neutrophil cytoplasmic antibody) and ASCA (anti-Saccharomyces cerevisiae antibody) are useful for further refining the diagnosis. The aim of our study was to compare two different fecal calprotectin quick tests with respect to discriminating between different types of IBD. The relationship between calprotectin, ANCA, ASCA and MMP-9 in IBD was also investigated. 40 patients were tested for this study. 26 patients have been diagnosed with IBD: 5 with colitis ulcerosa and 21 with Crohn's disease. 14 patients suffered from other gastrointestinal diseases such as dysbacteriosis, colon polypus etc. An immunochromatographic method was compared to lateral flow assay of calprotectin extracted from feces. Fecal MMP-9, serum ANCA and ASCA were measured by ELISA. MMP-9 was able to discriminate between IBD and non-IBD diseases especially in case of colitis ulcerosa. The results of two calprotectin tests were similar with slight differences due to different cut-off values, especially in the "grey" zone. The negative calprotectin tests were fully reflected in MMP-9 values whereas from 14 calprotectin-positive tests only 8 were MMP-9 positive. Serum ANCA and ASCA were less specific in IBD patients but both can give additional information on the possible autoimmune origin of the disease. These biomarkers in feces offer new opportunities to find patients suffering from IBD at the early stage of disease. The real usefulness of these tests in clinical practice requires further evaluation.

SE7.4

Drug level monitoring in clinical toxicology

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In our institute we perform clinical and forensic toxicology testing since 2000. Usually, clinical toxicology means qualitative analysis, but if it is necessary we can use semiquantitative or quantitative methods to measure the concentration of drugs in sera. The exact concentration of any kind of drugs may be important in toxic cases.

The Shimadzu Prominence TOX.I.S II HPLC-DAD equipment installed at the institute is developed for the identification of many different drugs, but with proper calibration it could be used to evaluate the concentration of rare drugs and drug agents. In practice up to now we could track the alkaline and neutral drugs (e.g.: benzodiazepines, antipsychotics, antiepileptic drugs, beta blockers). The poisoning with these chemicals are relatively frequent, however, there are cases when we miss another method for acidic drugs (particularly barbiturates, acetaminophen, NSAIDs, ACE blockers etc.). With the system upgrade performed this year it became possible for us to determine the acidic drugs from sera as well.

Therefore, we are able to get a comprehensive and complex picture of the extent of the poisoning, which can support the work of the clinicians. Moreover now we have a broader picture about the drugs used by suicide patient.

SE8.1

Quality assurance and external quality assessment: a necessary evil or a useful tool in the hands of laboratory management?

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Each organization implements quality management system (hereinafter QMS) for various reasons, but basically they can be classified into two main categories: (1) results are expected from it or (2) a wish for formal recognition.

Establishing the QMS sometimes makes the routine operation more difficult and requires several various resources, but the properly functioning system, in which external quality assessment (hereinafter EQA) has an outstanding importance, might provide several benefits, and serve unique source of information which can not be ensured from any other way.

In Hungary law prescribes the operation of QMS for health service providers, and also the participation in EQA schemes for in vitro medical diagnostic laboratories. During the 20 years of its operation QualiCont went through significant development, which can be characterized with series of quantitative and qualitative changes.

In this presentation examples will be shown based on the long-term analysis data deriving from its specific EQA schemes, which may confirm the practical use of EQA and can be proved that it provides objective evidence of laboratory competence for the users of their service, the accreditation bodies and other stakeholders. In health care the ultimate aim of quality assurance is the continuous ensuring and improvement of patient safety, in which EQA has an outstanding role: opportunity and tool in maintaining and developing the quality and reliability of laboratory work and in the continuous training of laboratory staff. The further development possibilities and challenges of EQA can be deduced from the claim of quality control covering the Total Testing Process, the continuously developing technical opportunities and the changes in patient care.

SE8.2

Improving patient safety: How to manage the laboratory results interfered by hemolysis?

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In vitro hemolysis as a frequent pre-analytical error highly impacts laboratory results and consequential patient safety. The use of automatic hemolysis estimation (HI) - together with lipemic and icteric indices - overcomes the limitations of visual inspection by providing more objective data. Although lots of authors described the expectations towards the manufacturers and demanded harmonization regarding of hemolysis, in the meantime laboratories should take the responsibility to manage their hemolytic specimen and develop objective rules of sample rejection. Our laboratory also introduced HI in the routine workflow on two chemistry platforms AU Beckman and Modular Roche. To verify HI measurement we 1. made comparison studies of automatic HI with the hemoglobin (Hb) reference method; 2. determined biases due to hemolysis by spiking of samples with serial Hb dilutions in 22 analytes; 3. set up test rejection cutoffs. Our results demonstrated that the detection limits were 0.5 and 0.05 g/l on AU and Modular respectively. We found that the limit of AU was not sensitive enough to detect significant interferences for some tests e.g. lactate dehydrogenase, potassium and direct bilirubin. In these cases the lowest detection HI limit (0.5g/l) was considered as cutoff value. At the setting up the cutoffs of the remaining analytes we adopted the IFCC working group recommendations and a bias was considered significant if it exceeded more than 50% of desirable total error based on the biological variations of an analyte. Conclusion: Only consistent rules based on harmonization and/or home settings prevent producing unreliable laboratory results that can lead diagnostic errors. These rules can convince the physicians about the importance of the second specimen sending in case of significant hemolysis as well.

SE8.3

Quality Assessment of Preanalytical Phase: What is the Situation in Hungary?

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Quality assurance (QA) is much less developed for preanalytical than for analytical processes, despite the fact that ISO 15189:2012 standard (Medical laboratories – Requirements for quality and competence) expresses the need for definition of QA for all laboratory processes, including pre-examination phase, too. Although the number of published QA literature dealing with preanalytical phase increased, there is still no consensus on quality indicators to be used in general for its assessment. An IFCC working group model recommends 34 quality indicators (QI) for the preanalytical phase from the total number of 56 QI. At the QualiCont Forum 2016 two questionnaires were launched to get information about current practices for documentation and handling of preanalytical errors in Hungarian laboratories and to estimate the demands of the laboratories for the ongoing design of a preanalytical survey this year. 100 completed questionnaires of the handling of haemolysis and 152 items regarding general preanalytical issues were evaluated. In the majority of the laboratories the documentation of preanalytical errors is regulated, while still several factors affecting sample quality (e.g. haemolysis: 93.8%, clotting: 86.9%), quantity and handling of sample data should be identified as possible sources of errors. The evaluation of answers draws attention to the need of improvement in detection and treatment of errors. This lecture will present the results of the collected data in detail along with a review of available literature, guidelines, and standard regulations for the preanalytical phase to support laboratories during preparing for accreditation.

SE8.4

Mixing by a vortex to obtain correct platelet count result in the case of EDTA-dependent platelet clumping

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EDTA-dependent clumping is the most common cause of falsely low platelet counts by automated hematology analyzers. In such cases a good laboratory practice is to analyze a new specimen from citrated blood. Our aim was to examine an alternative approach to obtain reliable automated platelet counts from the original, clumped samples after mixing them by vortex and rerunning the samples.

We used 93 EDTA-anticoagulated, routine blood specimens (from ambulatory and hospitalized patients) after having been analyzed by CELL-DYN Sapphire (Abbott) hematology analyzer, and judged by the Mono Poly I cytogram as EDTA-dependent clumping. We prepared MGG stained smears to confirm the platelet aggregates. These specimens were subjected to a vortex for 1.5 minutes at the maximum speed and repeated analysis. We prepared another MGG smear to confirm the disaggregation. This procedure gave complete platelet clump

disaggregation in 61% (n=57) and partial disaggregation in 33% (n=31) of specimens. The platelet clumps were dissociated in the complete and partial group with a median increased platelet count of 41% (range: 1.8-927.9%) and 120.8% (range: 21.6-746.6%), respectively. There was no significant change in WBC count and RDW. Mixing caused a small but statistically significant decrease in the White Blood Cell Viability Fraction parameter, showing minimal mechanic effect on WBC population without significant change in cell morphology. This method can be applied as the first step in the case of EDTA-dependent pseudothrombocytopenia and EDTA-dependent clumping (without thrombocytopenia). There is just a minimal negative effect on the WBC morphology.

Gulati GL, Asselta A, Chen C. Lab Med 1997; 28: 665-7

SE8.5

Managing quality improvement in the Hungarian medical laboratories

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In Hungary, the medical laboratory services (total number of laboratories are between 280 and 315 in last years) are integrated with the 3-tier public health care system: at the outpatient clinics, hospital and clinical laboratories, and to provide services for complex and special tests. The private sector affords laboratory support at all levels of health care both in rural and urban areas. Each medical laboratory should identify the scope, functions and the capacity of the services offered by it and appropriate infrastructure with requisite biosafety measures should be planned. Qualified and trained staff should be employed with periodic up-gradation of their skills. No concern is of greater importance in laboratory medicine than quality assurance (QA). Managing the quality of Hungarian medical laboratories engages oneself defining what you want in terms of quality goals, requirements and performance specification, measuring what you get in terms of performance characteristics, analyzing whether it is fit for its intended purpose. Furthermore, it also involves improving the process, if necessary, and then controlling and monitoring the process in a manner that ensures that it continues to be fit for purpose by establishing performance indicators and operating specifications.

Classical external quality assessment (EQA) is well established in laboratory medicine, however, the need for quality improvement services recently emerged, among others to empower Hungarian clinical laboratories for future tasks, e.g., contribution to the development and implementation of global health-care policies. In Hungary, successful participation in EQA schemes (minimum 4-times/year, at least 80% of accepted results) is a pre-requisite for clinical laboratory services for obtaining license.

SE9.1

Prevalence of gastrointestinal disease and clinical features of *Clostridium difficile*-associated infections

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The epidemiology of *Clostridium difficile* infections (CDIs) has changed dramatically worldwide during the last ten years. Infection rates have increased markedly in most countries with detailed surveillance data. There have been clear changes in clinical presentation, response to treatment and outcome of CDIs. These changes have been driven to a major degree by the emergence and epidemic spread of a novel strain, known as PCR ribotype 027 (BI/NAP1/027). Community-acquired CDI (CA-CDIs) has also emerged, although the evidence for this as a distinct new entity is less clear. There are new data on the aetiology and potential risk factors for CDI; controversial issues include specific antimicrobial agents, gastric acid suppressants, potential animal and food sources of *C. difficile*. Recent spread of severe cases of *C. difficile*-associated diarrhoea (CDAD) reported in different parts of Hungary has emphasized an ongoing epidemiological surveillance of this disease. Recent reports have documented that *C. difficile* infections are occurring among patients without traditional risk factors. Objectives of this presentation are to show diagnostic algorithms, different diagnostic opportunities and to determine the epidemiology of CDAD according to the available data in Hungary. We would like to compare these data with our local data by estimating the incidence of CA-CDIs and hospital-acquired CDIs (HA-CDIs), and identifying patient-related risk factors.

SE9.2

Investigation of antibiotic susceptibility data of Hungarian clinically relevant *Bacteroides* isolates

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In the human clinical samples the most commonly isolated anaerobe genus is the *Bacteroides*. This genus is the most resistant one among the anaerobic bacteria, and there is a threatening antibiotic resistance rate according to the international literature. Because of the lack of

Hungarian multicenter study of antibiotic susceptibility of *Bacteroides*, our aim was to check these data of 300 clinical isolates of 3 Hungarian clinical microbiology centers. The collected isolates were stored at -70 C°, cultured on solid anaerobic blood agar plate among anaerobic conditions, identified with a special, mass spectrometry-based identification (Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry, MALDI TOF Biotyper, Bruker Ltd., Germany). The antibiotic susceptibility tests were performed with agar dilution method by the guideline of CLSI (Clinical and Laboratory Standards Institute, USA) for 10 antibiotics. After 48 h incubation at anaerobic conditions we read the MIC (Minimal Inhibitory Concentration) values of each isolate. Based on the breakpoints of the guidelines of international organizations of CLSI and EUCAST (European Committee on Antibiotic Susceptibility Testing) we evaluated the results by categories of susceptible, moderately susceptible and resistant. As controls we used *B. fragilis* ATCC 25285 és a *B. thetaiotaomicron* ATCC 29741. In the case of ampicillin, moxifloxacin, clindamycin and tetracyclin we found high resistant rates, like in the international literature, but the rate of meropenem resistant isolates is threatening. Tigecyclin, metronidazole and chloramphenicol remained the most effective antibiotics.

SE9.3

Possibility of rapid diagnosis of mycobacterial infections directly from clinical specimens

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Species level identification of mycobacteria based on phenotypic and biochemical characteristics is labor-intensive and time consuming. Hence, laboratories try to apply quick methods e.g. HPLC, PCR or commercially available kits. On the basis of the above-mentioned problems, our aim was to apply an earlier described PCR method for the direct detection of NTM (non-tuberculous mycobacteria) from human clinical samples. Modified *hsp65* nested-PCR described by Bascunana and Belák was used for direct amplification of mycobacteria from various types of clinical samples after DNA isolation with QIAamp DNA mini kit (Qiagen). PCR-RFLPs or sequencing were performed for identification. More cases were found with the help of this method. The first case was a 61-year-old woman with a cutaneous infection caused by *M. chelonae* in a patient treated with long-term methylprednisolone for rheumatoid arthritis. The second case was a 17-month-old child with a history of enlarged axillary lymph node after BCG (Bacillus Calmette-Guerin) vaccination. It revealed that it was a *M. bovis* infection derived from the BCG vaccination. The third case was a *M. terrae* complex infection of the gluteal region of a 32-year-old man with a medical history of hydradenitis and TNF inhibitor treatment. Setting up *hsp65* nested-PCR for identification of mycobacteria was successful. Despite of high sensitivity of this method, additional sequences and RFLP patterns are inevitable, because a wide database is required for the precise application. The above described cases justify the necessity of a quicker and accurate method for the species level identification of mycobacteria.

SE9.4

Screening for cytomegalovirus infection in pregnancy, early findings of a single-center prospective survey

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Cytomegalovirus (CMV) is one of the most important causes of congenital infection. The number of children with congenital CMV is similar to other conditions such as Down syndrome, however the community awareness in case of CMV is sparse. In the majority of countries, routine universal cytomegalovirus screening is not recommended during pregnancy, mainly because of the costs of investigations. While in some countries, universal screening is used to identify seropositive women who are not at risk of primary CMV and to identify seronegative women who are susceptible to CMV infection. Beside the universal screening, screening can be used in those women who are at increased risk for CMV infection or in some studies, 'once off' serology with IgG avidity testing is used at around 20 weeks to identify primary infections occurred early in pregnancy. Another possibility is to use ultrasound for the identification of malformations associated with congenital CMV infection.

Our aims were to use universal CMV screening till 16 weeks, to get valuable information on CMV epidemiology, to follow seronegative women during the pregnancy, to give hygiene advice to seronegative women, to determine the willingness of pregnant women to participate in this survey, and to educate healthcare providers about the importance and the correct diagnostic algorithm of CMV infection.

About 700 pregnant women were planned to test for the presence of CMV specific IgM and IgG using Cytomegalovirus ELISA IgM capture (Vircell, Spain) and Cytomegalovirus ELISA IgG (Vircell, Spain). CMV screening is offered for pregnant women participating in routine investigations in outpatient departments of Obstetrics and Gynecology. In case of negative serology, routine screening during the pregnancy is recommended. Epidemiological data (age, number of pregnancy, number of children, marital status, knowledge about CMV etc.) are collected from all patients.

SE9.5

Characteristics of *Pasteurella* spp. infections between 2002 and 2015, a single-center survey

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Background: *Pasteurella* spp. are one of the most important pathogens which can be associated with acute or chronic infections in animals and in humans. Our aims were to retrospectively summarize the prevalence of infections caused by *Pasteurella* spp. in a local University Hospital, between 2002 and 2015.

Materials/methods: Clinical specimens from inpatients and outpatients were collected in local, university hospitals in Szeged. Specimens were cultured simultaneously for aerobic, anaerobic bacteria and fungi. Species level identification was carried out using VITEK 1 and 2 (bio-Mérieux, Marcy l'Etoile, France) and after 2012 using MALDI-TOF (Bruker Corporation). Antibiotic susceptibility testing and interpretation of the results were performed according to CLSI or EUCAST recommendations.

Results: Between 2002 and 2015, 205 *Pasteurella* spp. were isolated from human clinical specimens. These samples were collected from 162 patients. The majority of specimens were collected from various wounds and abscesses (150 specimens), and 21 specimens came from the respiratory tract (nasopharyngeal swabs or endotracheal aspirates). 4 specimens from the ear and also 4 conjunctival specimens proved to be positive for *Pasteurella* spp. Interestingly, other specimens such as, maxillar specimens, CSF, abdominal abscesses, dialysis catheter were also positive for *Pasteurella* spp. In the majority of samples, *P. multocida* (141) were isolated, while in smaller number, *P. canis* (36), *P. pneumotropica* (10), *P. dogmatis* (2), *P. stomatis* (1) and *P. aerogenes* (1) were identified. In case of 14 strains, only genus level identification could be achieved. From year to year, the number of identified *Pasteurella* spp. is getting higher and higher.

Conclusion: It is well known that the popularity of cats, dogs and exotic animals is increasing thus we have to face with increasing tendency in the number of zoonoses. In addition to this, because of the high number of immunosuppressed patients, these infections is getting more and more severe, or manifests as unusual presentations. These can be represented in the epidemiology of infections associated with *Pasteurella* spp.

SE9.6

Controversial results of HBV diagnosis in Hungary 2015

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Background: Separation of acute and chronic HBV infection is well distinct according to the diagnostic algorithm. Nevertheless, more and more often we meet diagnostic results, which deter a clear diagnosis. The diagnosis of occult HBV includes high HBsAg titers with no detectable HBV DNA, detectable high aHBs antibody and HBsAg titers at the same time.

Materials/methods: 1./ In the case of occult HBV infection (only aHBc antibody detectable) HBV viral load detection is recommended. 2./ The investigation protocol for the chronic HBV infected patients prescribe to determine the HBV Viral load, but not the HBsAg titer. 3./ Many patients had HBsAg positive, aHBc antibody positive and inexplicable aHBs antibody positive results at the same time.

Results: 1./ In the case of occult HBV infection the range of detected HBV VL was from targeted low to 9 million IU/ml 2./ Patients without measurable HBV DNA had variable HBsAg titer results : <5 IU/ml to >1000IU/ml, 3./ 14 patients had aHBs titer between 20-100 IU/ml, parallel high aHBc and HBsAg and HBV DNA Viral load titer.

Conclusion: The diagnosis of HBV is the most complex. It seems that behind the strange, uninterpretable results - mutations, escape mutations, and new genotypes appearance – could be assumed.

SE10.1

Consultation in laboratory medicine

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Laboratory medicine is a medical discipline evolving rapidly and playing a more and more important role in modern healthcare. Medically reasonable, accurate and precise measurements are essential for diagnosis, risk assessment, treatment and follow-up of patients. The diagnostic laboratory uses nowadays more than 1.000 different tests. There is an erosion of traditional boundaries between laboratory medicine and other diagnostic specialities with consequences for education and future job roles. Although advancements in the science and technology offer exciting opportunities for our patients, we are faced with some significant challenges in the delivery of that care. Professionals in

laboratory medicine have been long focusing on the analytical part of the testing process. The integration of laboratory diagnostics and the modern techniques of molecular biology will result in individual treatments based on specific diagnostic tests. With the new possibilities of personalized medicine, the diagnostic laboratory will add a new quality to clinical medicine. Appropriate test selection and correct interpretation of test results are the most important areas of such a consultative approach, especially since patient safety risks associated with incorrect laboratory test selection and misinterpretation of test results have been largely neglected. Laboratory consultation in Hungary, however, is less common and laboratory professionals are seen as somewhat inaccessible for such a role. Re-integration of the diagnostic laboratory into clinical medicine, into the daily decision-making of the clinicians will in essence be the main future goal of our discipline.

SE10.2

The importance of communication between clinicians and the laboratory

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In modern medicine laboratory plays a significant role in the diagnosis, monitoring and treatment of diseases. Many therapies (e.g. cytostatics, anticoagulants) are guided by the test results. Errors can occur in each (praeanalytic, analytic and postanalytic) phase of laboratory diagnosis.

Especially praeanalytic and postanalytic phases are “clinician-sensitive.” An explanation might be the wide spectrum of laboratory results not readily understandable for a non field-expert as it can happen especially with the new emergency ward system. The same problem might occur when the selection and ordering of a test is inadequate, due to the lack of knowledge. The laboratory “panel-sirens” tempt for overrequest. A very condemnable but unfortunately existing practice is when tests are not ordered by the physicians themselves, but by students or other members of the staff unfamiliar with the test or the patient. It has to be emphasized, however that the final decision/responsibility belongs to the clinician therefore laboratory suggestions for further examinations automatically turning up on the discharge summary might be uncomfortable.

The communication between laboratory and clinical health workers have several advantages. Laboratory staff should be ready to ask additional information when needed; recognize *in vitro* the *in vivo* patient while clinicians should be familiar with the availability, importance, value and labor demand of the tests and provide sufficient patient information.

Guidelines and electronic order forms with mandatory minimal data reduce failures but do not replace personal communication (e.g. face-to-face or telephone) and problem-solving workshops. Clinicians and laboratory workers should recognise and accept their complementary roles in the team since this attitude is the prerequisite of a cost effective *and* human medical care.

SE10.3

How to use autoimmune serology professionally and prudently?

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The author first presents a very brief clinical overview about systemic autoimmune diseases, including the most important symptoms and organ manifestations. The heterogeneity and variability of these diseases is emphasised. In the following, the value of the generally used autoantibody determinations and other autoimmune serological methods will be presented from a clinician’s point of view. Instead of thinking in panels, the importance of the selected analysis of autoimmune tests, always based upon the clinical investigation and the suspected diagnosis is stressed. This obviously requires that a previous clinical assessment and routine laboratory tests must be available and be performed by an immunologist or a physician with documented experience in the field of autoimmunity. The value of various autoantibody determinations in the follow-up of patients, and consequently, the rationale of the repetition of the tests is also discussed.

SE10.4

Laboratory from a GP point of view

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Background: Zákányszék is a village with population of 3000 inhabitants, located 25 km away from Szeged. Roughly 50% of the residents live in farms. The main user of the laboratory diagnostics is the modern Hungarian GP society. Laboratory diagnostics is our only and expansively

available primary diagnostic tool. However, the information technology, economics and legal of the present issues call for a reinterpretation of the relationship and communication between primary health care and laboratory diagnostics.

-Present: Having a role in the pre- and post-analytic phases of blood tests. Taking blood samples weekly at the GP surgery or at the patient's house. Transporting the samples to the contracted laboratory and on the following day receiving the validated results at the surgery. The evaluation of the results at the follow-up appointments. The analytic phase takes place at the Albert Szent-Györgyi Health Centre in Szeged.

-Concept: 1. The close to home GP service as the novel public health approach requires the development of a well-thought, well-structured system, which is based on the satisfaction of its patients. It is important to emphasize that any kind of mistakes are made in the 3 phases mentioned above may lead to misdiagnosis. According to an article on the www.medicalonline.hu [1] 70-80% of the errors occur in the pre-analytic phase (exchange of samples, unexperienced professional, not providing enough samples for the analyst, the usage of the inappropriate blood sample tubes or the misidentification of these tubes). 2. The aim is the expansion of professional competence. From one side this requires high quality primary care and prevention reinforcement (PSA, TSH, T3, and T4), on the other side it minimizes the avoidable and not grounded laboratory diagnostics requests (tumor markers, RF). 3. Further aim is to develop in the patients to take responsibility of their own health and to manage the unduly requests for certain laboratory diagnostics arising from patients due to information heard in the media and from out-of-date guidelines. 4. This coordinated system can only be achievable if all the individuals involved are competent both in professionally and ethically and work in collaboration with each other at every stage.

[1] http://www.medicalonline.hu/gyogyitas/cikk/a_haziorvosi_gyakorlat_szamara_fontos_laboratoriumi_preanalitikai_tenyezok

SE10.5

Interpreting procalcitonin at the bedside

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One of the most challenging tasks in critical care medicine is the treatment of serious infection related multiple organ dysfunction, termed in general as sepsis and septic shock. Early detection of infection and the immediate start of resuscitation parallel with adequate antimicrobial therapy undoubtedly give the best possible chance for survival and received strong recommendation by the Surviving Sepsis Campaign guidelines. However, while recognizing organ failure via objective signs is relatively easy, diagnosing infection as possible underlying cause remains a challenge. Due to the non-specific properties of conventional signs of infection, such as body temperature and white cell count, biomarkers have been searched to aid diagnosis for decades. One of the most studied biomarkers is procalcitonin (PCT). Its role in assisting antibiotic therapy has been studied extensively, with contradicting results.

Every biomarker, including PCT, has its own merit and limitations. Biomarkers can support decision making but they will never be able to differentiate between inflammatory response for infection from host response for non-infectious insults with a 100% sensitivity and specificity due to the complex, overlapping pathomechanism.

Nevertheless, a multimodal, individualized approach, consisting of a) recognizing organ dysfunction, b) identifying the possible source, c) following the clinical picture, d) evaluating microbiological results and d) taking PCT and PCT-kinetics into account, and putting all that into context, is necessary to make the most out of your PCT and to do the best of your patients in your everyday practice.

YF1

Immunogenicity profile and predictors of trough levels and anti-drug antibody development of biosimilar infliximab

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Introduction: Biosimilar infliximab CT-P13 received EMA approval in June 2013 for all indications of the originator product. Its use is mandatory in all anti-TNF naïve IBD patients in Hungary since May 2014. In the present study we aimed to prospectively evaluate the immunogenicity profile of the biosimilar infliximab and predictors of trough levels and anti-drug antibody development in IBD in a nationwide, multicentre cohort.

Methods: Demographic data were collected and a harmonized monitoring strategy was applied. Clinical and biochemical activity were evaluated at weeks 14, 30 and 54. Trough level (TL) and anti-drug antibody (ADA) concentration were measured by ELISA (LT-005, Theradiag, France) at baseline, at week 2,6,14, 30 and 54 weeks right before anti-TNF administration.

Results: 291 consecutive IBD patients (184 Crohn's disease [CD] patients and 107 ulcerative colitis [UC] patients) were included in the present cohort. Mean TLs were 20.1, 14.7 and 5.1 µg/ml at weeks 2, 6 and 14 (n=124, 86 and 158). Cumulative ADA positivity rates were 8.7%, 19.3%, and

28.0% in IBD patients at weeks 0, 14, and 30 ($n_{\text{total}} = 229, 192 \text{ and } 143$). In CD early TLs at week 2 were predicting short term (week 14 response/remission, $AUC_{\text{TLweek2}} = 0.72/0.72$, $p=0.05/0.005$), but not medium-term (week 30 or 54) clinical efficacy. In UC TLs at week 2 were predicting short and medium term clinical efficacy (week 14 response/remission, $AUC_{\text{TLweek2}} = 0.81/0.79$, $P=0.001/0.001$, week 30 response/remission, $AUC_{\text{TLweek2}} = 0.79/0.74$, $P=0.002/0.006$). TLs measured at week 6/14 were not predicting either short or medium-term clinical outcome. In addition, early ADA status by week 14 ($p=0.04-0.05$, OR: 2.1-2.6 for week 14 and 30), early clinical response ($p<0.001$, OR: 7.7-42.8 for week 30/54) and normal CRP at week 14 ($p=0.005-0.0001$, OR: 3.2-7.8 for week 14 and 30) and previous anti-TNF exposure ($p=0.03-0.0001$, OR: 2.22-6.25, for week 14, 30 and 54) were associated with short and medium-term clinical outcomes (response and remission) in CD. In UC only previous anti-TNF exposure and ADA status were associated with clinical outcomes.

Conclusions: Early TLs were only associated with short-term clinical outcomes. ADA development by week 14, early clinical response, normal CRP at week 14 and previous anti-TNF exposure were predicting medium-term clinical outcomes.

YF2

First Hungarian pilot study on point of care INR-meter-driven management of vitamin K antagonist therapy in primary care

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Introduction: Oral anticoagulation therapy (OAC) with vitamin K antagonists (VKA) is widely used in secondary prevention of thromboembolic diseases. Patients on VKA should be regularly monitored by INR measurements, which can be done either in central laboratories (CL) or by point of care (POC) INR testing at general practitioner's (GP) offices. The later is very little practiced in Hungarian health care.

The aim of the study was to pilot test INR monitoring in primary care.

Methods: Six GPs with large number of VKA patients were invited to the study. CoaguChek XS Pro (Roche) POC-INR-meter was implemented in their offices. All potential users got training, passed an exam and certified. 102 patients were enrolled who were subjected to standard INR measurement in CL and, simultaneously, to POC INR tests in GP's offices. Clinical decisions (eg. modification of therapy) were based on POC INR results. During the 14 months follow-up period POC and CL INR values were compared both by aspects of their analytical and clinical performance.

Results: 900 simultaneous INR measurements were analysed. The total analytical deviation between POC-INRs and CL-INRs met all the analytical requirement of the ISO17593:2007 standard. In 148 cases (16% of all measurements) the two INR results could have induced slightly different therapeutic decisions (eg. slight VKA dose elevation by POC INR while no change in dosage by CL-INR). However, none of the differences indicated opposite therapeutic decisions. During the 14 months follow-up no thrombotic or bleeding adverse event occurred in any patient. Time in therapeutic range of patients with more than 8 visits during the follow-up was 50%. POC INRs were available 6 hours earlier for GP than CL results on average.

Conclusions: The findings of the first Hungarian pilot study of INR monitoring by POC meters in GP's offices corresponded well with the international experiences. Quality and competence based POC INR monitoring of VKA treatment in primary care is a safe and effective alternative to that based on CL.

YF3

Hemophagocytic lymphohistiocytosis: case study of an uncommon syndrome

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Hemophagocytic syndromes are extremely rare (1case/1 million/year) diseases featured by a "cytokine storm" resulting phagocytosis of red blood cells (RBC), platelets (PLT) and white blood cells (WBC) by macrophages. There are two main types of the disease: the primary (hereditary), which shows autosomal recessive inheritance, and the secondary (acquired), which can be triggered by infections, autoimmunity or malignancies. Both of them lead to death if untreated. The disease is characterized by a nonmalignant aggressive proliferation of activated macrophages and histiocytes. The typical sites of their action are: bone marrow, liver, skin, membranes of the central nervous system. The symptoms are not specific: fever, hepatosplenomegaly, jaundice, skin rash, neurologic signs. Laboratory findings are mostly also not specific: low RBC, low PLT, low WBC, low fibrinogen level, elevated triglyceride, bilirubin, and liver enzymes. Extremely high ferritin can be a hallmark of the disease if requested. Characteristic finding can be the presence of phagocytosis in the bone marrow or in other affected tissues. Because of the uncharacteristic symptoms and laboratory findings it can be easily misdiagnosed. Our aim is to demonstrate the difficulty of the disease's differential diagnosis through the example of a 53 years old patient presented with gastroenterological symptoms in our Emergency Unit.

YF4

Neonatal leukemia – a special case study

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Leukemia is the second most common neonatal malignancy. In UK and USA, the incidence is approximately one in every 12,500-27,500 live births. Leukemia occurring in the newborn period has different features from those that occur later in childhood (1;2). V.K. (female) was born at 40th weeks via normal vaginal delivery after undisturbed II/2 pregnancy. Cardiopulmonary adaptation was undisturbed. In the newborn unit of Petz Aladár County Hospital Győr (Hungary) she seemed pale before discharge, so her doctor indicated a stat CBC examination. We measured an extreme leukocytosis (125 G/L) with 42% lymphoblasts (MGG blood smear). Due to these abnormal laboratory values she has been urgently referred to II. Department of Pediatrics Semmelweis University of Medicine, Budapest, where additional tests (flow cytometry, histology, cytogenetic and FISH) confirmed newborn precursor B-cell ALL with MLL translocation. After unsuccessful chemotherapy hematopoietic cell transplantation (HCT) (3) was administered, successfully. We present this case because of its extreme rarity.

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YF5

Reducing patient risks during the post-post-analytical processes

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In 2013 the SYNLAB Hungary Kft. has set a goal to increase patient safety. Patient safety risks were estimated and compared with the application of FMEA (Failure Mode and Effects Analysis) method. In laboratory medicine we should apply professionally controlled reliable methods to find those processes where the quality can be monitored and/or improved by scientifically proven indicators. Because information based on laboratory test data obtained from patients by a relevant, reliable and reproducible way, play a deterministic role in more than 70% of medical decision making, therefore in 2015-2016 we studied the post-post analytical process in regard of possible errors. Post-post-analytical questionnaire containing 13 questions was constructed and sent out for the clinicians. 360 pieces of questionnaire data were processed. We examined if the doctors considered necessary to indicate age adjusted reference ranges in the laboratory report. 20% of the respondents thought it to be unnecessary. Medical practitioners were asked about the significance of factors influencing the results (lipemia, hemolysis, icterus) by more than 10%. The majority (68%) believes that in all cases the test results should include LIH remarks as well. It was considered to be a potential patient risk if the parameter name by the laboratories was not clear (for example: abbreviations, the same test with multiple names, etc.). We wondered how often the clinicians perceived this non-compliance. 24% of them already had this experience. Based on the results we aimed to extend our pilot study for the clinical chemistry laboratories of our network. In order to correctly inform the clients, the company's management intends to improve the home page by brief description of the tests, as well as pre- and post-analytical guidance.

YF6

Systematic analysis of the effect of maternal cell contamination on prenatal molecular testing

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Prenatal molecular diagnosis is now available for a large series of severe Mendelian disorders. Specimens for analysis, amniotic fluid (AF) or chorionic villus samples (CVS), are obtained by invasive techniques. Maternal cell contamination (MCC) in fetal samples has to be considered as a preanalytical risk for each prenatal molecular analysis and may lead to false genotyping. It is recommended to determine the routinely used prenatal diagnostic tests' sensitivity to MCC.

In this study the sensitivity of three different diagnostic methods to MCC was tested. These were: Sanger DNA sequencing, multiplex ligation-dependent probe amplification (MLPA) and a next-generation sequencing methodology (NGS). The experiments were performed by mixing a wild type DNA sample ('fetal') with a heterozygote DNA sample ('maternal') simulating different levels (1, 5, 10, 20, 30 and 40%) of MCC.

The limit of detection of the mutant allele by Sanger sequencing was 10% (20% MCC), above this level the fetal genotype may be obscured. MLPA sensitivity testing showed that 1, 5, 10, 20 and 30% MCC had no effect on genotyping result. Forty percent MCC resulted in a maximum of 30% reduction in relative peak height of relevant probes leading to diagnostic uncertainty (heterozygosity is defined by 35-50% reduction). The NGS method detected as low as 0.5% mutant ('maternal') allele in experiments where high coverage was obtained and accurate quantification in the range of 0.5-20% which did not lead to false genotyping.

Our results show that the level of MCC affecting the results of diagnostic prenatal tests is highly dependent on the applied method. One should also emphasize that the diagnostic test results can be interpreted correctly only in conjunction with simultaneously determined MCC level.

YF7

A novel and more efficient review protocol for screening patient samples with hematological alterations

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Even in today's almost fully-automated laboratories manual peripheral blood smear reviews retained their key role in the diagnosis of hematological malignancies. In 2005 a standardized protocol on smear performance was recommended by an international consensus group and it was emphasized that the parameters suggested should be validated by each laboratory for their platform. We aimed to evaluate the validity of a new review protocol based on international guidelines by testing it on several patient groups and hematology analyzers (Siemens Advia and Sysmex XE).

Patients were assigned to ALL (n=63 children and 13 adults), AML (n=17 children and 70 adults), CLPD (n=41 adults) and CML (n=18 adults) groups on the basis of flow cytometry and cytogenetic examinations. Then we tested and refined our protocol based on the results from the patient groups. Finally, the new parameters of the protocol were analyzed using retrospective data of the General Laboratory Information Management System (GLIMS). This approach provided information not only about the utility of the protocol but also about the difference in performance of two hematology analyzers.

With the application of the new protocol, true positive cases were less frequently overlooked (12,2% versus 3,2%) and mean alterations in the different patient groups were higher (2,5 vs 3). As several parameters are reviewed simultaneously and more than one criteria should be fulfilled to prepare smear, the number of unnecessarily reviewed negative cases did not increase.

The novel review protocol introduced here can be applied as a standard method to identify more efficiently patients with hematological alterations without increasing the superfluous review of negative cases.

YF8

Serum HE4 is a suitable inflammatory biomarker in cystic fibrosis

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Increased human epididymis protein 4 (HE4) expression was previously observed in lung biopsy specimens of cystic fibrosis (CF). Accordingly, we presumed that serum HE4 concentrations were also elevated in CF, and might be used as a biomarker.

In this study, 77 children with CF and 57 adult CF patients were enrolled. In parallel, 94 individuals with non-CF lung diseases, and 117 normal controls without pulmonary disorders were analyzed. Serum HE4 was measured by chemiluminescent microparticle immunoassay (Architect[®], Abbott). HE4 expression was further investigated via the quantification of HE4 mRNA using RT-qPCR in CF versus non-CF respiratory epithelium biopsies. The expression of the potential regulator miR-140-5p was analyzed using an UPL-based RT-qPCR assay (Roche). In addition, HE4 was measured in the supernatants from unpolarized and polarized cystic fibrosis bronchial epithelial (CFBE) cells expressing WT- or F508del-CFTR.

Serum HE4 levels were significantly elevated ($P < 0.0001$) in both CF children (99.5 [73.1-128.9] pmol/L) and CF adults (115.7 [77.8-148.7] pmol/L) compared to controls (36.3 [31.1-43.4] pmol/L). In contrast, abnormal but lower HE4 concentrations were found in cases of severe bronchitis, asthma, pneumonia or bronchiectasis. HE4 concentrations positively correlated with disease severity and C-reactive protein

concentrations in CF, while a significant inverse relationship was found between HE4 and the spirometric FEV₁ value. Relative HE4 mRNA levels were significantly augmented (P=0.011) in the presence of decreased miR-140-5p expression (P=0.020) in CF versus non-CF airway biopsies. Finally, 2-fold higher HE4 concentrations were measured in the supernatants of polarized F508del-CFTR CFBE cells compared to WT cells.

In conclusion, serum HE4 positively correlates with the overall severity of CF and the degree of pulmonary dysfunction. Thus, HE4 may be used as a novel inflammatory biomarker and for the treatment efficacy in this lung disease.

YF9

Hunting driver clones by real-time polymerase chain reaction and next generation sequencing in myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN) are defined by the presence of mutually exclusive mutations affecting *BCR-ABL1* (in chronic myeloid leukemia), *JAK2* or *CALRETICULIN* genes (in essential thrombocythemia, primary myelofibrosis). The concomitant occurrence of two diseases with two driver mutations in the same patient in an extremely rare case. Here we report the comprehensive genetic analysis of sequential samples (4-38 samples/patients) obtained from 7 patients with concurrent *BCR-ABL1* (n=7) and *JAK2* V617F (n=5) or *CALR* (n=3) mutations. (In one case *JAK2* V617F and *CALR* mutations could be simultaneously detected.) The quantity of *BCR-ABL1* fusion transcript and *JAK2* V617F mutation were monitored by real-time PCR analysis, while the burden of the *CALR* mutated clone was followed semiquantitatively by fragment analysis. Further 10 genes frequently mutated in MPNs (*ASXL1*, *EZH2*, *SRSF2*, *IDH1*, *IDH2*, *LNK*, *CBL*, *TET2*, *DNMT3A*, *TP53*) was analyzed using next generation sequencing (NGS: AmpliSeq technology with an IonTorrent instrument at an average depth of 1000x). The quantitative analyses of the mutations suggested that driver mutations occurred in different clones in 5 of 7 cases, and in the same clone in 2 cases. NGS identified additional somatic mutations in 3 cases (*DNMT3A* R882C, P904L in 2 cases, *ASXL1* W583X in a single case). *TET2* mutations were identified in 3 cases, but the differentiation between inherited and somatic variants is still in progress. Molecular testing for the presence of the *BCR-ABL1*, *JAK2* and *CALR* mutations has become a frontline test for the investigation of neutrophilia or thrombocytosis. Our analysis suggested that the *BCR-ABL1* and *JAK2* or *CALR* mutations occur in different clones in majority of cases with frequent acquisition of additional phenotype modifying genes including *TET2*, *DNMT3A* and *ASXL1*. The frequency of additional mutations is not higher in MPN cases with double drivers compared to *BCR-ABL1* negative primary myelofibrosis cases.

YF10

Hemocompatibility test of magnetic fluids - what does the microscope see?

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A wide variety of core-shell magnetite nanoparticles (MNPs) dispersed in water has been designed up to now with diagnostic (magnetic resonance imaging, MRI; magnetic particle imaging, MPI) and/or therapeutic aims (e.g. magnetic hyperthermia and targeted drug delivery). Core-shell structured MNPs consist of a protective layer around the magnetic core, which is needed to prevent the aggregation of nanoparticles, to stabilize the dispersion and to hinder the chemical and biological degradation of nanomagnets. There are various strategies to prepare surface functionalized iron oxide nanoparticles with different structure and magnetic properties. Based on our previous studies, the MNP's colloid stability exhibited at simulated physiological conditions (i.e. salt tolerance test) can predict their hemocompatibility [1]. In this work, we examined how the different chemical composition and physico-chemical properties of magnetic fluids alter microscopically the blood cells in vitro. Four aqueous magnetic fluids with various chemical composition/top shell prepared and characterized in multiple chemical labs were studied. K-EDTA anticoagulated blood from three healthy donors was used for the tests. As references, smears from donors' native blood and from blood-distilled water mixtures were prepared. During the evaluation of smears particularly the cell death caused by MFs, the change in the ratio of various white blood cell subsets, the possible aggregation of MNPs and the relationship between physico-chemical characteristics of nanomagnets and their hemocompatibility were investigated. Our results clearly demonstrated that the studied magnetic fluids behave differently in contact with blood depending on their characteristics. The smear evaluation showed strong correlation between the physico-chemical properties of the magnetic fluids and their hemocompatibility.

Reference:

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YF11**Monitoring of chimerism after allogeneic hematopoietic stem cell transplantation by short tandem repeats**

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Allogeneic hematopoietic stem cell transplantation (HSCT) has become an important, but a high-risk treatment for malignant and non-malignant disorders. While it is a potential for cure, but may have life-threatening complications (e.g. infections, graft-versus-host disease, graft failure or disease relapse). Monitoring the relative mixture of donor and recipient hematopoiesis (the so called "chimerism") allows early prediction of engraftment, graft rejection or relapse after HSCT. The most commonly used technique for chimerism analysis is the fluorescently labeled polymerase chain reaction of short tandem repeats (STR) followed by capillary electrophoresis (Beckman Coulter CEQ 8000 Genetic Analysis System), a similar technique used in forensic genetics. In case of sex-mismatch between the recipient-donor pair, chimerism can be analyzed by using fluorescent *in situ* hybridization (FISH). In 2015, we investigated 110 recipient-donor pairs before HSCT and identified at least one among 12 STR markers (GenomeLab Human STR Primer Set), which allowed differentiation between recipient and donor hematopoiesis after HSCT. In the same year, we performed chimerism monitoring of 823 samples from 388 patients after HSCT. A total of 123 peripheral blood or bone marrow samples were analyzed by both STR and FISH techniques at the same timepoint. Complete donor chimerism observed concordantly by both tests was present in 86 (70%) samples. Mixed chimerism with less than 10% difference between the results of the two techniques was reported in 32 (26%) samples. We identified >10% difference between STR and FISH results in 5 (4%) samples. The exploration of the discrepancies (e.g. altered sample source, stutter peaks or preferential amplification) is in progress. In one sample (male recipient, female donor) we observed 94% XX (donor), 6% XO (recipient) karyotype without chromosome Y specific STR marker-amplification due to chromosome Y deletion in the leukemic cells. Although XY FISH and STR PCR showed a good concordance for chimerism analysis, our result indicates the need for carefully selected, multiple informative markers and using different methods of chimerism analysis.

YF12**An automated immune turbidimetric test for urinary orosomuroid: validation and clinical usage**

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Orosomuroid or alpha-1 acid glycoprotein is a positive acute-phase plasma protein. Due to its molecular mass of 42-43 kDa it is filtrated through the glomeruli. Former studies regarding inflammatory disorders suggested that urinary orosomuroid (u-ORM) may provide information on the severity of these diseases. However, the benefit of u-ORM determination has not been established yet. In our study, we would like to demonstrate the development and validation of a fully automated method for u-ORM measurements. Furthermore, our aim was to investigate u-ORM levels in patients with acute and chronic inflammatory diseases. A particle-enhanced immune turbidimetric assay was developed for a Cobas 8000/c502 analyzer to determine u-ORM levels. Spot urine samples from 72 healthy individuals, from 28 patients with Crohn's disease and from 30 severe septic patients were analyzed. We expressed our u-ORM data in u-ORM/creatinine ratios (mg/mmol) and in u-ORM/urinary total protein proportion (%). The detection limit of our assay was found to be 0.02 mg/L. The intra- and inter-assay imprecision CV% and also the inaccuracy were determined to be less than 5%. Reference values for u-ORM/creatinine ratio were established to be 0.08 (0.01-0.24) mg/mmol [median (2.5-97.5 percentiles)]. Compared to controls, u-ORM/creatinine ratios showed a 5-fold elevation in Crohn's disease and approximately 230-fold elevation in sepsis ($p < 0,001$) while serum ORM values increased only moderately (at about 2 fold).

We set up a highly sensitive, precise and accurate turbidimetric approach for ORM determination in urine. Our fully automated assay is ideal for routine utilization and our findings support that u-ORM measurements can be a novel laboratory test for diagnosis and monitoring of inflammatory processes.

YF13

Design of novel magnetic nanostructures for targeted tumour therapy - MagBioVin Project

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We present here some recent research advancements and opportunities within the FP7-ERA Chairs MagBioVin project. The project aims to design various novel magnetic nanoarchitectures (e.g. bimagnetic and polymeric core-shell systems, nanoparticles embedded in mesoporous silica and radiolabeled nanostructures) for application in targeted treatment and diagnostics of cancer. The magnetic core of these nanomaterials allows the selective treatment of tumor tissues (i.e. targeted drug-delivery, localized magnetic hyperthermia) by magnetic field. Attachment of radionuclides (e.g. ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{134}I) to the nanoparticles opens the possibilities for imaging and internal radiotherapy.

Magnetic nanoparticles (MNPs), i.e. iron oxides and spinel ferrites, were synthesized by different methods and coated by several compounds (e.g. citrate, polymers, silica, BSA) to increase their biocompatibility. The composition and morphology of the nanomaterials is characterized by XRD, TEM imaging and infrared spectroscopy, while their magnetic properties were studied by SQUID magnetometry and Mössbauer spectroscopy. Magnetic hyperthermia effects were monitored by DM100 device equipped with DM1, 2 and 3 applicators (nB nanoScale Biomagnetics). This unique setup allows us to monitor the heating efficiency development in cell cultures and small animals (e.g. mice, rats) as well. The current results showed that the MNPs can be successfully labeled with ^{90}Y and $^{99\text{m}}\text{Tc}$. The drug loading and release properties of MNPs are studied by HPLC using doxorubicin as the drug. *In vitro* and *in vivo* (animal model) applicability of the synthesized nanomaterials regarding toxicity, biodistribution and anti-cancer efficacy is explored for targeted cancer treatment.

P1

The impact of usage of Multiplate aggregometer on platelet concentrates utilization of a large Traumatology Department

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Platelet dysfunction contributes to bleeding complications during and after traumatic surgery. If injured patients receive ADP receptor inhibitors and the PLT inhibitory effect persists, urgent surgery may require the administration of platelet concentrates (PC). The PC is, however, very expensive. Its administration requires caution. Therefore a quick and efficient lab method like peri-operative point-of-care monitoring of platelet function may support the clinical decision making before the surgery. We assessed the impact of Multiplate platelet function analyser (MP) on utilization of PC in traumatic patients. A total of 511 MP tests have been done between May, 2013 May and the end of 2015. The number of tests performed for traumatology was 240. In parallel we calculated the annual consumption of PC and the total cost per traumatic intervention for the period of 2011-2015.

The ratio of PC consumption per traumatic surgery showed a decreasing trend after introducing MP tests in 2013. While the ratio of MP tests per traumatology surgery constantly increased for the same period. The total cost per surgery practically remained constant since 2013, probably due to the increased awareness and usage of MP by the physicians. We concluded that PC consumption and the use of pre-surgery MP tests associate in terms of the more frequently the MP test is used before surgery the less PC consumption per surgery is expected.

The application of Multiplate tests can provide qualitative and economic improvement in platelet concentrate utilization in hospital setting. In addition we recommend developing a hospital level guideline on adequate MP usage taking both the clinical and economic considerations into account.

P2

Evaluation of the analytical performance of two point of care INR meters

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Introduction: INR measurement from capillary blood using point of care INR meters is a relatively new approach in INR monitoring of patients under oral anticoagulant treatment (OAT) with vitamin K antagonists (VKA).

Aim: Analytical performance of two portable, IT connectable INR meters was evaluated in comparison with central laboratory (CL) INR measurements that were certified in UK-NEQAS external quality program.

Patients and methods: After informed consent 97 patients under VKA therapy was enrolled. In 57 patients INR was measured from venous and capillary blood in parallel, while on 20-20 patients two POC INR measurements were done at the same time. K₃-EDTA anticoagulated blood was taken from all patients for haemoglobin and haematocrit measurements to detect values that can interfere in POC INR methods.

CoaguChek XS Pro (Roche) and Xprecia Stride (Siemens) POC INR methods were evaluated. The reference INR method in CL was measured by Dia-PT (Diagon) on BCS XP (Siemens) coagulometer.

Imprecisions of each POC INR meters were calculated from parallel INR measurements of 20 patients. Then POC and CL INR values of 57 patients were correlated. In addition, it was also evaluated whether the performance of the two POC methods fulfils the analytical requirements of ISO 17593:2007 standard.

Results: The imprecision of both POC INR meters is acceptable ($CV_{\text{CoaguChek}}$: 8,9%, $CV_{\text{Xprecia Stride}}$: 6,6%). Both POC INR methods showed strong correlation with the reference CL method. The equation of correlation between CoaguChek XS Pro and CL method were as follows $y=1.1345x-0.2435$ ($r=0.95$). The equation of correlation between Xprecia Stride and CL method were as follows $y=0.8973x-0.0108$ ($r=0,91$). Both INR meters fulfilled analytical requirements of ISO 17593:2007.

Conclusions: The analytical performance of both POC INR meters was in conform with ISO 17593:2007, which makes both meters suitable and reliable for INR monitoring in primary care or in outpatient services.

P2

Effect of storage and pneumatic-tube transport system on platelet indices

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Type of anticoagulant for routine measurement of complete blood count in hematology laboratory has a significant effect on the results. The use of EDTA change platelet volume in a time dependent manner. On the other hand, citrate anticoagulant influence platelet volume to a less extent incubation. Pneumatic tube transport system (PTS) possibly could activate the platelets by agitation and shaking of the samples. The aims of this study were to examine the impact of PTS, and EDTA and citrate anticoagulants on the platelet indices measured at different time-points following sampling.

Forty-two (25 female, 17 male) apparently healthy individuals were enrolled in this study. Blood was drawn after at least eight hours fasting in sample tubes containing EDTA or citrate as an anticoagulant. The Sysmex XT-2000i analyzer was used and blood cell count measurements were done immediately, then 2, 3, 4 and 7 hours intervals for all samples at room temperature. The same samples were transported by PTS to the central laboratory and measured at the identical times.

We analyzed platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR). PLT and PDW did not change during the period of incubation neither for EDTA samples nor for citrated samples ($P>0.05$). Pneumatic-tube system handling did not affect the results. The same outcomes were observed for MPV and P-LCR with citrate anticoagulant. MPV and P-LCR from samples anticoagulated with EDTA demonstrated statistically significant elevation after two hours of incubation ($P<0.05$). The difference between zero time and 2-hour time for MPV was on the border line of clinically relevant difference; the further incubation increased the difference.

Citrated samples showed lower results for all measurements compare with similar EDTA samples. All measurements after PTS handling demonstrated no influence on platelet indices and this is independent of the type of anticoagulant. To increase the chance of standardization for the MPV results, EDTA samples should be measured within 120 min. after phlebotomy.

P4

Transient atypical lymphocytosis in adults

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A reactive lymphocytosis with an absolute lymphocyte count greater than $5 \times 10^9/l$ is common in children and young adults and is usually related to infectious mononucleosis or other viral infections. Benign lymphocytosis in older adults is unusual, because lymphocytosis in this age group frequently indicates chronic lymphocytic leukemia or another lymphoproliferative disorder. Therefore, we evaluate blood smear in all adult patients, who have absolute lymphocytosis without relevant diagnosis connected with lymphoproliferative diseases.

During last 3 months we identified 23 cases, characterised by transient lymphocytosis, and atypical-appearing lymphocytes in the blood smear. 15 patients presented with serious cardiac conditions - with- or without cardiac arrest, and reanimation - 8 patients presented with other critical conditions including surgical intervention, anaphylactic reactions, hypertensive crisis and traumatic injuries. The lymphocytosis was probably secondary as a consequence of administration of epinephrine, or as the exposure to a severe stress. Epinephrine is known to cause a nearly immediate increase in numbers of circulating lymphocytes. It was observed a pleomorphic population of lymphocytes in peripheral blood smears. Some of them were small, mature-appearing lymphocytes with condensed nuclear chromatin and a small rim of cytoplasm. However, majority of them were large reactive atypical lymphocytes. Atypical lymphocytes ranged 30-90% of the lymphocyte populations. Most of the atypical lymphocytes were large cells with abundant pale cytoplasm, and round or oval nuclei. Typical prominent asuophilic granules of large granular lymphocytes were present in approximately 15-25% of the lymphocyte population. In two blood smears we identified hairy cells either. After 24 hours numerical and morphological abnormalities were disappeared in all cases.

P5

Classification and treatment of the thrombotic microangiopathies

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Thrombotic microangiopathy is a group of disorders, which is characterized by thrombocytopenia and microangiopathic hemolytic anemia (intravascular hemolysis and presence of schistocytes), neurological symptoms and renal dysfunction. The damage can occur in the smallest blood vessels – most commonly in the kidney and the brain. To make an accurate diagnosis, the clinician must differentiate between thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS).

HUS represents a heterogeneous group of disorders with variable etiologies that result in differences in presentation, management, and outcome. In recent years, better understanding of HUS, especially of mutations in the genes encoding proteins of the alternative complement pathway, has provided an update on the terminology, classification, and treatment of the disease. Based on the clinical and laboratory data, three main forms of HUS can be distinguished: diarrhea-associated HUS, atypical non-familial HUS and atypical familial HUS.

Atypical hemolytic uremic syndrome (aHUS) is a rare disorder caused by dysregulation of the complement alternative pathway, and associated with mutations in genes of complement components and regulators.

Most cases of TTP arise from autoantibody-mediated inhibition of the enzyme ADAMTS13, a metalloprotease responsible for cleaving large multimers of von Willebrand factor (vWF) into smaller units. In rare cases, mutations in the *ADAMTS13* gene cause congenital deficiency of the protease and result in familial recessive form of TTP.

In our laboratory we investigated altogether 425 TMA patients since 2008. From that time we measure complement parameters, ADAMTS13 activity and also perform the screening of the complement genes. In our presentation I would like to review the correct classification of different TMA types based on clinical symptoms and laboratory results.

P6

The presence of a rare haemoglobin variant in a family

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Hemoglobinopathies (HP) are the most prevalent monogenic autosomal recessive hereditary disorders worldwide. They are caused by mutations in the coding, non-coding or regulatory sequence of globin genes, resulting an unstable hemoglobin molecule. In our country the prevalence of the disease is very rare, clinicians often do not think about them, and, therefore, these diseases are usually misdiagnosed. In the case of suspected HP the algorithm of investigation in our laboratory is: complete blood count, reticulocyte count, calculating the Mentzer-index, iron homeostasis tests, laboratory tests for detecting haemolysis, blood smear examination (May-Grünwald-Giemsa stain), detection of Heinz-bodies with supravital staining, haemoglobin instability tests (isopropanol-, heat stability-test), capillary haemoglobin electrophoresis. The verifying test should be a genetic test confirming the mutation. HPs cause usually hypochrom microcytic anaemia and are inherited in an autosomal recessive manner. But there are some exceptions as haemoglobin Köln, in which hyperchrom macrocytic anaemia and haemolysis are present and shows autosomal dominant inheritance. Therefore it's hardly recognised and is often misdiagnosed. Our aim is to present the algorithm of laboratory investigation of this disease through the presentation of a family.

P7**Introducing the Beckman-Coulter DXH-800 Hematology Automated Analyzer at the Central Laboratory of Hospital of Siófok**

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In fields of healthcare the laboratory hematology measurements are very important since multiple diseases can be diagnosed based on hematology measurement parameters. In case of the introduction of a new automated analyzer a careful procedure of comparison is required because the right analyzer is essential for accurate results.

We selected randomly 177 samples from that arrive to our laboratory and we have measured them on two hematology automated analyzers: Abott Cell-Dyn 3700 (CD3700) and Beckman Coulter DXH-800 (DXH800). The Pearson's correlations showed strong significant relationship between all measured hematological parameters. Measurements of three leveled control sera demonstrated the following intervals of coefficient of variations (CV): CD3700 = 0.7 – 9.1 % and DXH800 = 0.6 – 1.95 %. White blood cell, red blood cell and platelet counts were comparable using hemoglobin tercilis. In the anemic hemoglobin level subgroup the following most frequent diagnoses were present: chronic kidney failure, pregnancy, child-birth and joint disease. Comparing two parallel measurements of 29 patients' samples measured between 30 and 60 minutes after sampling we found very strong relationships ($r = 0,996 - 0,999$, $p < 0,01$).

Our study demonstrated that (i) the newly introduced DXH800 hematology automated analyzer's measurements correlated well with those obtained by CD3700 automated analyzer, (ii) based on the CVs DXH800 is accurate and reliable and (iii) the correlations between measurements on the new automated analyzer seems to be strong. In the future we plan to continue our comparisons using peripheral blood smear and to compare graphical cell differentiations and flags from our patients' samples.

P8**The report of a special case of thalassemia**

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Thalassemias are inherited disorders of hemoglobin (Hb) synthesis caused by mutations and/or deletions in the α - or β -globin genes, resulting in anemia. Clinical severity of thalassemias varies widely; it may range from asymptomatic forms to severe or even fatal entities.

There are relatively large number of patients with thalassemia in Fejér county in Hungary, particularly near to Sárbogárd, a region where big marshlands were located long time ago. Some hemoglobinopathies, such as sickle cell anaemia, thalassemia provide protection against malaria, which explains the high prevalence of thalassemia in marshland areas. Most of the thalassemias are beta thalassemia with or without minor symptoms in Hungary.

A by-product of migration we identified some interesting hemoglobinopathies in our laboratory, such as sickle cell anaemia and unusual appearance of thalassemia. A 15 years old boy from Iraq and lived in the reception camp of Bicske town arrived to the emergency department of our hospital. Complete blood count did not indicate thalassemia minor trait, because his red blood count was 3.7 T/L, Hb was 78 g/L, MCV 77 fL, RDW-CV 28%. Hypochromia and microcytosis, aniso-poikilocytosis, target cells, basophil punctations, Howell-Jolly bodies and a great many normoblasts (750 per 100 white blood cells) were observed. Reticulocyte count was very high (193‰). Other chemical parameters were as follows: serum iron 41 $\mu\text{mol/L}$, ferritin 2750 $\mu\text{g/L}$, lactate dehydrogenase 541 U/L. Blood sample of this boy was sent to Department of Laboratory Medicine of the University of Debrecen, where thalassemia specific HPLC examination was done with Bio-Rad Variant Turbo II HPLC system (β -Thalassemia short program). On the basis of results of HPLC analysis (HbF 13.8% and HbA2 4.9%) and other parameters the boy has probably β -thalassemia major. The prevalence of β -thalassemia major will likely increase in Hungary because of migration.

P9**Development of new lupus anticoagulant algorithm**

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Lupus anticoagulant (LA) testing is important for evaluating patients with antiphospholipid syndromes and hypercoagulable conditions. LAs are heterogeneous circulating autoantibodies, directed against epitopes found on negatively charged phospholipid-binding proteins, which

inhibit phospholipid-dependent coagulation reactions in vitro. Paradoxically, except in rare instances, LAs are associated with increased risk of arterial and venous thrombosis, and not with bleeding.

LA-sensitive APTT (activated partial thromboplastin time) based screening test (Diagnostica Stago) and diluted prothrombin time (dPT) (Siemens AG) as confirmatory test were used for LA testing in our laboratory earlier. CLSI 2014 guideline recommends in sequence to start LA testing with two screening tests, each challenging a different coagulation pathway. In this study 96 patients' plasma were tested for LA. LA-sensitive APTT screening assay and dPT confirmatory assay were compared with paired dRVVT (diluted Russell's viper venom time; Siemens AG) test system (screening and confirmatory tests use the same principle).

We identified 23 LA positive results with two types of test system, 9 of them LA positivity were shown with both test systems. 11 plasmas were LA positive with paired dRVVT system (9.4%), but these samples were LA negative with APTT/dPT system. On the other hand only 3 samples (3.1%) were LA positive with APTT/dPT, but were negative with dRVVT system. Our results agree with the results of other studies, because it is known that different type of coagulation assays for LA, such as intrinsic, extrinsic or common pathways differ from each other in sensitivity to LAs. Out of patients with LA positivity based on APTT/dPT system 3-4 months earlier a large number of them presented LA positivity with paired dRVVT system now. According to results dRVVT system seems to be more sensitive for LAs than the other one.

We have possibility for using only one type of screening assay, therefore on the basis of the CLSI 2014 guideline recommendations an algorithm was derived and will be presented for the detection of LAs.

P10

Comparison of routine hemostasis test results obtained by CoagXL with those of Siemens BCS XP in a university lab

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CoagXL (Diagon Company, Hungary) is an increasingly used system for high throughput performance of hemostasis tests. At the Department of Laboratory Medicine, Semmelweis University we compared test results obtained by CoagXL with those of Siemens BCS XP.

During our work we measured prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (F), thrombin time (TT) and D-dimer (DD) levels with the two systems simultaneously in 481, 311, 99, 118, 120 and 158 consecutive samples, respectively. The values measured by the two systems were well comparable for aPTT, (provided the Diagon APTT liquid was compared with Siemens Actin FS) TT and DD values. PT values in terms of INR and aPTT-TR values in terms of seconds differed systematically in high INR range. The PT correlation between the results was $r=0.91$. INR results measured by Innovin reagent on Siemens BCS XP tended to be higher near to the end of linearity, but the systemic difference had minor clinical significance in the majority of patients.

Some references in the literature found similar tendency between the tissue originated and recombinant thromboplastin reagents.

The difference in INR value was less than 0.5 in 97% of sample; just 10 of the 481 samples exhibited higher (+1-+2) INR values by BCS than by CoagXL.

Our conclusion is that for the purpose of routine hemostasis tests CoagXL system is a fully appropriate instrument and fulfills the needs of a routine clinical laboratory.

P11

Epidemiology of invasive infections caused by *S. pneumoniae* isolated in the Department of Clinical Microbiology at the University of Szeged between 2010 and 2015

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Introduction: Despite of the vaccination against pneumococcal infections, *S. pneumoniae* is still one of the leading causes of community-acquired invasive infections. In this study, we examined retrospectively *S. pneumoniae* strains which were isolated from invasive samples in our laboratory between 2010 and 2015. We compared the serotypes of these strains isolated to the serotypes of the vaccines.

Materials and methods: For retrospective analysis, we used our microbiological database. Collected specimens were cultured by conventional method and antimicrobial susceptibility tests were carried out according to EUCAST recommendation. All of the invasive *S. pneumococcus* strains were sent to the National Center for Epidemiology where the serotypes of these strains were determined.

Results: During the study period, 114 *S. pneumoniae* strains were isolated from invasive samples in our laboratory. 91 of 114 strains were isolated from blood culture, 12 from cerebrospinal fluid and 11 from pleural fluid. The mean age of the patients was 55.4 years. 77 (84%) of 114

patients were older than 50 years. The most frequently isolated serotype was the serotype 3 (32 strains (28%). 16 strains (14%) were intermediate resistant to penicillin. Only two strains were resistant to penicillin. 19% of the strains were resistant to macrolides and 17.5% were resistant to clindamycin. 17.5% of the strains were resistant to fluoroquinolones.

Discussion: Compared the vaccine strains to the strains isolated in our laboratory, 21 strains isolated from patients were not among the vaccine strains. 14 of these 21 patients were older than 50 years. According to the recommendation of the Hungarian Infectious Diseases and Clinical Microbiology Society, 55% of these invasive infections could have been prevented with vaccination.

P12

Molecular diagnostic of gastrointestinal Human Cytomegalovirus infection in inflammatory bowel disease

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Inflammatory bowel disease (IBD), including ulcerative colitis (CU) and Crohn's disease (CD), is chronic inflammation of colonic mucosa. In most cases, genetic and environmental factors, as well as immunoregulatory disorders play a role its development. As one of the main targets of the tissue-invasive human cytomegalovirus (CMV) infection is gastrointestinal (GI) tract, CMV infection is considered as a major complicating factor in IBD. Rapid and exact detection of CMV seems to be essential in CU and CD, and especially critical in immuno-compromised individuals. CMV infection of the GI tract cannot be diagnosed solely by endoscopy and histology. Human cytomegalovirus (HHV-5) is a member of the herpesvirus family and is prone to persist in latent form throughout the host's lifetime after primary infection. Partial reactivation of CMV can be detected in the inflamed colonic mucosa only by specific Polymerase Chain Reaction (PCR), as immediate early genes are expressed primarily in the course of local reactivation of CMV. Therefore, it is important to distinguish between general and local virus infection in the GI tract. The presence of CMV in the intestinal mucosa causes resistance to steroid treatment, especially in CU patients. A quantitative PCR assay (Geneproof CMV PCR assay, specificity 100%, sensitivity 95%) helps monitoring the progress of the infection, as well as therapy. Our method allows to process different clinical sample types (blood, urine, colon-, rectum- or sigma-bowel biopsy tissues). In our laboratory a total of 286 clinical samples, obtained from 136 patients treated in Gastroenterology Unit (age of 40±14 years) were analysed for CMV real-time PCR from 2012 to 2015. Of them, 157 (54.9%) were collected from blood, 112 (39.2%) from tissue and 17 (5.9%) from urine. 76 (26.5 %) samples of 40 patients were positive for CMV DNA, so we had a chance to monitor the therapy in some patients, as well. The distribution of the 76 CMV-DNA positive samples was: 46 (60.5%) tissue; 27 (35.5%) blood, and 3 (3.9%) urine. Although we could prove the local gastrointestinal CMV infection only in 27 (19.8%) patients, it is worth mentioning that it made 67.5% of the 40 CMV positive patients. 39 CMV-DNA positive cases were treated with ganciclovir, resulting improvement of the clinical symptoms in 19 (56.4%) patients. In summary, early and accurate diagnosis of gastrointestinal CMV infection is essential for the proper treatment of IBD. For IBD patients, quantitative PCR assay is useful to detect and quantify CMV-DNA in clinical tissue samples.

P13

Assessment of hepatitis E seropositivity among patients with acute hepatic injury in the United Szent István and Szent László Hospitals

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The hepatitis E virus (HEV) is one of the most common causes of acute hepatitis worldwide, predominantly in developing countries. It occurs sporadically in most European countries, including Hungary, and most of the cases are supposed to be of zoonotic (swine) origin. We reviewed the results of the serological tests carried out in 2015 in the Molecular Biology Laboratory of our hospital that were carried out presumably to specify the cause of acute hepatic injury. Our aim was to assess the possible benefits of expanding our diagnostic capacity (regarding both the number of processed samples and serologic tests available). Due to the aspecificity of BNO (international classification of diseases) diagnosis codes we enrolled the results of those tested for at least HEV-IgM and/or two other markers specific for acute infections with hepatotropic viruses (HAV-IgM/HAV-total, HBSAg, anti-HBc, anti-HCV, CMV-IgM, EBV-IgM). In total we analyzed the data of 1761 patients. A clear diagnosis was made in 757 cases, out of which 30 patients were found to be HEV-IgM positive (3.96%). However, 8.9% of all HEV-IgM ELISA tests (n=337) were positive. In only 20.7% of the eventually non-diagnostic panels HEV-IgM was also determined, although in nearly third of them four or more markers were included. Our results suggest that the prevalence of hepatitis E in our hospital among patients with acute liver injury is comparable to EBV and CMV infections. Furthermore, up to half of the cases might remain unrecognized. After ruling out the most common causes of acute hepatitis considering the possibility of hepatitis E is advisable, and the evaluation of the serologic status of currently asymptomatic individuals might be considered in selected cases (particularly in pregnancy).

P14

Enterobiosis diagnosed during colonoscopy in adults

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We describe two cases of *Enterobius vermicularis* infection in adults, diagnosed by colonoscopy. In October 2015, two female patients (aged 60 and 73 years) were admitted to the Gastroenterology Department of Hungarian Defense Forces Medical Centre, Military Hospital with unspecific but relevant gastrointestinal symptoms such as lower abdominal discomfort, changing in stool habits. The patients had different underlying diseases, but there were no actual weight loss, contact with pets or travel abroad in the anamnesis. Physical examination and initial laboratory findings were normal, except mild anaemia. Clinical specimens of each patient were sent to faecal bacteriological culture and parasitological stool examination. All of the results were negative. According to our protocol and considering the patient's age they had undergone colonoscopy.

In both patients, during the colonoscopy approx. 10 mm long whitish helminths appeared in the sigma. The worms were caught with the forceps of the colonoscope and sent to the hospital's Laboratory. In the samples injured worm-parts have been found and several typical ova of *E. vermicularis* in the sediment of the transport fluid were identified.

The findings in these cases suggest that infection caused by *E. vermicularis* should not be neglected in adults with atypical gastrointestinal symptoms, and in some cases it could be diagnosed by colonoscopy.

P15

Use of MALDI-TOF method for the identification of yeast isolates

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Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry is a very useful, inexpensive and rapid technique to identify bacteria and mycobacteria. The aims of our study were to examine its' availability in the identification of fungal isolates in routine microbiological laboratory diagnostics and to compare the effectivity of fast formic acid (FFA) and complete formic acid/acetonitrile extractions (CFAAE) pretreatment procedures. Fungal identification was performed from yeast strains cultured from human clinical samples by MALDI-TOF and compared with conventional methods: (CHROMagar, rice agar) based on micro-and macromorphology properties and/or biochemical methods. We examined 100 isolates: *Candida albicans* (5), *C. dubliniensis* (1), *C. glabrata* (38), *C. inconspicua* (5), *C. kefyr* (6), *C. krusei* (7), *C. lusitaniae* (4), *C. parapsilosis* (10), *C. tropicalis* (11), *Geotrichum capitatum* (1), *G. sylvicola* (2), *Rhodotorula mucilaginosa* (1), *Saccharomyces cerevisiae* (9). During our study the LogScore values of CFAAE were between (1.436-2.454), of the FFA were between (1.199-2.155). Based on the average LogScores values the CFAAE (2.017) had better effectivity than the FFA (1.734) pretreatment procedure. According to our experience the effectivity of MALDI-TOF for fungal identification is not as good as in case of bacteria. Analysis by MALDI-TOF results indicates the usefulness of complete formic acid/acetonitrile extractions pretreatment procedure.

P16

HBV vaccination and actual immunity among pregnant women

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Infections caused by Hepatitis B (HBV) are one of the most widespread infectious diseases in the world. 2-20% of the European population is already infected with the virus, and because every 10% of the infection becomes chronic, the ratio of cirrhosis and liver cancer (HCC) caused by chronic infection is 0.2–2% in Europe. Vaccination against HBV is the most effective way of preventing the infection. Since the introduction of vaccination, the number of infected persons has decreased to 1% or below in Europe and in Hungary during the past 15-20 years. After recognizing the importance of prevention of hepatitis B infection, teenagers have been compulsorily vaccinated since 1999 in Hungary. As a consequence of this, 95% of the 14-31 years old population is „theoretically” protected against the disease. The marker for successful immunization is the anti-HBs, which is an antibody developing after hepatitis B infection and after successful vaccination. After vaccination and in case of successful immunization only the anti-HBs antibody can be detected, other markers are negative.

The source of infection: asymptomatic people carrying the virus or patients with known acute or chronic symptoms.

Transmission: parenteral, sexual contact, blood, blood derivatives, medical equipment and instruments contaminated with blood, tissue fluid, secretions, transplantation, intravenous drug users, and from infected mothers to new-borns. Children of those young mothers, who were tested and found positive for HBsAg in the 16th week of their pregnancy according to the Hungarian practice, will be given active and passive immunization, but the actual immunity of the mothers against HBV is not tested.

Materials and methods: At the Department of Molecular Biology we analysed anti-HBs level in 150 selected cases between January 2015 and March 2015.

Results and conclusions: Our results show that no immunity was developed in 18% of pregnant women receiving vaccination, a booster vaccination would be necessary for 39% of the women. Furthermore, another very interesting finding of our study was recognized during the examination of the anti-HBc IgG. Among the 107 pregnant women who have already been vaccinated we found positive anti-HBc IgG result in the case of seven women (5.6). In summary we can say that whenever it is possible the determination of anti-HBs level with additional serological markers should be considered to determine the exact Hepatitis B status of pregnant women.

P17

Application of MALDI-TOF MS for detection of highly virulent *Streptococcus agalactiae* ST-17 and ST-1 clones in Group B *Streptococcus* screening of pregnant women

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Streptococcus agalactiae (Group B *Streptococcus*, GBS) is considered as one of the leading causes of neonatal sepsis and meningitis. This Gram-positive β -haemolytic bacterium may cause invasive diseases in new-borns or pregnant and postpartum women. Vaginal or rectal GBS colonization is detected by a culture-based screening strategy to indicate intrapartum antibiotic prophylaxis. The aim of the present study was to analyse the results of GBS screening among pregnant women at Albert Szent-Györgyi Clinical Center between 01 October 2015 and 05 January 2016 and to present our experiences in relation to the application of MALDI-TOF MS in the GBS screening.

S. agalactiae colonization status was determined by collecting vaginal or cervical specimens. Screening for GBS colonization was performed according to the recommendations of CDC 2010. Antimicrobial susceptibility testing was performed according to the recommendation of EUCAST. MALDI-TOF MS analysis was performed for identification of *S. agalactiae* and rapid detection of highly virulent Group B *Streptococcus* ST-17 and emerging ST-1 clones.

During the examined period, we tested vaginal or cervical samples of 1326 pregnant women for *S. agalactiae*. GBS-colonization was detected in 198 cases (14.9%). For each positive strain, the antibiotic susceptibility was examined. All isolated strains proved to be susceptible to penicillin, β -lactams and vancomycin, while 72% and 69% of the strains were susceptible to clindamycin and erythromycin, respectively. At the same time, 28% and 31% proved to be resistant to the same two compounds, respectively. Mass spectra of 100 isolated *S. agalactiae* strains identified by MALDI-TOF MS were analysed for detection of highly virulent Group B *Streptococcus* ST-17 and emerging ST-1 clones. 23% and 30% of the examined GBS strains proved to be ST1 and ST 17 clones, respectively.

Detection of the GBS colonization status and the antibiotic susceptibility of the isolated strains are important data for the determination of the appropriate intrapartum antibiotic prophylaxis. Application of MALDI-TOF MS in *S. agalactiae* screening is a rapid possibility to detect highly virulent emerging ST1 and ST17 GBS-clones and helps to manage prevention of invasive GBS infection.

P18

Alimentary related (Pho soup) Hepatitis A infections in September 2015

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The inflammation of the liver called hepatitis can be caused by hepatitis viruses A, B, C, D and E. These viral infections have common symptoms: jaundice, nausea, vomiting, fever and abdominal pain. However, hepatitis A (HAV) infection appears only with a few, or no clinical symptoms, especially at younger ages. This virus produces fulminant hepatitis and death only in a very small proportion of patients. Hepatitis A is a non-enveloped, positive stranded RNA virus, which belongs to the Hepatovirus genus of the Picornavirus family. The virus can be transmitted from person to person mainly via the fecal-oral route. In September 2015, we have noticed an increased number of HAV IgM positive patients. 27 from 55 new case have been proved and further 24 patients had contact with a Far-Eastern Restaurant, where the possibly source of the infection was the Pho soup. All of these patients had eaten from this Vietnamese food during August 2015. Typical

clinical signs were usually liver inflammation accompanied with jaundice, liver-function damage and low-grade fever. Positive serological data were paired with significant bilirubin and liver-enzyme level elevations (SGPT, SGOT, alkaline phosphatase, gamma GT, and LDH). The average of HAV IgM factor positivity (s/co) was 5.7 (positive sample is greater than 2) and the average of liver enzyme and bilirubin levels were elevated (SGPT=1692 U/L; SGOT=838.8 U/L; alkaline phosphatase =744.6 U/L; Gamma GT=337 U/L; LDH= 459 U/L; bilirubin 142.6 $\mu\text{mol/L}$).

In conclusion, HAV epidemics in Hungary are usually caused by poor sanitary conditions, whereas our cases were clearly food-derived infections.

P19

Prevalence and typing of ESBL-producing and carbapenem resistant *Klebsiella pneumoniae* strains between inpatient- and outpatient units of J6sa Andr6s hospital, in 2015

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The spread of carbapenemase-producing *Enterobacteriaceae* is a global problem. Infections due to these microorganisms limit treatment options for patients and are associated with longer hospitalization, and increased morbidity and mortality. Between January and December 2015 extended-spectrum β -lactamase (ESBL) producing and carbapenem resistant (KPC) *Klebsiella pneumoniae* were isolated from 57 samples of 57 children in perinatal intensive care unit and pathological premature children unit (49 perianal swabs, 3 tubes, 2 canullas, 1 blood culture, 1 abdominal and 1 environmental sample). At the same time, in adult patient group ESBL-producing KPC strains from 38 samples of 36 patients (18 urines, 10 BAL-s, 5 wounds, 1 ascites, 1 sputum, 1 perianal swab, 1 catheter and 1 blood culture) were also isolated. 7 samples were obtained from outpatient care unit. This pathogen was isolated in intensive care unit from 12 samples of 10 patients. The antibiotic resistance pattern was determined by EUCAST disc diffusion method or E test or VITEK2 resistance system. ESBL-production of *K. pneumoniae* was determined by double disc synergy test (DDST) or VITEK2 system. The percentage of KPC resistant (R), intermediate (I) and susceptible (S) to carbapenem antibiotics isolated from children was as follows: ertapenem 91.4%, 8.6%, 0%, imipenem 10.3%, 43.1%, 46.6%, meropenem 10.3%, 12.1%, 77.6%, respectively. The percentage of KPC resistant (R), intermediate (I) and susceptible (S) to carbapenem antibiotics isolated from adults of was as follows: ertapenem 88.6 %, 11.4%, 0%, imipenem 0%, 25.7%, 74.3%, meropenem 0%, 11.4%, 88.6%, respectively. All of the investigated strains proved to be ertapenem non-susceptible. The verification of carbapenemase production and its type was confirmed by the National Center for Epidemiology. Except one, every clinical isolate was identified as VIM type metallo- β -lactamase producer. It is known that VIM type carbapenemase producers have usually low level of carbapenem resistance so that the carbapenemase detection can be very difficult. CarbaNP test confirms carbapenemase production relatively short period of time hence this may give a therapeutic help for clinicians, but all suspected isolates need to verify with molecular method.

P20

Real-time automated PCR for the detection of Cytomegalovirus (CMV) viraemia and monitoring after bone marrow transplantation

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Objective: The objective of this study was to determine that the monitoring and the surveillance of the cytomegalovirus (CMV) reactivation with quantitative real-time PCR can assist for the confirmation of the clinical diagnosis.

Methods: Retrospective epidemiological analysis of the patient records from the adult and children bone marrow recipients, during the period of 01/01/2015 to 30/06/2016. The examinations were carried out with the COBAS® Ampliprep/COBAS® TaqMan® (Roche) and the QIASymphony® (QIAGEN) systems.

Results: In our results we are discussing the followings:

- how big the rate of the virus reactivation is between the children and the adult patients
- how the virus reactivation can predestinate the further increase of the copy number
- after the first reactivation, what the probability is for detecting the presence of the virus again

Conclusion: The quantitative RT-PCR screening for the CMV viral load is crucial after the bone marrow transplantation. Following the exact copy numbers, repeated sampling is required within four days, as it is provided in our hospital methodology.

P21**Associations between genetic variants in DAB, PRKAG, and DACH genes and chronic kidney disease by multiplex high resolution melting**

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A recent genome-wide association study demonstrated the association between the prevalence of chronic kidney disease (CKD) and rs11959928, rs626277, and rs7805747 polymorphisms. In this study, we investigated the association between CKD and these polymorphisms in patients and controls according to gender. High resolution melting (HRM) analysis was performed to detect DAB2 rs11959928, DACH1 rs626277, and PRKAG2 rs7805747 single nucleotide polymorphisms. Genomic DNA was extracted from buffy coat of 200 patients with chronic renal disease and 250 control individuals. Ten percent of the results were also randomly confirmed by direct DNA sequencing.

Multivariable logistic regression analysis with adjustment for confounders showed that rs7805747 (dominant model) has statistically significant protective effect in females, and rs11959928 (additive and dominant models) was significantly associated with the prevalence of CKD in males. rs7805747 (recessive model) was significantly associated with the prevalence of CKD in males.

The very same genetic variants have different effects in males and females, separately. Our results warrant the need of similar studies in larger cohorts.

P22**Diacylglycerol kinase epsilon (DGKE) mutations in two patients with atypical hemolytic uremic syndrome**

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Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy (TMA) characterized by hemolytic anemia, thrombocytopenia and acute renal failure. Atypical HUS (aHUS) in which the pathogenesis is related to dysregulation of the alternative complement pathway, leads to uncontrolled activation of the complement system, resulting in endothelial cell damage and TMA. In about 50-70% of aHUS patients, mutations can be identified in genes of complement components and regulators. In addition, homozygous or compound heterozygous variations in the *DGKE* gene were recently described in aHUS patients. *DGKE* is expressed in endothelium, platelets and podocytes but the exact mechanism how loss-of expression is associated with development of aHUS is not known.

We report histories of two patients with aHUS (2 girls, 7 and 17 months old), whose clinical symptoms were characteristic for TMA, but at presentation they also had nephroso-nephritis syndrome with heavy proteinuria and hematuria. Molecular genetic analysis identified mutations in *DGKE* gene (one patient was found to carry heterozygous variations, one in exon 6 (c.966G>A), causing the generation of a premature stop codon at amino acid position 322 (p.W322X) and one in exon 3 (c.559delA), the other patient was homozygous for the p.W322X variation). The patients were initially treated with plasma infusions, one patient responded well whereas the other patient was treated subsequently with eculizumab. However, after identification of *DGKE* variations only supportive therapy was given, and the patients showed finally resolution of kidney damage.

P23**Using traditional and polymerase chain reaction based biodosimetric tools in case of disaster recovery**

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Tumor disease can emerge years after the exposure to ionizing radiation. The DNA damaging effect of radiation can be detected just after the event with biological dosimetry, in order to give a chance to prevent the further damages. For many years the dicentric chromosome assay and cytokinesis-block micronuclei test were the most frequently used methods. In these tests the lymphocytes, which are non-dividing cells

in the peripheral blood need induction and a few days incubation for formation of chromosomes. Analysis of specimens is performed with conventional microscopy by trained personnel, so the result can be subjective. Finding new and quicker methods to complete our palette, the mitochondrial DNA deletion seems to be a useful test. The polymerase chain reaction (PCR) technique provides a new tool for dosimetry in order to avoid the time consuming cell culturing and microscopic analysis. As the mitochondrial genome possesses weak repair properties, it can be a sensitive indicator of the stochastic damages even at low dose radiation. Ionizing radiation damages not only the nuclear DNA, but the mtDNA as well. Exposure to ionizing radiation is followed by enhanced leakage of reactive oxygen species leading to different mtDNA deletion formations, the most frequent out of these deletions is the „common deletion”. Our laboratory is equipped with an appropriate PCR device for quantifications of these deletions, hereby the assessment of the general damage of DNA in the cell. Though measuring the ratio of mitochondrial deletion alone is not enough for accurate determination of the absorbed dose, however it provides a result quickly. This PCR based technique can be crucial at triage in case of emergency.

P24

Immune cell-derived extracellular vesicles differ in their RNA content depending on the cellular state and EV subtype

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Introduction: Extracellular vesicles (EVs) are membrane-surrounded structures secreted by all types of cells, having an important role in intercellular communication, via e.g. horizontal transfer of RNAs. Here, we looked at the RNA content of EV subpopulations secreted by immune cells in different functional states.

Methods: EV subpopulations - exosomes, microvesicles (MV) and apoptotic bodies – were isolated from the supernatant of steady state or activated (PMA, LPS or ConA) human monocytes and mouse T-cells. In some cases, nuclear export of RNA was inhibited by Leptomycin B. EVs were isolated by differential centrifugation and gravity-driven size-based filtration. Following RNA isolation, Agilent Pico and Small chips were used to determine the quality and profile of RNA that was further analyzed by fragment analysis and Taqman assays.

Results: Substantial differences were found in the RNA cargo of different EV subtypes. Exosomes had the highest small RNA/total RNA ratio and the lowest rRNA/total RNA ratio. The small RNA (≤ 200 nt) content of MVs was inducible. The small RNA profiles of the different EV subtypes were more similar to each other than to those of the releasing cells.

Conclusion: Our data suggest that variation of the RNA cargo (depending on the EV subtype and the functional state of the releasing cells), may endow EVs with an important role in the epigenetic regulation of the immune system.

P25

Depressed level of platelet miR-223, miR-26b and miR-140 induced by hyperglycemia may affect platelet activation in type 2 diabetes mellitus

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Platelet microRNAs are considered as novel regulators of platelet activation. Here we analyzed platelet miR-223, miR-26b and miR-140 in type 2 diabetes mellitus (DM2). MiRNAs were isolated from leukocyte-depleted platelets obtained from 28 obese DM2, 19 non-DM obese and 23 healthy individuals. The effect of hyperglycemia on miRNAs was also evaluated in MEG-01 megakaryocytes under hyperglycemic conditions up to 4 weeks. Quantitation of mature miRNA levels was performed by UPL-based RT-qPCR, while pre-miRNAs and target mRNA levels (P2RY12 and SELP) were measured by RT-qPCR. To prove the association of miR-26b/miR-140 with SELP (P-selectin) mRNA level, overexpression of these miRNAs was performed using miRNA mimics in MEG-01 cells for 24 hours. Platelet activation was evaluated via P-selectin by flow cytometry. Mature and pre-forms of miR-223, miR-26b and miR-140 were significantly depressed in DM2 platelets, and P2RY12 and SELP mRNA levels were augmented by 2-fold at increased platelet activation based on P-selectin vs. obese or healthy counterparts. Gradually and significantly reduced miRNA expressions were observed in MEG-01 cells induced by hyperglycemia vs. baseline values, while miR-26b and miR-140 mimics resulted in decreased SELP mRNA level. In conclusion, these miRNA alterations may contribute to abnormal platelet function.

P26

Regulation of plasma factor XIII levels in healthy individuals; a major impact by subunit B intron K F13B:c.1952+144C>G polymorphism

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Blood coagulation factor XIII (FXIII) is a pro-transglutaminase, consisting of two catalytic A (FXIII-A) and two carrier/inhibitory B (FXIII-B) subunits. There is only scarce information on the effect of the environmental and genetic factors on FXIII levels. The aim of this study was to investigate the relationship of FXIII levels with age, sex, smoking and FXIII polymorphisms. 268 apparently healthy young and middle-aged individuals from Eastern Hungary were enrolled in the study. FXIII activity, FXIII A₂B₂ antigen, FXIII-B antigen and fibrinogen levels were measured. FXIII-A p.Val34Leu, FXIII-B p.His95Arg, FXIII-B Intron K (F13B:c.1952+144 C>G) polymorphisms were determined. Age and fibrinogen levels significantly correlated with FXIII levels. There was also a significant correlation between FXIII-B antigen levels and FXIII activity, as well as FXIII A₂B₂ antigen levels. The presence of FXIII-B Intron K G allele resulted in significantly lower FXIII activity, FXIII A₂B₂ and FXIII-B antigen levels. The presence of the FXIII-B R95 allele elevated the FXIII activity, FXIII A₂B₂ and FXIII-B antigen levels significantly. FXIII-B Intron K polymorphism decreased FXIII-B antigen levels, and FXIII A₂B₂ antigen levels, thereby decreasing FXIII activity. Carriers of both the FXIII-A Leu34 and FXIII-B Intron K G allele had significantly lower FXIII activity and FXIII A₂B₂ antigen levels than wild type individuals. Age, unadjusted and adjusted fibrinogen levels significantly correlated with FXIII levels. There was also a significant correlation between FXIII-B antigen levels and FXIII activity, as well as FXIII A₂B₂ antigen levels. The presence of FXIII-B Intron K G allele resulted in significantly lower FXIII activity, FXIII A₂B₂ and FXIII-B antigen levels. The presence of the FXIII-B R95 allele elevated the FXIII activity, FXIII A₂B₂ and FXIII-B antigen levels significantly. FXIII-B Intron K polymorphism decreased FXIII-B antigen levels, and FXIII A₂B₂ antigen levels, thereby decreasing FXIII activity. Carriers of both the FXIII-A Leu34 and FXIII-B Intron K G allele had significantly lower FXIII activity and FXIII A₂B₂ antigen levels than individuals with the combination of intron K C homozygosity with either of the FXIII-A p.Val34Leu variants.

P27

Effects of antithrombin, protein C, protein S and EPCR polymorphisms on the plasma levels of natural anticoagulants in healthy individuals

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Antithrombin (AT), protein C and protein S (PC and PS) are natural anticoagulants. The endothelial protein C receptor (EPCR) plays a role in PC activation. Several single nucleotide polymorphisms (SNPs) have been reported in the genes encoding AT, PC and PS (*SERPINC1*, *PROC* and *PROS1*) with uncertain consequences. A multiplex PCR primer extension assay has been established by us to detect 12 SNPs simultaneously. The aim of this work was to investigate whether these polymorphisms have an effect on AT, PC and PS plasma levels in healthy individuals. The median age of the recruited persons (n=366) involved in the study was of 36 years (range 18-85 years), the proportion of women was 58.1%. AT activity was determined by progressive activity (p-AT) and heparin cofactor assay (hc-AT), PC activity was measured by chromogenic assay; PS was measured for free PS antigen levels. The presence of *PROC* promoter SNPs rs1799809 and rs1799808 increased, while the rs1799810 reduced PC activity, other *PROC* SNPs had no effect on it. The *PROCR* rs8119351 and rs867186 increased the PC level and the PC rs867186 homozygous individuals (n=2) had a particularly high PC activity (160 and 180%). The *PROS1* rs121918472 reduced PS levels (106 vs 77%, p=0.012). In conclusion, the PC levels are affected by some *PROC* and *PROCR* SNPs. The *PROS1* rs121918472 (Heerlen polymorphism) significantly reduces the concentration of free PS level. Exploration of the clinical relevance of these laboratory observations may be interesting from the point of view of thrombotic risk assessment.

P28

Study of TSH circadian rhythm in hypothyroidism or other disease under clinical treatment conditions

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Background: In Hungary hypothyroidism is one of the most common diseases, which is usually treated by thyroxine therapy. In some cases, we found considerable differences between TSH levels of treated patients sampled at different time in our laboratory. The aim of this study

was to investigate the changes of TSH circadian rhythm in thyroxine-treated patients as compared to non-treated control group and to detect the effect of the individual differences on this process.

Methods: Data were collected with the help of the staff of I. Department of Internal Medicine in Pest Country Flór Ferenc Hospital. In this work 25 treated patients and 27 non-treated patients were studied. Blood was taken at 6.00 am and 6.00 pm. Inter-, intra-assay variability of the method was calculated, data were analyzed using Medcalc Microsoft Excel program.

Results: The quality control 1 and 2 of inter-assay variability (CV%) were 1.69 ± 0.07 , and 8.44 ± 0.28 , respectively. In case of intra-assay variability the quality control 1 was 1.64 ± 0.03 and the quality control 2 was 8.41 ± 0.12 . The average age of control group was 58 ± 14 years and of the treated group was 65 ± 8 years. The mean values of TSH level of the control group were 1.69 ± 1.13 mIU/L in the morning and 1.44 ± 1.10 mIU/L in the evening. In case of the treated group these values were 3.31 ± 2.33 mIU/L and 2.81 ± 2.26 mIU/L, respectively. Although higher mean values of TSH were obtained during the morning sampling in both groups, this difference was not significant (control $p = 0.40$, treated $p = 0.85$). On the other hand TSH variability in respect of gender, age and body weight showed significant differences in both groups (control group: age $p < 0.0001$, gender $p < 0.0001$, body weight $p < 0.0001$, treated group: age $p < 0.0001$, gender $p < 0.0001$, body weight $p < 0.0001$).

Conclusions: These results suggest that the time of sampling does not influence the clinical decision-making but the individual variability must be considered.

P29

Evaluation of the laboratory method of public health screening program for colorectal cancer screening in Hungary

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Public health screening programs are cancer mortality reduction tools and as such crucial components of the National Public Health Program launched in 2003. The screening project for colorectal cancer, started in 2004 has been suspended in 2007 by the Health Government. A multidisciplinary Working Group (WG) reviewed literature and guidelines to define evidence-based recommendations, and the program has been re-established in 2009. The automated, quantitative fecal immunochemical tests (FIT) were purchased in seven consecutive tendering processes by the National Public Health and Medical Officer Service (NPHMOS). During the period between June 2009 and October 2015, the examination of 83,889 samples of 44,645 inhabitants was performed in the limited screening program by the use of three different quantitative FIT-s, in successive periods. In the first period (FOB Gold; Sentinel, Italy) in response to the invitation letters 17,306 inhabitants returned 32,837 fecal samples. 12.97% of the samples proved to be positive (negatives 79.84%, inappropriate samples 5.41%) Of the positives 801 patients were directed to endoscopic examination, 399 of those participated in the test. The results of the colonoscopy were 213 negative cases (53.38%), 76 cases of identified adenoma (19.05%) and 14 cases of diagnosed carcinoma (3.51%). In the second period (FOB-Check 2; Vedalab, France) 12,669 inhabitants returned 22,892 fecal samples. 28.14% of the samples proved to be positive (negatives 61.29%, inappropriate samples 10.68%). There are no data about endoscopic examinations (because of confusion caused by the high positivity rate). In the third period (OC Sensor; Eiken, Japan) 14,640 inhabitants returned 28,150 fecal samples. 11.96% of the samples proved to be positive (negatives 84.66%, inappropriate samples 3.98%). Of the 1,675 positive patients from the 2013-2014 period directed to endoscopic examination 306 participated in the test. The results of the colonoscopy were 69 negative cases (22.55%), 153 cases of identified adenoma (50.00%) and 13 cases of diagnosed carcinoma (4.25%). Based on the three different FITs experiences it was concluded that the two fully-automated quantitative tests (FOB Gold and OC Sensor) technical applicability regarding the analytical and clinical performance index both proved to be suitable for population screening tasks, in accordance with the European guideline and international experiences.

P30

Novel urinary protein markers in sepsis

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Diagnosis and monitoring of sepsis is challenging even nowadays. Although proteinuria is a well-known phenomenon in sepsis, the clinical usefulness of urinary proteins has not been explored yet. In the present work, we investigated different urinary proteins as diagnostic and severity markers of the septic process. The acute phase protein orosomuroid, the cell component actin and cystatin-c were measured in urine samples of septic ($n=35$) and control ($n=30$) patients. Urinary orosomuroid (u-ORM) and cystatin-c (u-CYSC) were measured by automated immune turbidimetry, while urinary actin (u-ACT) levels were determined by quantitative western blot. We referred these concentration data to urinary creatinine levels. Our data are presented as medians.

240-fold higher u-ORM values were found in sepsis than in controls (19.31 vs 0.08 mg/mmol, $p < 0.001$) and additional extreme u-ORM levels were measured in dialyzed septic patients.

We could not detect u-ACT in urine samples of controls in contrast to sepsis. Significantly elevated u-ACT was found in samples of patients with sepsis-related acute kidney injury (AKI) compared to non-AKI patients (1.67 vs 1.05 ng/mmol, $p < 0.05$).

U-CYSC levels were higher in septic patients compared to controls as well (0.234 vs 0.007 mg/mmol, $p < 0.005$).

The early and relevant increase of u-ORM suggests that it might be a promising novel diagnostic marker of sepsis. U-ACT concentrations might indicate acute kidney injury. U-CYSC is a reliable marker of tubular damage. These novel parameters provide useful information on the septic process and could help the clinicians in rapid decision making.

P31

Serum Gc globulin and gelsolin as sepsis markers

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For successful therapy of sepsis, clinicians need early and predictive biomarkers. We investigated the predictive value of two actin binding proteins: serum group-specific component (Gc globulin) and gelsolin (GSN) in SIRS and in sepsis. Serum samples of 32 septic and 12 SIRS patients from our university's Intensive Care Unit were obtained on day 1, 3, 5, after clinical diagnosis. The control group consisted of 28 ophthalmologic patients. Serum Gc globulin measurements were performed by an immune turbidimetric assay on Cobas 8000/c502 analyzer (Roche), GSN levels were estimated by quantitative chemiluminescence Western blot. PCT, hsCRP levels were determined by automated routine laboratory techniques and first-day clinical scores were also calculated. Data were expressed as medians and interquartile ranges (IQR). Septic and SIRS patients exhibited significantly lower first-day Gc globulin [sepsis: 158.2 (48.6-220.6) mg/L; SIRS: 195.5 (158.9-251.7) mg/L; $p < 0.01$] and GSN levels [sepsis: 15.2 (5.8-24.1) mg/L; SIRS: 31.6 (23.6-40.8) mg/L; $p = 0.001$] compared to controls [Gc: 341.2 (269.6-362) mg/L; GSN: 65.1 (38.8-89.2) mg/L]. First-day GSN levels were significantly higher ($p < 0.01$) in SIRS than in sepsis. Septic patients with 7-day mortality showed lower ($p < 0.05$) first-day Gc globulin (46.7 mg/L) and GSN concentrations (8.9 mg/L) than surviving septic patients (Gc: 194.9 mg/L; GSN: 16.9 mg/L). ROC area under curve of Gc globulin was 0.79 ($p < 0.05$) for predicting 7-day mortality in sepsis compared to PCT (0.75) and GSN (0.74). Based on our results, both Gc globulin and GSN are promising predictive markers in sepsis.

P32

Examination of the cell-mediated immunity in unsuccessful in vitro fertilization

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Reproductive problems, recurrent spontaneous abortions affect millions of women worldwide. It is called infertility in case the pregnancy does not occur within one year despite regular sexual life without using birth control methods, or in case the pregnancy ends several times with spontaneous miscarriage. One approach for treatment of infertility is the in vitro fertilization (IVF). However, this treatment is a great burden for families psychologically and financially as well. For these reasons, it is important to find the possible cause of the recurrent failure. One opportunity of the laboratory testing is to examine the cell-mediated immune response. In this study the cell-mediated immunity has been examined in 197 women who have undergone at least two unsuccessful IVF. The T-cell subpopulations (helper, suppressor and activated T-cells), the B and NK-cell ratio have been tested. Furthermore the NK-function also has been evaluated. The measurements were performed by flow cytometry and the results were analyzed by statistical tests. In the background of unsuccessful IVF the following differences have been found: increased NK-cell ratio, enhanced NK-function and decreased helper/suppressor ratio. These differences might disturb the early stage of embryogenesis, the implantation and the development of the transferred embryo.

P33

Changes in lipid and platelet parameters in Hungarian patients with subclinical hypothyroidism

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Background: Subclinical hypothyroidism (SCH) is diagnosed when peripheral thyroid hormone levels are within the reference range but serum thyroid-stimulating hormone (TSH) levels are mildly elevated. This condition occurs in 3% to 8% of the general population. SCH is more common in women than men and its prevalence increases with age. 80% of the patients with SCH have a serum TSH of < 10 mIU/L. The

most important implication of SCH is the high likelihood of its progression to clinical hypothyroidism. SCH is also a known risk factor for cardiovascular disease.

Aim: We investigated the relationships between thyroid, lipid and platelet parameters in SCH patients who were tested in our laboratory.

Patients and methods: 420 retrospectively selected patients with subclinical hypothyroidism and 283 euthyroid healthy subjects matched for age and gender were enrolled in the study. We created two groups, one with TSH level between 4-5 mIU/L (220 patients) and another with TSH level between 4-10 mIU/L (420 patients). We measured the hormones and the lipid parameters serum cholesterol, serum triglyceride, high and low density lipoprotein cholesterol on Beckman Coulter DXI 600 and Olympus 640 analysers, and the blood count on Abbott CellDyn Zaphire instrument. For the evaluation we used SPSS and ANOVA statistical analysis.

Results: In subclinical hypothyroidism when the TSH level was 4-5 mIU/L, there was a weak negative correlation $r = -0.25$, $p < 0.0001$ between TSH and platelet count, but the MPV (Mean Platelet Volume) was significantly higher (9.2 ± 3.6 fl) than in the control group (8.3 ± 1.4 fl). At the TSH level of 4-10 mIU/L we found a weak correlation between TSH and cholesterol ($r = 0.21$, $p < 0.0001$). MPV was significantly higher in this group compared to control group (9.2 ± 1.4 fl vs. 8.3 ± 1.4 fl.). There was no connection between TSH and other lipid parameters.

Conclusion: Our data show that SCH patients have increased MPV values probably caused by their increased platelet activation. Higher serum cholesterol is another risk factor, which contributes to the increased risk of cardiovascular complications in Hungarian patients. Further research is needed to find the reason of the negative correlation between TSH and platelet count, in patients with subclinical hypothyroidism.

P34

Kappa free light chain in the central nervous system

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Several studies recently indicated the potential diagnostic value of kappa free light chains (KFLCs) in Multiple Sclerosis (MS) from cerebrospinal fluid. Beside intact immunoglobulins, plasma cells secrete an excess of free light chains, which accumulate in the cerebrospinal fluid (CSF) in case of intrathecal B cell activity.

CSF samples and paired serum were collected by lumbar puncture. The KFLC values, albumin and IgG were measured by nephelometry (SIEMENS, The Binding Site). IgG oligoclonal bands were detected by isoelectric focusing and immunostaining (SEBIA).

Samples were excluded:

- CSF samples with blood contamination (>500 erythrocytes/ μ l)
- blood brain barrier (BBB) dysfunction
- 5. type oligoclonal IgG bands (OGP)

There are some limitations of this method. It has been stated that KFLC is not a specific marker of inflammation. The quantitative KFLC values depend on the existence of intrathecal humoral immunoresponse only and are independent of the clonality. It is important, that the value of KFLC correlate well with the activity of disease and serum and CSF KFLC values correlate with age.

The findings support the diagnostic value of KFLC synthesis, although this is not the alternative of OGP detection. The detection of intrathecal KFLC synthesis could have clinical significance when the oligoclonal IgG test is negative or borderline.

Conclusions: The measurement of CSF KFLC is a rapid, quantitative and easy to standardize tool and it is almost equal but not superior to OGP with regard to diagnostic sensitivity and specificity in patients with MS. Further investigations need to explore the connections with other central nervous system (CNS) diseases.

P35

Detection and evaluation of human specific Thyroglobulin autoantibody

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The presence of Thyroglobulin – autoantibodies (TgAb) in the systemic circulation can be an outcome of Thyroglobulin (Tg) leakage into the blood stream, as a consequence of thyroid gland diseases. Determination of TgAb is useful adjunct in the diagnosis of autoimmune thyroid disease such as Hashimoto disease, postpartum thyroiditis, neonatal hypothyroidism, and Graves disease. Unfortunately the circulating TgAb interferes with Tg level and causes a falsely low or undetectable serum Tg that can have serious consequences masking the presence of the disease. For patients with differentiated thyroid cancer (DTC) serum Tg level is primarily measured as a postoperative tumor marker. Therefore not only in the above-mentioned diseases, but in case of DTC it is essential to determine an accurate TgAb value. In our Hospital radioimmunoassay (RIA) (Thermo scientific) and enzyme-linked immunosorbent assay (ELISA) (Hycor) are available to determine serum TgAb. The goal of this study was to explain the background of deviation of the data. 107 samples were measured parallel with RIA and ELISA methods, 58 patients with suspected thyroid cancer and 49 patients with suspected autoimmune disease. Statistical analysis was performed by SPSS

statistical software (Chicago, IL). In accordance with the literature our results were not independent from the used techniques. Results of our two different TgAb methods confirmed that current assays are quantitatively variable and cannot be used interchangeably. The relationships between the two methods were specimen dependent and varied 100-fold. The discordant relationship between TgAb values reported from the same specimen using different methods likely reflect serum TgAb heterogeneity compounded by differences in the specificity of circulating TgAb for Tg antigen, assay reagents, and the standards used by various methods. The follow up of patients should always be based on the same methodological technique. In the future we plan to investigate further if the TgAb measured by RIA and used parallel to Tg as a tumor marker would be more specific for Thyroid disease than the ELISA method.

P36

The role of automated microscopes and computer aided pattern recognition in autoantibody detection by indirect immunofluorescence assays

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Indirect immunofluorescence assays (IFA) are versatile and sensitive solid phase tests for detecting autoantibodies needed to confirm the diagnosis of autoimmune diseases. Utilization of cells or tissue sections as the antigen source makes these assays capable of detecting antibodies to delicate epitopes masked in other tests such as enzyme linked immunosorbent assay or immunoblot. However, conventional microscopic evaluation of the fluorescent patterns is time consuming and prone to transcription error.

In our work we compared four automated fluorescence microscopes (Helios-Aesku Diagnostics, NovaView-Werfen Group, Europattern-Euroimmun, Image Navigator-Immunoconcept) regarding the number of available antigen substrates, specifications of the image capture and analysis system, extent of the automation, patient safety and impact on the daily routine workflow.

All four systems are able to detect antinuclear (ANA) and anti-cytoplasmic antibodies on HEp-2 epithelial cells (positive/negative discrimination only) while Europattern and NovaView also help with the recognition of the most frequent ANA patterns. The sensitivity of ANA positive/negative discrimination is rather similar between the systems (around 95%), while the specificity varies between 85 to 95%. The capability for detection of anti-neutrophil cytoplasmic antibodies (ANCA) and image capture from other substrates like rat liver-kidney-stomach (LKS) shows variability between the different analyzers. Similarly, the throughput, titer-estimation and physical parameters differ significantly.

Automation of the indirect immunofluorescence autoantibody testing is beneficial. In addition to slide processing, several fluorescence microscopes are available now which are able to digitize and archive IFA images. Their image analysis software helps to evaluate samples, making the indirect immunofluorescence method less laborious and error-prone.

P37

Diary of a "QualiCont Forum" maniac attendant

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QualiCont is a 20-years-old External Quality Assessment Organisation. It held its 18th annual meeting this year. As a regular participant of its workshops, I would like to share some of my professional and personal experiences that I recorded in my diary.

P38

Problems with reference ranges suggested by manufacturers in clinical chemistry

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Roche Modular clinical chemistry analyzer was exchanged to Beckman Coulter AU5800 analyzer in our laboratory in Autumn, 2015. In relation to the new system we had to perform comparative measurements for 33 parameters (enzymes, metabolites, electrolytes, inorganic parameters and specific proteins) of 87 serum samples healthy subjects and patients.

Based on our examinations, results on Modular and AU5800 instruments correlated very well for most of the analytes ($R^2 > 0.99$ for 20 parameters and $R^2 > 0.95$ for 9 ones). Correlation for Ca, Mg and HDL-cholesterol R^2 were 0.937, 0.935 and 0.932, respectively. Only LDL-cholesterol results showed less correlation with values higher on AU5800 ($R^2 = 0.807$). Although, in case of the most analytes correlation tests results were similar between analyzers, we realized significant differences between lactate dehydrogenase (LDH) and creatine kinase (CK) reference intervals suggested by manufacturers (in spite that both tests are based on the same measurement principle).

LDH values in the same samples measured by Beckman Coulter analyzer were higher than those measured by Roche. However, the adult reference range by Beckman Coulter is lower (208-378 U/L) than that is recommended by Roche (240-480 U/L). We noted many false positive results due to the lower reference interval of Beckman Coulter and the higher values measured by AU5800 analyzer. On the basis of reference ranges suggested by Beckman Coulter, 37% of the tested samples were false positive.

Similar anomaly was seen in case of CK: female reference values of Beckman and Roche are 26-145 U/L, and 26-192 U/L, respectively. A similar trend was observed for men: 39-171 U/L and 39-308 U/L for Beckman and Roche, respectively. According to the compared values (33 men, 54 women), results of 15.1% and 3.7% were false positive in men and women, respectively. The higher ratio of false positive results in men is probably due to the greater deviation in reference ranges. After reconsidering reference ranges suggested by manufacturers and comparison those with literature data, we decided to use reference intervals suggested by Roche for these two parameters, because of our results showed better agreement with that values clinically.

Discovered discrepancies between reagents indicate that in addition to the correlation of methods the clinical relevance of results should be also considered when methods are changed.

P39

Emergency Medical Services – changes in the chemical section of the Clinical Laboratory Department

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The Emergency Medicine Department (SBO – abbreviated in Hungarian) has been operating since September 2014 at the Borsod-Abaúj-Zemplén County Hospital. Since then the number of day-time emergency and on-call duty test requests gradually increased. The demand for more faster but still accurate test result led to the gradual establishment of the emergency section of the Laboratory with respect to preanalyticals, analyticals and postanalyticals. Our goal is to show our laboratory efforts meet the ever growing challenges.

Preanalytical developments: 1) sample transport is performed by the hospital's central pneumatic tube system, 2) automatic puck removal, 3) sorting by two Sample Managers of the stadd. Analytical processing has become faster by 1) the replacement of the Olympus Au 640 chemistry analyzer with a Siemens Advia 2400 chemistry analyzer in the emergency laboratory, and, the most important step forward, 2) by setting up a series of Advia (-1, -2, -3) chemistry analyzers along an automatic sample moving track in the integrated chemical laboratory. In postanalytical phase: by displaying the automatic and technical validations together on the validation screen used by our colleagues with diploma.

The number of urgent test requested was higher by 29.46 % between September and December, 2014 compared to the same period in 2015. Specifically, hs-Troponin-I, D-dimer and lipase tests increased by 1.24-, 2.11- and 155-fold, respectively. The median turn around time (TAT) of selected tests changed just slightly during the same period: sodium: 58'24" → 1h, carbamide: 57'11" → 1h 1'36", ASAT : 56'24" → 1h 4", CRP : 58'39" → 59'30".

Our results demonstrate that with the increasing number of test requests we could still keep the TAT of emergency chemical tests within the proper time intervals. The technological developments meet the demand of the Emergency Medical Services for fast results.

P40

Possible sources of pre-analytical and analytical errors in determination of the amount of *in vivo* circulating platelet-granulocyte complexes

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The importance of circulating platelet-granulocyte complexes in various clinical settings like in angina pectoris, allergic inflammation, severe sepsis, etc. was demonstrated by several investigators. Flow cytometry is the most convenient *in vitro* method to measure the amount of platelet granulocyte complexes in anticoagulated blood. However, to determine the real amount of *in vivo* circulating platelet-granulocyte

complexes some pre-analytical as well as analytical errors need to be taken into account. According to our experiences the main sources of the pre-analytical errors are the followings: time elapsed from the blood sampling and type of the anticoagulant. Heparin anticoagulant induces fast, almost complete, *in vitro* complex formation. Sodium-citrate induces *in vitro* complex formation as well, however, it is less pronounced than that of heparin. To the contrary EDTA inhibits complex formation *in vitro*. Considering the size and count differences between granulocytes and platelets coincidence of the two cell types in the flow cytometer may falsify the determination of real amount of *in vivo* circulating complexes. This problem can be overcome by the method elaborated by in our laboratory earlier (cf. references). Furthermore, for flow cytometry both platelets and granulocytes need to be labeled with fluorescent antibodies. This labeling procedure can be another source of analytical error.

Bihari P., Fent J., et al. J Biochem Biophys Methods 2008;70:1080–1085

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P41

Analytical evaluation of high-sensitivity cardiac troponin I assay on Architect i2000SR analyzer

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Background: High-sensitivity cardiac troponin (hs-cTn) assays are recommended over less sensitive ones according to the latest European Society of Cardiology (ESC) guideline for the management of acute coronary syndrome. The high-sensitivity cardiac troponin I (hs-cTnI) assay was introduced in 2015 in our laboratory. Before its introduction we evaluated the analytical performance of Abbott Architect STAT high-sensitivity troponin I immunoassay.

Methods: For the evaluation process we followed CLSI guidelines EP5-A2 for precision, EP17-A for detection limits and EP6-A for linearity on Abbott Architect i2000SR immunoanalyzer. We also compared patient results of Architect STAT high-sensitive troponin I assay with Architect STAT troponin I assay using 87 specimens with cTnI concentrations across the dynamic range of assay.

Results: Three troponin I controls were assayed and the total imprecision ranged from 9,3% to 9,8% and was lowest for the medium control. The observed limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) assumed values of 0,8, 1,7 and 5,8 ng/L, respectively. Hemolysis interference and sample dilution did not affect hs-TnI results. Method comparison showed a good correlation across the assay range of 60 and 50000 ng/L ($R^2= 0,9796$), but below 60 ng/L the correlation was rather poor ($R^2= 0,7889$). In these samples ($n=52$) cTnI was detectable in 98% by hs-cTnI assay and in 59,6% by cTnI assay.

Conclusions: The Architect STAT hs-cTnI assay with improved analytical features met the criteria of high sensitivity cTn test. Based on this feature a novel three-hour diagnostic algorithm has been established for the early rule-in and rule-out of acute coronary syndrome in our hospital.

P42

Total error versus measurement uncertainty

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Preparing for ISO 15189 accreditation it was important for us to clarify the difference and usefulness between total error (TE) and measurement uncertainty (MU).

Total error (TE) was introduced in the 1970s to provide a quantitative method to judge whether an analytical method has acceptable precision and accuracy. Measurement uncertainty (MU) was introduced in the 1990s due to the lack of consensus on how to express the quality of measurement results.

TE and MU appear as two sides of the same coin: TE defines a region around the “true” value where measured analytical results can be found with a certain probability. MU defines an interval around the measured analytical result where the “true” value can be found with a defined probability.

Daily quality management requires the use of TE. It was helpful for validating the performance of examination procedures, designing statistical quality controls, assessing quality on the sigma-scale, and satisfying regulatory requirements for successful participation in EQA programs.

Our laboratory focuses on the needs of the end users. Calculation of MU represents an important opportunity to improve the quality of the services of our laboratory to the diagnosis and monitoring the effects of treatment of patients. MU must be determined by the laboratory to inform physicians of the known variability of test results.

P43

Analysis of proficiency testing results support the improvement of laboratory performance

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Background: External quality assessment (EQA) or proficiency testing (PT) serves as an educational tool and, also, helps to monitor and improve the performance of the laboratory. Even if our results are acceptable we can determine any possible trend that could result in a future failure. The simplest way to detect problems is to monitor the Standard Deviation Index (SDI) results.

Methods: We examined our results from clinical chemistry program of QualiCont (Hungarian proficiency test provider). Performances from the last six cycles for the same analytes were reviewed. We used z-score graphs from the available charts for analysis. The measurements were done on Olympus AU400 chemistry analyzer.

Results: Two main types of graph patterns were revealed in the course of analysis: 1. All results were on one side of the mean (close to or at variable distances from mean). The glucose PT results showed this pattern: all z-scores exceeded +1 but there were no unacceptable results. The use of a new vial or a new lot of calibrator should reduce the bias. 2. Low level results were under the target value but high level results were above the mean or vice versa. The creatinine PT results had this pattern. The previously used two-point calibration (AB: reagent blank and high level of calibrator) was exchanged to another type of two-point calibration (AA: low and high level of calibrator). Due to the AA type of calibration the bias decreased from 7% to 3%.

Conclusions: The discovered errors were all systematic. Z-score graph can provide a quick interpretation and it should be examined for unacceptable results, unusual trends, sudden shifts, or other problems.

P44

Laboratory requests: statistical comparison of three urban hospitals laboratories

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The daily routine laboratory work includes the analytical processing of hundreds of samples and communicating the findings toward the requesting doctors. The quality control is also a routine task; during this we provide a workflow in accordance with the rules set in the standard operation procedures. We are convinced to compare regularly the laboratory requests from urban laboratories situated geographically in different counties.

Our goal was the statistical comparison of three years (2013-2015) lab requests of Mohács, Dombóvár and Siófok central laboratories.

The results showed that the most requested lab tests over the defined period of time in all laboratories in case of outpatients were: urea, glucose, ion measurements, cholesterol, general urinalysis, liver enzymes, complete blood count (CBC) and prothrombin. Regarding regional laboratory services the lab requests showed a number of similarities, but the following tests were outstandingly often requested: CRP (Siófok), LDH (Dombóvár) and amylase (Mohács). Adjusting the number of lab requests of inpatients to the number of beds in hospital we found the following differences: in Mohács less CRP, blood sugar and more bilirubin tests were requested, in Dombóvár less liver enzyme tests were done, while in the Siófok Laboratory a significant number of CBC's, prothrombin and urinalysis were requested. The number of lab requests are increasing by each year: for outpatients, the number of 13 lab test types and all lab test number, while for inpatients only the number of 5 lab tests increased.

This analysis indicated a common trend that in all three laboratories the number of lab tests was continuously increasing in every year, particularly when ordered for outpatients.

P45

Moving the Lab: logistics of transport and organization of a new structure for functioning

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The creation of the Central Laboratory Facilities at Semmelweis University provided an opportunity for integration of four labs previously located at different sites. The authors present the procedures and their efforts that resulted in the establishment of Koranyi Lab that includes functions of earlier Central Lab Pest, Lab of Immunology, Lab of Microbiology and Tuberculosis Lab.

The organization of moving was separated for consecutive phases. During the 'planning phase' the future localization of analysators was identified with a specific emphasis of functions to be integrated. Two novel facilities were established; Molecular Biology Lab runs all the molecular biology techniques used in the institute, while Cellular Immunology Lab provides cellular tests for allergy and immune deficiencies. Some functions with higher turn-around-time including electrophoresis were translocated to another site of the Institute (Kútvölgyi lab) with a parallel integration of endocrine tests into Korányi Lab. The critical 'transportation phase' was 1 week without any discontinuation of lab services. The final 'settling phase' was the time when the local situation was tailored for the actual needs. The staff was actively involved in this phase. Under the mentoring of an expert in Industry Efficiency Programs Kaizen meetings were organized.

During these meetings problems resulting any loss in test result production procedures were identified by small groups of staff members and the adequate response to solve these problems was developed.

P46

Determination interference limits of hemolysis index when open access tests are set on analysers with automated HI determination

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Background: Haemolysis is the release of free haemoglobin (fHb) from erythrocytes to the surrounding plasma. It can cause interference in laboratory assays through several mechanism. fHb concentrations (fHbcc) causing clinically relevant interference (fHbcc-s that cause $\geq 10\%$ deviation compared to the fHb free sample) in laboratory tests can be established by CLSI Guideline: EP7-A2 standard, in a parameter and method specific way. Modern chemistry and immunochemistry analysers automatically measure fHbcc and convert them into a dimensionless parameter called Hemolysis Index (HI). HI causing clinically relevant interference in measurement is also provided for each analyte by manufacturer. However, when these analysers used in an open access mode, method specific interference limits of HI should be determined by the user.

The aim of our study was to determine interference limits of HI for Total protein assay (TP) (Human, Hungary), Alkaline phosphatase and Creatinine assays (Diagnosticum, Hungary) on Modular analyser (Roche, Switzerland). HI values of Modular were calibrated using 5-point dilution of locally prepared haemoglobin standard and compared to the calibration provided by Roche, in all three assays. fHbcc-s that cause $\geq 10\%$ deviation compared to the fHb free sample were determined by CLSI Guideline: EP7-A2 method on serum pools representing normal concentrations of the analysed laboratory parameters. HIs of the samples with $\geq 10\%$ deviation compared to the Hb free sample were recorded and set as Interference limits of HI-s.

Results: Manual calibration of HI values of each 3 tests resulted in very good agreement with the one provided by manufacturer. fHbcc that caused $\geq 10\%$ deviation compared to the fHb free sample was in good agreement with the reagent's manufacturers in the case of TP: 2.45 versus 2.5 g/L, respectively. However, in case of ALP and CREAT our results indicated lower fHbcc-s inducing clinically relevant interference: 5.35 versus 10 g/L and 3.45 versus 5 g/L, respectively. Interference limits of HI-s of the three parameters were set as follows: TP: 152, ALP:332, CREAT:241.

Conclusions: Evaluating fHbcc-s resulting in clinically relevant interference in laboratory testing by CLSI method with subsequent HI interference-limit determination is a useful approach when open access tests are set on analysers with automated HI determination. This concept worked nicely on Modular analyser and resulted in lower HI values than those provided by manufacturers in two of the three analysed laboratory tests.

P47

Keep your eyes on HbA1c curves...

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Generally there are 200-250 glycated hemoglobin (HbA1c) measurements a day in Synlab Laboratory Székesfehérvár. The HbA1c values are determined by ion exchange HPLC method, which is the reference method for HbA1c in diabetic control. Our results presented enhance the significant importance of technical validation of HbA1c curves because many interfering factors can cause false diagnostic interpretation. The HPLC Bio-Rad Variant Turbo II system working with double wavelength detection (415 and 690 nm) used by our laboratory allows the

separation of different hemoglobin (Hb) components in real time. During the technical validation of the results, several chromatograms differed from the standards: HbA1c identification problems or numerical deviation were observed.

In the last six month of the total 24000 HbA1c measurements 9 were sent for further testing to the Department of Laboratory Medicine of the University of Debrecen. Using the Bio-Rad β -Thalassemia short program on a Variant II HPLC, different hemoglobin variants were detected. Subsequent molecular genetic analysis (PCR amplification of the beta globin gene exons and Sanger sequencing) identified the mutations. The interfering effects of the Hb variants with the HbA1c peaks are shown in our special chromatograms. In the case of the Hb Graz variant, an additional peak appeared on the chromatograms but none of them corresponded to HbA1c. Due to the presence of the Hb Sherwood Forest variant the HbA1c peak area gave an extremely high value (517 mmol/mol; 49.4 %), which obviously was uninterpretable. In case of three german patients (a mother and her two daughters), on the chromatograms the HbA1c and LA1c (labile HbA1c) peaks were superponated on each other. Sequencing revealed that all three of them were carriers of the Hb Sitia variant.

HbA1c is a key parameter in the early detection of diabetes and the reliable monitoring of the disease. Our results highlight the importance of correct technical validation of HbA1c results by HPLC methods. Knowledge of the interfering factors prevents the release of false results and allows the detection of some rare hemoglobinopathies. Lastly, due to the interfering effects of the Hb variants on the results of HbA1c, the measurement of HbA1c should be performed by another method in the presented cases.

P48

HbA1c Separation with Capillary electrophoresis and High Performance Liquid Chromatography-Comparative Study

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Our goal was to compare quantitative determination of HbA1c on a High Performance Liquid Chromatography (Arkray ADAMS A1c HA-8180V) and with a Capillary Electrophoresis (CAPILLARYS 2 FLEX-PIERCING) instrument, knowing that HPLC is the “gold standard” method of HbA1c measurement.

Apart from the quantitative determination we were curious also to know if the capillary electrophoresis gives more information, if so what these are.

The CAPILLARYS 2 FLEX-PIERCING instrument uses the principle of capillary electrophoresis to determine the complete hemoglobin profile for the quantitative analysis. In many aspects the method is an intermediary type of technique between classical zone electrophoresis and liquid chromatography.

We completed analysis on almost 400 samples both on HPLC and on Capillary Electrophoresis instruments and evaluated the measured parameters.

As a result we could drive consequences for the accuracy of the measurements. Detecting normal and abnormal hemoglobins it can draw attention to the possible presence of hemoglobin variants.

P49

Microchip electrophoretic analysis of acid soluble serum proteins of patients

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Recently, in the Department of Laboratory Medicine a perchloric acid (PCA) precipitation method was worked out and adapted for isolation and characterization of acid soluble serum proteins less than 67 kDa. Orosomuroid (an acidic alpha-1-glycoprotein, 42 kDa - AGP) known as an acute phase protein could be detected in the supernate of PCA treated sera even in healthy individuals. Comparing the amounts and patterns of acid soluble proteins of healthy individuals with those of patients' samples suffering from systemic inflammatory diseases, striking differences were found. Our method has proved that in malignancies as well, the amount of acid soluble proteins is increased and characteristic changes in the protein patterns are detected too. The major goal of the research is further characterization of perchloric acid soluble proteins in bacterial sepsis and in Crohn's disease with the outcome of finding and identifying disease specific molecules or molecular patterns. The applied methods are: one- and two dimensional gel electrophoresis (PAGE) and microchip electrophoresis. The labeled protein complexes are analyzed in the Agilent 2100 Bioanalyzer microchip electrophoresis system applying the High Sensitivity Protein 250 LabChip kit with minor modifications. These chip electrophoretic methods are able to complete the conventional SDS-PAGE, with the advantage of better sensitivity, high speed and being capable of quantification. As a methodological development we plan to work out a quantitative measurement to test the acid soluble proteins suitable for routine automated analysis.

Reference:

Int. J. Cancer 1980; 25: 281-288.

The research was supported by the grants OTKA K-100667 and TÁMOP-4.2.2/A-11/1/KONV-2012-0065.

P50**Total Laboratory Automation – Does it a children playground?**

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Total laboratory Automation (TLA) was 5.4 billion USD worth of business worldwide in 2013 according to Kalorama market research and according the report of Technavio, will reach the 12 billion USD by 2016. Laboratory automation business definitely requires experts at vendors' side, but also at customers' side, who express clear needs, understand the benefits and disadvantages of different solutions and are capable to negotiate the most custom tailored version which fits the best to the laboratory workflow.

Choices are almost endless. Two basic questions shall be asked first: Task Target Automation (TTA) or TLA, (ii) Single vendor solution or open automation concept?

TTA utilizes the supreme of automation at different laboratory procedures by stand alone units. TTA offers the versatile choice of capacity in order to improve Turn Around Time (TAT) most intensively. TLA on the other hand is a compromise on loosing on TAT but gaining on workflow management. Taking TLA is not only the question of daily sample traffic, but the investment into the improvement in result security and reliability.

However for better understanding we shall clear several myths around TLA, which very often give false expectations from customers and cloudy offers from vendors' side. In the presentation you will learn about

- (1) Why open automation gives more flexibility and more scalable solution?
2. Why aliquoting is overestimated by certain vendors while others handling this as a secondary or even marginal issue?
3. Why a rack sample delivery method does not a real TLA solution?
4. Why you really need dual way track in the TLA?
5. Why the TAT becomes longer with TLA?
6. What is the major labor paradigm in TLA, less people but more qualified?
7. How to handle off line testing?
8. TLA is valuable solution for laboratories, nothing is free of cost!
9. Without full sample barcoding procedure all TLA is only able to give one armed support.
10. Why the corner stone of the proper operation of any TLA is the applied middleware solution?

P51**Second generation antiepileptics' parallel determination with a HPLC method**

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Among the newer antiepileptics Lamotrigine (LTG) and Oxcarbazepine (MHD metabolite) is increasingly used in bitherapy or tritherapy with Lacosamide (LAC), Zonisamide (ZON), Valproate. Rufinamide (RUF) and Felbamate (FBM) as a co-medication. In these cases separation is solved with solvent gradient elution. With a liquid-liquid extraction on Extrelut NT, an inert, wide-pore kieselguhr, we routinely use 250 μ L plasma for measurements. Cartridge column is conditioned with 1.0 mL phosphate buffer (10 mmol/L) and 25 μ L internal standard (butabarbital 200 mg/L) is added. Washing is with 3 \times 2 mL dichloromethane. Reduced in water bath. Residue is resolved in (20:80, v/v) acetonitrile, 10 mmol/L phosphate buffer. 10 μ L is injected to column. Waters 1525 Binari Pump was used with a Waters Breeze Software for creating a gradient. The sample is eluted onto a Luna C8 column (75 \times 4.6 mm) filled with 3 nm particles. Mobile phase A (60:40, v/v) acetonitrile, phosphate buffer (10mmol/L), mobile phase B (20:80, v/v) acetonitrile, phosphate buffer (10 mmol/L). Detection was performed on a Waters 2487 UV/VIS dual wavelength detector (195 nm, 215 nm).

The method is linear in the range of 0.5% - 200% of the lower and upper limits of therapeutic ranges. Detection limit is 0.7 mg/L, 1.7 mg/L, 0.5 mg/L, 2.8 mg/L, 1.1 mg/L, 3.7 mg/L for LTG, MHD, LAC, ZON, RUF, FBM respectively. This is a selective method with accurate determination for the above listed co-medicated antiepileptics.

P52

Catecholamine measurements in the urine of drug users

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Recently, there has been an increasing variety of new recreational drugs called “new psychoactive substances”. Some of them are legal, others are already illegal drugs. These drugs alter the catecholamine balance in the central nervous system. We would like to examine if alteration caused by stimulants can be detected in the catecholamine concentrations excreted in urine or not.

We used 20 urine samples of stimulant drug-users, and 16 control samples. The drugs were identified by Shimadzu Prominence TOX.I.S II HPLC-DAD equipment. The catecholamine measurements were performed on the Shimadzu HPLC system with ANTEC EC detector by the HPLC kit of Chromsystems.

We found that the level of epinephrine was significantly higher and the level of dopamine was significantly lower in the urine of drug users than in the control urines. We could not find significant difference in the norepinephrine level.

P53

Examination of Classical Galactosemia by a Modified Quantitative Beutler Test

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The Classical Galactosemia is an autosomal recessive inherited disease, which is caused by enzymatic deficiency of galactose-1-phosphate uridylyltransferase (GALT). This enzyme is the third participant of the galactose metabolism, which takes care of the conversion of the galactose-1-phosphate and uridine diphosphate glucose (UDPglucose) to uridine diphosphate galactose (UDPgalactose) and glucose-1-phosphate, as described by Leloir.

The determination method is based on the qualitative Beutler enzyme spot test, which is an effective assay for examination of galactosemia. The main advantage of the test: immediate detection of GALT deficiency, but has some disadvantages: for example, the assay is dependent on visual evaluation therefore it is quite subjective. Fujimoto et al. (1) modified the macroscopic visual Beutler enzyme spot test by adding extraction of blood components from filter paper, deproteinization with acetone/methanol. The deproteinization made it possible to evaluate the fluorescence intensity (FI) quantitatively by a fluorometric microplate reader and personal computer. We also examined the recovery efficiency of the method by measuring hemoglobin concentration in the extracts. After the first extraction we measured the residual hemoglobin content of the dried blood spot two more times. The efficiency was more than 97 % by the extraction and the following centrifugation. For the more precise detection of the GALT enzyme activity, the obtained FI were converted to effective quantities by preparing a nicotin-amid adenin dinucleotide (reduced form of NAD⁺) standard curve. In order to obtain accurate quantitative results, the NADPH concentrations were corrected to the hemoglobin content. The samples for the examination of classical galactosemia were obtained from the newborn screening (NBS) program. In contrast to the Beutler test the modified quantitative test by this way gives an exact GALT enzyme activity, so could help the differential diagnosis of those cases which were positive in the NBS. Furthermore by this test we can measure at least eighty-four specimens the same time per plate. Moreover, the assay can help us to separate the compound heterozygotes (D/G, Duart/classical) from the healthy subjects. In addition this method helps to understand the connection between the biochemical phenotype and genotype of the GALT enzyme.

1, Fujimoto A, Okana Y, Miyagi T, Isshiki G, Oura T. Clin Chem 2000;46:806-10.

P54

Exam period stress in medical students: Monitoring psychological and somatic parameters

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We studied the effect of 2-months-long exam period stress on the psychological and somatic status of medical students.

The study involved 23 students and had 3 time points for measurements: 1) before the exam period; 2) at the end of the exam period; 3) after 6 weeks recovery of the exam period. The psychological response of the students was assessed by self-report questionnaires:

General Health Questionnaire 12; Beck Anxiety Scale; Beck Depression Scale; Medical Students Stress Questionnaire and Philadelphia Sleep Quality Index. The somatic response to stress was investigated by measurements of plasma proteins including inflammatory cytokines and analysis of blood pictures. We also made functional MRI measurements to assess the activity of the amygdala in response to exam stress.

As expected, exam stress had pronounced effect on the psychological status of the students, they were more anxious, depressed and had sleep disturbances at the end of the exam period. Furthermore, exam stress resulted in a significant reduction in the count of the monocytes, eosinophil granulocytes and platelets. The analysis of the cytokine levels and the fMRI-analysis is still in progress.

In sum, we report here that a 2-month long exam stress has a significant effect both on the psychological and on the somatic status of the students as well.

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P55

25-hydroxyvitamin D levels in serum, dried serum spots and dried blood spots

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Dried blood spot analysis has gained increasing popularity and provides advantages for the evaluation of the vitamin D status. However, neither the impacts of the methodology, the sample matrix and the physiological factors on the results obtained, nor the utility of the serum reference ranges have been elucidated. Our aim was to compare serum 25-hydroxyvitamin D results delivered by a widely used immunoassay platform with those obtained in dried serum (DSS) and dried blood spots (DBS) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The potential associations between the levels of 8 relevant endogenous substances and 25-hydroxyvitamin D (25OHD) were also evaluated.

Samples obtained from 73 adult patients were assayed. Serum 25OHD was evaluated using direct competitive chemiluminescent immunoassay. The LC-MS/MS analysis of 25-hydroxyergocalciferol (25OHD₂) and 25-hydroxycholecalciferol (25OHD₃) were performed employing a validated in-house method.

Although there was a strong correlation between the concentrations of total 25OHD obtained in each pair of matrices, there were significant differences between the clinical classification of serum versus DSS values and serum versus DBS results. The clinical classification of DSS and DBS test results showed no significant difference. 25OHD levels showed a positive correlation with HDL cholesterol and, in DSS and DBS samples, with triglyceride concentrations.

DBS and DSS can be employed for the evaluation of the vitamin D status, however, the establishment of the method- and matrix-related reference ranges is imperative, and lipid biomarkers should also be assessed.

P56

Comparison of the analytical and clinical performance of 25-hydroxyvitamin D assays

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We report a study conducted to compare both the analytical and clinical performance of six automated 25-hydroxyvitamin D (25OHD) assays and an in-house liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Serum samples obtained from 165 patients (except for the Siemens Advia Centaur assay with n=116) were analyzed for 25OHD and the results were assessed pairwise for each combination of methods. The agreement of analytical performance was evaluated performing Passing-Bablok regression and Bland-Altman analysis. The clinical performance was compared using a hypothesis test (McNemar's), the direct pairwise assessment of the clinical classification of results.

The comparison of analytical performance led to the identification of a lack of agreement in assay outputs for all method pairs (concordance correlation coefficients: 0.499-0.876). Nevertheless, the evaluation did not yield any information on the differences in the clinical performance. Hypothesis tests also failed to make a difference between methods in agreement from those delivering clinically non-equivalent results. Employing a cut-off level of 20 ng/mL for hypovitaminosis D, the agreement in the clinical classification of results ranged between 66.6% (DiaSorin Liaison vs IDS-iSYS DS) and 87.4% (IDS-iSYS DS vs LC-MS/MS).

The direct comparison of the clinical classification of 25-hydroxyvitamin D results is the recommended approach for assessing the agreement of assays prior to a method transfer in the clinical laboratory. Performing the comparison on a sample set requiring not more than 200 runs is cost-efficient and results in a reliable estimate of the percentage agreement with a narrow confidence interval, delivering outputs which are easy to interpret by clinicians requesting the tests.

P57

Comparison of estimated glomerular filtration rate values calculated by cystatin C and creatinine concentration-based equations

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Introduction of the European reference material ERM-DA471/IFCC made harmonisation of different cystatin C methods possible. This also supported wider spread of cystatin C - based estimated glomerular filtration rates ($eGFR_{cys}$) calculated by the newest formulas^(1,2) in every day clinical use.

Our aim was to calculate $eGFR_{cys}$ values by CKD-EPI⁽¹⁾ and CAPA⁽²⁾ formulas, using cystatin C results from our laboratory information system database and subsequently compare them to creatinine concentration-based estimated glomerular filtration rates (EPI- $eGFR_{crea}$). Our database in 2015 contained 1043 patients' records - 350 males, 693 females with an average age of 52 and 65 years, respectively - who had both serum creatinine and cystatin C results measured. $eGFR_{crea}$ was found $<90\text{ml}/\text{min}/1,73\text{m}^2$ in 420 cases. Using the G1-G5 $eGFR$ based classification of chronic kidney disease (CKD), the 1043 cases resulted in similar CKD classification pattern by the two $eGFR_{cys}$ formulas with exception of the fact that between-genders difference in the range of $<90\text{ml}/\text{min}/1,73\text{m}^2$ could be observed. This is very likely due to the fact that the CAPA formula lacks to use any gender-dependent parameter. However, between $eGFR_{cys}$ and EPI- $eGFR_{crea}$ some discrepancies could be observed. Namely, the number of records classified into G1 stage ($\geq 90\text{ml}/\text{min}/1,73\text{m}^2$) were found somewhat higher, while the number of records classified into G3 stage ($<60\text{ml}/\text{min}/1,73\text{m}^2$) somewhat lower using $eGFR_{crea}$ formula compared to that seen in the two $eGFR_{cys}$.

(1) Inker LA, et.al, N Engl J Med 2012;367:20-29

(2) Grubb A. et.al, Clin Chem 2014;60:974-986.