Abstracts*)

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M. Ferrari
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The effects of natural and synthetic polyhydroxyphenolic compounds against inflammation and tumor cells

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Resveratrol (RV) is a polyhydroxystilbene compound, which was made responsible for the so called “French Paradox”. This is the fact, that the incidence of heart infarction is much lower in France in comparison with other western countries (60% lower) despite an unhealthy diet. This effect was contributed to the red wine consumption in France. Therefore different ingredients of wine were analysed in the search for the most beneficial compound and RV was identified as one candidate.

Our group synthesized a number of analogues of RV in order to further enhance the free radical scavenging, anti-inflammatory and anticancer effects of RV. We could identify a structure activity relationship and further investigated the effects of the most promising of these compounds, which turned out to be hexyhydroxystilbene (M8).

One important mechanism of action is the inhibition of the key enzyme of de novo dNTP (deoxynucleosidetriphospahte) synthesis, Ribonucleotide Reductase (RR). RR activity is significantly up-regulated in tumour cells in comparison to non-malignant cell populations, and is therefore considered to be an important target for anticancer therapy. In addition, we could show a very good correlation between the free radical scavenging capacity, inhibition of cyclooxygenase activity, inhibition of RR activity and growth inhibition of tumour cells.

M8 was then tested in animals and in two different melanoma animal models and was shown to effectively inhibit tumour growth.

Based on our findings we further investigated a number of natural compounds, such as gallic acid, and also synthesized novel compounds, such as digalloylresveratrol in the search for more effective anticancer compounds.

The compounds were tested in different settings, including combination with well established anticancer drugs, in order to find additional promising treatment options for tumours such as pancreatic carcinoma or leukaemia.

Elucidation of structure activity relationships and synthesis of analogues of natural compounds with enhanced activity might help to develop new antitumour compounds with activity against otherwise resistant tumour entities.

PL.3

The Hungarian JENDRASSIK-award lecture

Laboratory assisted quality improvement of the preanalytical phase in emergency medicine

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Total Quality Management is a commitment to improve health care processes with main focus on the costumers’ (patients’) outcome. Diagnostic professions serve patients and physicians, who request laboratory tests, in parallel, therefore play a central role in health care processes. Measuring customer satisfaction in health care is not only to identify patients’ expectations and satisfaction but also to understand customer satisfaction in all supportive services. Measuring customer satisfaction in supportive departments (medical laboratory diagnostics, radiology, central pharmacy) in our hospital has a long tradition.

We demonstrate a questionnaire-based customer satisfaction survey on laboratory service organized in emergency medicine department (ED), which was combined with a project on improvement of the preanalytical phase of laboratory testing process in ED. Questions of costumer satisfaction survey were set, indicators of preanalytical errors were selected and local protocol for ED was established by the
It is widely acknowledged that clinical laboratories play a valuable role in providing data for an early and accurate diagnosis, for screening tests needed for preventive care, and that lab results are vital in monitoring disease progression and treatment efficacy. Clinical laboratories have always undergone major changes because of technological advances and economic pressures. Laboratories are an easy target for economic restrictions because of their technological characteristics. The funding position of clinical laboratories in Hungary is becoming critical. In clinical laboratories, cost savings have frequently been realised by consolidation of laboratory sections. Unfortunately, this "technological" approach to lowering costs per assay has frequently been used to undermine the influence of laboratory professionals and to further isolate them from clinical problems. Often forgotten is the value of clinical information associated with clinical laboratory testing. As healthcare reforms necessitate a shift to value-based laboratory models, the real question is whether the laboratory offers enough value in service and speed of results to support clinical care. The future survival of laboratory medicine depends on the ability to add value to the care of patients. In the future, the integration of hematology, transfusion laboratory models, the real question is whether the laboratory offers enough value in service and speed of results to support clinical care. The future forgotten is the value of clinical information associated with clinical laboratory testing. As healthcare reforms necessitate a shift to value-based cost savings have frequently been realised by consolidation of laboratory sections. Unfortunately, this "technological" approach to lowering costs per assay has frequently been used to undermine the influence of laboratory professionals and to further isolate them from clinical problems. Often forgotten is the value of clinical information associated with clinical laboratory testing. As healthcare reforms necessitate a shift to value-based laboratory models, the real question is whether the laboratory offers enough value in service and speed of results to support clinical care. The future survival of laboratory medicine depends on the ability to add value to the care of patients. In the future, the integration of hematology, transfusion
medicine, biochemistry and immunology into a unified blood sciences discipline will have been achieved. The era of ‘personalized medicine’ offers enormous opportunities for laboratory medicine disciplines in the future, particularly in the area of devices, remote patient monitoring and custom-designed DNA, RNA and protein assays. New emerging technologies, including robotics, humanoid technology, lab-on-chip devices, nanodevices and patient ‘smart’ implants, will in the future offer unique opportunities for laboratories to develop new business areas. Strategically, laboratory medicine departments at the medical universities should even now be planning for future to develop translational cores in laboratory medicine.

**SY1.2**

**Next generation sequencing – modern molecular methods**

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The molecular genetic testing of an individual’s genetic make-up is currently on its transitional phase. The very recent technical advancement in the field of DNA sequencing resulted in the development of next-generation sequencing (NGS) methods, which are capable to sequence hitherto unprecedented amount of genetic material. NGS has quickly reaching the clinical use. There are several different strategies of the development of NGS assay systems. Targeted sequencing is performed where a single gene is tested quickly and cost-effectively (BRCA1, CFTR) or when a group of genes are analyzed in case of monogenic diseases with overlapping phenotypes (i.e. retinitis pigmentosa, monogenic diabetes). Frequently, where a Mendelian disease is suspected with unknown cause, the entire coding region, the exome of the individual is tested. By exome sequencing, depending on the settings, app. 20,000 single nucleotide variations are detected. It is also possible to sequence the entire genome of an individual resulting in app. 3,000,000 variations. This amount of data, the ethical questions, the complexity of the analysis and result interpretation requires a multidisciplinary team consists of technology experts, clinical geneticists, clinical laboratory geneticists and bioinformaticians. Although there are limitations of the available techniques (method validation, standardization, quality assurance, reference materials, mutation databases) and serious concerns of the use of the genetic data, the tremendous potential provided by NGS (genetic screening, non-invasive prenatal testing, pharmacogenomics) will clearly change the way of genetic testing.

**SY1.3**

**Molecular profiles in disease diagnosis: epitome profiling in cancer**

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Molecular profiling represents global, high resolution and parallel detection of an entire class of molecular analytes in clinical samples and results in the myriad of biomarkers not necessarily applicable to routine diagnostics. At the same time, smaller groups of disease condition specific analytes, SNPs, qPCR detected gene expression or multiple proteins provide useful multivariate diagnostics which are in clinical use. Transition to the use of multivariate index based diagnostics is halted by barriers today, but it will change the current medical paradigm inevitably. Here, I will attempt to provide a short overview of the evolution of multivariate indices from discovery to clinical use. Technical aspects and future directions will also be discussed. Special attention will be paid to affinity reagent based protein panel diagnostics in cancer and one form of this, monoclonal antibody (mAb) proteomics. Profiling the human plasma proteome with epitope specific minimally redundant mAb libraries delivered unexpectedly high specificity and sensitivity (>0.9 AUC in ROC analysis) for the detection of lung, breast, colon, prostate and ovarian cancer.

Our current focus includes extending our approach to tumor tissue and bringing the technology to closer to patients.

**SY1.4**

**Novel techniques in lab: the human brain will be essential**

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Laboratory techniques develop in an unprecedented scale and speed. This lecture provides a short overview on emerging techniques such as genetic testing, flow cytometry and mass spectrometry; and indicates their current position in routine laboratory investigations. The number of data generated by these tests are exponentially increasing. The large amount of information raises novel risks and challenges for correct interpretation. The interpretation of genotype, phenotype and metabolomic data requires novel approaches and the involvement of biostatisticians.

While the majority of applications are currently under development, it is reasonable to postulate that the professionals of the future labs and their partners in clinical care will routinely use the information derived with novel techniques for diagnostic and therapeutic decision making purposes.
The purpose of diagnostic algorithms in Clinical Microbiology is to identify the disease cause and to characterize it from the point of therapeutic intervention. Either phenotypical or genotypical characteristics or both are determined in the lab and they have to be organized in algorithms to predict the complex phenotypic profile of the microorganism. MALDI-TOF mass spectrometric identification is based on the very stable ribosomal proteins and the spectrum is compared to a database of those of several millions of microorganisms. The system is suitable to identify from homogeneous microbial populations, rarely from mixture of two microbes and not from mixture of more than two. Based on these properties, the major application of MALDI-TOF is the rapid identification of isolated bacterial colonies from agar plates. Broth cultures, hemocultures and clinical specimens directly before enrichment by culture can be tested if they are monobacterial.

Advantages: Time is saved in the lab, rapid information for the expert, cost effective organization of further analysis, labour needs for pure colonies and operation costs in long term are similar to conventional methods. Two-year experience on pure colonies in our lab revealed >95% identification of species and inability to identify microorganisms at least even at genus level is approximately 1%.

Limitations: No automatic clinical interpretation, only clinical microbiologist can translate the rapid information to clinical use through consultation. In general, the independent antimicrobial sensitivity testing remains the key determinant of turn around time for written report. Uncertain differentiation within alpha-hemolytic streptococci.

Serological antibodies in inflammatory bowel disease

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Backgrounds: Serological antibodies have a potential role to assist the diagnosis, disease stratification and to predict the prognosis in inflammatory bowel disease (IBD). Understanding antibody formation might provide insight into the disregulated immunological response to gut microbiota in IBD. This review summarizes recent evidence regarding the role of serology in IBD.

Recent findings: There is accumulating evidence from cross-sectional and longitudinal studies, and from recent meta-analyses that support the value of serological markers in identifying patients with complicated disease phenotype and need for surgery in patients with Crohn’s disease (CD). ASCA remains the most accurate single marker, while recently-identified targets of exocrine pancreas antibodies (GP2 and CUZD1) provide for the first time evidence for a role of serological antibodies in the pathogenesis of CD.

Summary: Despite these developments, the use of the serological antibodies remains complementary in the clinical practice with IBD patients. New markers are currently evaluated and recent data support an active role of some of these markers in the pathogenesis of IBD.

The diagnostic uses of hepcidin: wishes and limits

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The 25 AA long peptide hormone hepcidin is believed to play a key role in iron homeostasis. The hormone is generated by the hepatocytes and regulates iron homeostasis through the inhibition of ferroportin, therefore its elevated levels are associated with decreased iron uptake into the liver, enterocytes and macrophages.

Both hepcidin and its 60AA long pre-form are found in the circulation and their elevated levels were associated with various anemia, inflammatory and iron overload diseases. Despite, the determination of the hormone and prehormone remains problematic, and therefore their diagnostic uses are limited.

In the present report we will try to sum the possible uses of hepcidin measurements and to present the obstacles which prevent efficient diagnostic uses.
SE1.3

**The immunomodulatory role of bile acids**

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Enzymatic oxidation of cholesterol generates numerous distinct bile acids that function both as detergents that facilitate digestion and adsorption of dietary lipids, and as hormones that activate five distinct receptors. Activation of these receptors alters gene expression in multiple tissues, leading to changes not only in bile acid metabolism but also in glucose homeostasis, lipid and lipoprotein metabolism, energy expenditure, intestinal motility and bacterial growth, inflammation and in the functions of liver-gut axis. This review sums up and discusses the main results and facts of the present knowledge about the physiologic and pathologic role of bile acids. A special attention is paid to the connection between bacterial lipopolysaccharides (“endotoxins”) and bile acids with some types of immunological disorders, because bile acids are natural and physiological inhibitors of the intestinal absorption of bacterial endotoxins. Thus, bile acids can take part in the regulation of innate immunity, various systemic inflammations like septic shock, inflammatory bowel diseases, allergy, psoriasis, cholestasis, obesity and metabolic syndrome.

SE1.4

**Autoantibody detection using acetylcholine receptor specific ELISA tests**

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The diagnosis of myasthenia gravis can be established by clinical and serologic testing. An immunologic assay to detect the presence of circulating acetylcholine receptor antibodies (AChR-Ab) is the first step in the laboratory confirmation of myasthenia. These antibodies are polyclonal and are present in approximately 80 to 90 percent of patients with generalized disease. AChR-Ab are responsible for failure of the neuromuscular junction in myasthenia gravis and their detection can be of considerable value in disease diagnosis and follow-up.

Recently two ELISA tests were introduced to replace the conventional RIA tests. One of them is a competition type ELISA (1.) (ACHR-Ab-Assay DLD GmBH), where the autoantibodies inhibit the binding of 3 AChR specific monoclonal antibodies to foetal and adult type AChR. The other is a simple indirect ELISA (2.) (anti-AChR, Euroimmun) based on the binding to recombinant AChR gamma and epsilon subunits.

First we compared the specificity and sensitivity of ELISA (1.) to RIA results of 21 patients. Then we compared the two ELISA tests by measuring 100 patients’ sera. There was a good correlation between the results of the two assays. Both ELISA tests showed 100% clinical specificity and 92-93% clinical sensitivity. No cross-reactions with other autoantibodies were observed.

AChR-Ab titers correlated poorly with disease severity, but in an individual patient, the titers tended to fall with successful immunomodulating therapy for myasthenia gravis, as such therapy can sometimes lead to apparent seronegativity.

SE1.5

**Presepsin: a new biomarker in the diagnosis and prognosis of bacterial infection in cirrhosis**

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The occurrence and severity of bacterial infection in cirrhosis is greater than in the population without cirrhosis. Bacterial infections represent one of the most important causes of progression of liver failure, development of liver-related complications, and mortality. The end-organ damaging effect of bacterial infection is greater in patients with cirrhosis and often culminates in acute-on-chronic liver failure (ACLF). However, in clinical practice the accurate identification of infectious episodes is challenging due to the disease specific characteristics in this patient population.

The aim of this study was to determine the diagnostic accuracy of presepsin – a novel sepsis marker – in identification of bacterial infections and also to assess its value for prediction of short-term mortality in a large cohort of patients with cirrhosis.
Coagulation factor XIII (FXIII) is a zymogen consisting of two catalytic A and two carrier/inhibitory B subunits (FXIII-A, B). It becomes converted into an active transglutaminase (FXIIIa) in the last phase of coagulation cascade by thrombin and Ca²⁺. The main function of FXIIIa is to strengthen fibrin by cross-linking its chains and to protect it against fibrinolysis by covalently attaching α, plasmin inhibitor to fibrin. FXIII is also involved in wound healing, and in maintaining pregnancy. Inherited FXIII deficiencies are classified as FXIII-A and FXIII-B deficiency. The former is usually a very severe bleeding diathesis and due to the high frequency of intracranial bleeding, it needs lifelong substitution therapy. Auto, or alloantibodies against one of the FXIII subunits usually result in very severe acquired FXIII deficiency. Milder deficiencies might be due to consumption coagulopathy or to decreased synthesis.

The aim of FXIII determination is: 1/ to diagnose inherited or acquired FXIII deficiency, 2/ to establish the severity of deficiency and indicate if there is a need for substitution, 3/ to monitor the effectiveness of prophylaxis or therapy, 4/ to detect and measure neutralizing autoantibody.

Guidelines for the diagnosis of FXIII deficiency emphasize the following points: 1/ neither clot solubility test nor FXIII antigen assay is recommended for screening FXIII deficiency; a quantitative functional assay based on ammonia release or on amine incorporation into a protein should be the first-line test, 2/ in the case of ammonia release assays a plasma blank is to be measured and subtracted from the results, 3/ the results of amine incorporation assays might be influenced by FXIIIA Val34Leu polymorphism, 4/ To detect neutralizing anti-FXIII-A antibody a mixing study and to measure its titer a Bethesda type assay should be used, 5/ The detection of a non-neutralizing antibody a protein should be the first-line test, 2/ in the case of ammonia release assays a plasma blank is to be measured and subtracted from the results, 3/ the results of amine incorporation assays might be influenced by FXIIIA Val34Leu polymorphism, 4/ To detect neutralizing anti-FXIII-A antibody a mixing study and to measure its titer a Bethesda type assay should be used, 5/ The detection of a non-neutralizing antibody a protein should be the first-line test.

In vitro

Inflammation, as well as cancer, smoking have been associated with increased citrullination. As citrulline is far more immunogenic than arginine, it drives immune responses including B-cell activation and the production of various anti-citrullinated protein/peptide antibodies (ACPA). Various ACPA modalities include antibodies to citrullinated fibrin (CF), vimentin (CV), collagen (CC) and other matrix proteins. In vitro ACPA is most commonly detected by ELISA measuring anti-cyclic citrullinated peptide (anti-CCP) antibodies.

ACPA is really a „Holy Grail” in inflammatory conditions, such as rheumatoid arthritis (RA) as it has been implicated in the pathogenesis, diagnostics and prognosis of the disease, as well as therapy responses. In genetically prone subjects, smoking and other environmental factors trigger the citrullination of synovial proteins, as well as ACPA production. These mechanisms exert a major role in the development of RA. With respect to diagnostics, various ACPA assays have sensitivity and specificity levels over 90-95%. It has been widely acknowledged that two major phenotypes of RA could be distinguished. ACPA seropositive and seronegative patients may have different genetic background and ACPA positivity has been associated with more aggressive disease course, worse prognosis and increased frequency of comorbidities, such as cardiovascular disease. Finally, some data also suggest that responses to anti-rheumatic agents, primarily biologics, may be related to the ACPA status. Therefore, the determination of ACPA may be also important with respect to personalized treatment and patient management.
SE2.3

Functional alterations of T-cells in patients with rheumatoid arthritis

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SE2.4

Multiparametric luminescent viability assays in cellular toxicology models

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Cell viability testing is a widely used technique in studying toxic and/or cytoprotective effects of natural and synthetic molecules as well, using tissue culture models. A vast majority of the applied approaches rely on chemiluminescent and fluorescent techniques including intracellular ATP measurements, a variety of cell permeability tests based on visualization of DNA or detection of the end products of enzymatic processes undergoing only in living cells. However, it is often difficult to interpret viability data because of the non-uniform behavior of different cell lines and also on poor understanding of the mechanisms producing the signal.

In our work we applied NaF glycolysis inhibitor (1-20 mM), NaN₃ metabolic poison (1-10 μM) and ochratoxin A (OTA) mycotoxin (5-20 μM) treatments in monolayer HepG2 and MDCK cell cultures. Calcein fluorescent esterase probe, bioluminescent ATP measurement and intracellular protein determinations were applied. A novel perchloric acid extraction method was used to obtain ATP, protein and esterase activity parameters from the same sample.

We showed that the esterase activity was independent of the ATP contents and reflected the cell number (including ATP depleted ones) in the samples rather than cellular viability. ATP decreased in a dose dependent manner (10 mM NaF → 25% ATP, 20 μM OTA → 60% ATP). However, ATP/protein ratios did not change in a parallel manner. OTA caused a 30% while NaF a 70% decrease in ATP/protein ratios. NaN₃ did not have significant effects in the concentration range studied. HepG2 cells were more resistant to OTA and more sensitive to NaF than MDCK cells (p < 0.05). Our results suggest that measurement of a single viability parameter alone is often misleading when interpreting toxicity data in tissue culture models.

SE3.1

Establishing reference intervals for quantitative parameters in a Romanian Clinical Laboratory – methodology and limits

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SE3.2

How can we add value to laboratory tests?

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In the main focus of the everyday operation of laboratories is how to achieve and maintain the highest quality analytical test results. However, for the patient it is the impact of the laboratory test result on the clinical outcome that matters. Increasing understanding of patients’ needs lead to a better recognition of a concept called total testing process (TTP) within laboratory profession worldwide. TTP is an approach, when laboratories, beside their core analytical function, consider being responsible for extra-analytical phases of testing process from requesting of laboratory tests to the communication and interpretation of the results.
In order to influence extra-analytical phases successfully (where laboratories used to have a little control) laboratory professionals have to be able to show how the input of laboratory medicine is essential for best practice in these extra-analytical phases. This lecture will give examples on how laboratory professionals can assist their partners (clinicians) in better utilisation of laboratory tests in different steps of the TTP. These include: (1) Opportunities for laboratories in structuring their request forms and in educating their users on the importance of pretest probability estimation of the suspected disease during test requesting will be presented. (2) The importance of post-analytical management of pathological results of common laboratory tests and the influence of chosen laboratory actions on the duration of the whole diagnostic process and the timely causative treatment will be discussed. (3) How developing policies in shared responsibilities with clinicians for e.g. local alert result management or near patient testing can guarantee availability of reliable laboratory results requiring urgent/timely medical action will be also shown. (4) The usefulness of understanding analytical and biological variance of laboratory tests and critical difference concept by clinicians when interpreting laboratory results or changes between two subsequent results will be emphasized.

In order to provide more evidence-based ideas for laboratory professionals how to assist the extra-analytical phases more studies on extra-analytical laboratory activities should be organized. European Federation of Clinical Chemistry and Laboratory Medicine has been designated a Working Group (WG), WG on Postanalytical Phase, with the task of organizing such studies. Hungary actively participates in the WG’s work as well as in the international surveys organized by the WG.

**SE3.3**

**Critical result management in Hungary – Can we do better?**

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Although critical laboratory test results may have significant impact on medical decisions and on subsequent patient outcomes there is limited guidance on best practices of critical result (CR) management. Recognizing the need for harmonized standards in the field the Hungarian Society of Laboratory Medicine joined the international survey organized by the Task and Finish Group on Critical Results of the European Federation of Clinical Chemistry and Laboratory Medicine and the Australasian Association of Clinical Chemistry on CR management policies and alert thresholds of common biochemistry analytes. All Hungarian laboratories were invited to this international survey with a goal to collect existing practices in the management of CR countrywide and to compare the participants’ CR lists applied in everyday practice.

Forty-nine laboratories responded to the survey and twenty-four laboratories shared their CR lists, which was applied in three main clinical settings (adult out- and inpatients, pediatrics and obstetrics). We observed a great variation in CR management practices and detected a failure in reading back results when CR notification was done by telephone in 70% of the responding laboratories. The fundamental requirement for a shared policy between laboratory and clinical staff was often not fulfilled. Only 14% of respondents reported that relevant physicians were involved in decisions of which tests should be included in the laboratory’s critical result list. Alert thresholds were established in consultation with doctors in only 31% of the responding laboratories. Procedures for the maintenance and monitoring of the outcome of critical result management have also shown variations and shortcomings of current practices: 47% of respondents review their critical result list on a regular basis; 45% regularly monitor whether critical results are delivered within predetermined timeframes; and 18% of laboratories audit regularly their performance of delivering critical results.

Our results, reflecting Hungarian laboratory practices, confirm the large within country variations in CR management described in the literature and highlight the need for best practice recommendations for more efficient communication of CR.

**SE3.4**

**Correction and interpretation of laboratory test results**

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Today’s clinical laboratories work with numerous automated analyzers, which produce large (sometimes gigantic) amounts of results. On the one hand this is inevitable and provides great help in processing masses of biological samples within a limited length of time. On the other hand though the vast majority of the gained data are reliable and acceptable, in certain cases results either need to be corrected to become appropriate and real (A), or the true results need right interpretation by well-trained laboratory experts in cooperation with clinicians to become conceivable and useful (B).

(A) For technical reasons the results measured by the analyzers sometimes do not reflect the real conditions in the body. We collected a couple of demonstrative cases in our laboratory, when the intervention of a lab expert was necessary to be able to present appropriate results...
to the clinicians. These include the occurrence of hereditary or acquired myeloperoxidase deficiency detected by Advia haematology analyzers, cold agglutinins causing the reversible aggregation of red blood cells (RBC) leading to low RBC count (it might as well result in intensive anaemia treatment), haematologic malignancies, when blasts or other immature cells may be concealed in other cell populations and extreme high D-dimer values, which may not be measured by the analyzers even with the auto rerun function.

(B) We present two cases of patients with doubtful blood coagulation screening parameters. Patient 1 is a 59-year-old woman taking vitamin K antagonist. Despite her good compliance she found herself gasping for breath at the Emergency Department with extreme high INR and APTT. Patient 2 is also a woman, aged 83, taking platelet aggregation inhibitors because of previous stent implantation. During severe diarrhoea and worsening renal function her INR and APTT values were varying within a very broad range.

SE4.1
Forensic toxicology and its role in criminal proceedings
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In the recent past several branches of toxicology have evolved into independent scientific fields. Forensic toxicology – as an applied science – represents a rather peculiar discipline, in that it contributes to the application of a significant portion of criminal law and to the elucidation of legal disputes concerning the living and the deceased. The goal of the talk is to exhibit the most salient features of forensic toxicology:

– Its differences with respect to clinical toxicology,
– Its basic aims and goals,
– Cases for which a forensic toxicological examination must be carried out,
– Authorities who can request the examination,
– Persons who can perform the examination, what are the required qualifications for performing the examination (qualification and exclusivity regulations),
– Requirements for becoming a forensic toxicologist,
– Provisions of the “new Penal Code” which require a forensic toxicological examination,
– Domestic resources.

It is shown how the findings of a toxicological examination as detailed below contributed to the acquittal of a person charged with murder. Results of the examination: blood sample: 0.40 µg/ml 6-monoacetyl-morphine, 0.75 µg/ml free morphine, 0.46 µg/ml codeine. Urine sample: negative. Lethal concentrations in blood: 6-monoacetyl-morphine 0.19 µg/ml, free morphine 0.36 µg/ml.

SE4.2
Vitamin D determination: preanalytical and analytical considerations.
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The literature published over the two last decades indicates increasing awareness of vitamin D’s pleiotropic, multidirectional action in the human body. Evidence from large-scale studies contributed to the understanding that vitamin D deficiency may be a significant risk factor for many civilization diseases. There is recognized benefit of vitamin D for bone health based on both observational studies and randomized controlled trials. There is also evidence largely from cross-sectional, ecological, laboratory, and observational studies that vitamin D reduces risk of many types of cancer, cardiovascular disease, diabetes, autoimmune and metabolic disorders, infectious diseases linked to decreased immunity, and even some neuropsychiatric disorders. Based on the literature for non-skeletal effects of vitamin D, it appears that serum 25-hydroxyvitamin D (25-OH-D) concentrations between 75 and 125 nmol/L are associated with significantly reduced risk of such diseases. Therefore, a variety of practical and research activities are being undertaken worldwide to evaluate vitamin D deficiency and improve vitamin D status.

This fact makes its mandatory to follow strict preanalytical and analytical operating procedures in order to insure reliable 25-OH-D vitamin results. The author advocates the use of serum, rather than plasma, samples for the determination of 25-OH-D since the stability of the analyte in serum is far superior to that in plasma. Given the ever growing number of samples, automated analytic methodology is desired. Measurement of total (25-OH-D2+25-OH-D3) rather than 25-OH-D3 vitamin alone is insisted upon by all professional guidelines. The use of the >75 nmol/L value as sufficient 25-OH-D vitamin level is recommended by the 2nd Hungarian Vitamin D consensus statement and most international guidelines. Furthermore, participation in external quality assurance and acquirement of proficiency certification is strongly recommended for laboratories performing 25-OH-D examinations.
SE4.3

Accidents caused by intoxication

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In the case of different types of accidents (traffic crash, household or workplace misadventures, etc.) we have to pay attention to the influence of intoxication by chemicals. It is well-known that ethyl alcohol can dramatically decrease the driving abilities leading indirectly to serious injuries or even death. In a road accident it is obligatory to determine the blood alcohol concentration of the concerned persons. It is to be noted that other toxic drugs can cause alcohol like effects. The analysis of these substances in the biological fluids of victims is not so common in Hungary, yet. Our toxicology laboratory receives samples from traumatology several times to check the presence of suspected psychoactive substances because the injury is not in correlation with status of the patient. (i.e. when the patient is unconscious or disoriented.) We determine ethanol by an enzymological assay (Cobas Integra Roche) or by HS-GC-FID. We perform also semi-quantitative immunoassays for substance groups such as benzodiazepines, barbiturates and cannabinoids (Abbott Assym FPIA). For qualitative analysis we use HPLC-DAD with online extraction (Shimadzu Prominance TOX.I.S II.). This system is suitable to identify a broad spectrum of medical and illegal drugs. We examined more than 100 cases and found that investigated cases are mostly in connection with some intoxication. The most common poisons are ethanol, illicit drugs and psychiatric drugs. We represent two successfully solved cases in which the above-mentioned substances played a critical role. It is very important to consider the presence of the human performance decreasing drugs in persons involved in accidents.

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SE4.4

How to test the effect of aspirin and clopidogrel in patients on dual antiplatelet therapy?

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Dual antiplatelet therapy with clopidogrel and aspirin is currently the standard therapy in high-risk patients for the prevention of recurrent ischemic events. Our aim was to determine which laboratory tests, used for the detection of clopidogrel, are affected by aspirin and which laboratory tests, used for the detection of aspirin are influenced by clopidogrel. Study population included 111 patients with the history of ischemic stroke being on clopidogrel monotherapy for at least one month. Among these patients, 62 showed good response to the drug as demonstrated by flow cytometric analysis of vasodilator stimulated phosphoprotein (VASP) phosphorylation and a newly developed P2Y₁₃ receptor specific aggregation method in which ADP-induced aggregation is performed on prostaglandin E₁ treated platelets (ADP⁠[PGE₁]). Laboratory tests routinely used for the detection of aspirin effect (ADP-, collagen-, epinephrine-, arachidonic acid (AA) induced platelet aggregation and secretion, FPA-100 assay, VerifyNow ASA test and AA-induced thromboxane B₂ (TXB₂) production in platelet rich plasma) were carried out on samples obtained from these patients. The other arm of the study involved 52 patients with coronary artery disease being on aspirin monotherapy for at least one month. These patients showed good response to aspirin as demonstrated by the AA-induced platelet aggregation and TXB₂ production. Methods used for testing the effect of clopidogrel and aspirin were performed on samples obtained from these patients. Besides, all tests were carried out on samples from 160 healthy volunteers to determine diagnostic cut-offs.

Of the methods used for detecting the effect of aspirin, clopidogrel monotherapy significantly inhibited all aggregation and secretion tests including tests using AA as agonist. AA-induced TXB₂ production was also decreased (p<0.001). VASP phosphorylation and AA-induced platelet aggregation showed a surprisingly fair correlation in patients taking clopidogrel only (r=0.49, p<0.001). Clopidogrel therapy did not inhibit the VerifyNow ASA test. Of the methods used for testing clopidogrel resistance, aspirin monotherapy influenced ADP induced platelet aggregation and secretion (p<0.001), but did not have an effect on VASP phosphorylation and on the ADP⁠[PGE₁] aggregation test. According to our results, for testing patients on dual antiplatelet therapy, the VerifyNow ASA, VASP phosphorylation and ADP⁠[PGE₁] aggregation tests could be recommended.

YF.1

Experiences with a new D-dimer assay

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D-dimer test plays a very important role in diagnosis, and monitoring of thrombosis and other coagulation diseases. Primarily it has an overriding importance in the exclusion of venous thromboembolism, in particular deep vein thrombosis and pulmonary embolism. In clinical practice the specificity of currently used D-dimer assays is rather unsatisfactory, certain assays often show cross-reactivity with degradation products of fibrinogen. However, the harmonization of these assays poses several difficulties, it has a key importance in the reliable clinical interpretation of the results.
We have developed a reagent for assaying D-dimer that contains latex particles coated with D-dimer specific monoclonal antibodies. This is an immunoturbidimetric assay, based on latex-agglutination. In this work the possible cross-reactions of the used antibody with fibrinogen and degradation products were tested. The developed antibody gave no cross-reaction with fibrinogen, fibrin-E and fibrinogen-E fragments, but reacted with fibrin D and fibrinogen D fragments, and showed low cross-reaction with high molecular weight fragments fibrin X and Y.

A comparative study was made by measuring 100 frozen plasma samples with our self-developed reagent and with two other commercially available D-dimer assays. The results of the 3 assays showed significant differences. These alterations could be caused by the fact that the latex particles are coated with one or more kinds of antibodies, could be due to the different epitope specificity of the antibodies, and the different types of calibrations of each assays. Analyzing the harmonization recommendations and literature, a new approach of calibration was invented, which enabled us to ensure a better harmonization.

YF.2

The effect of factor XIII on the outcome of thrombolysis in ischemic stroke patients

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Thrombolysis by recombinant tissue plasminogen activator (rtPA) is the current most effective pharmacological therapy in acute ischemic stroke (IS). Little is known why in some patients thrombolytic therapy is less efficient and which are the factors promoting intracranial hemorrhage in others. Our aim was to investigate whether blood coagulation factor XIII (FXIII), a key regulator of fibrinolysis, is associated with the outcome of therapy. Blood samples of 133 IS patients, who underwent thrombolytic therapy within three hours of IS onset, were taken at admission, one hour and 24 hours after the initiation of rtPA infusion. Factor XIII (FXIII) activity and antigen levels were measured from all blood samples and patients were genotyped for FXIII-AVal34Leu, FXIII-A Tyr204Phe, FXIII-B His95Arg, FXIII-B intron K (IVS11+144) polymorphisms. Clinical data of patients using the National Institutes of Health Stroke Scale (NIHSS) score and results of imaging tests were registered at admission, on day 1 and day 7 after therapy. Unfavorable short-term outcome was defined as an increase in NIHSS score by at least 4 points on day 7. Long-term functional outcome was assessed according to the modified Rankin Scale (mRS) 3 months after the event.

FXIII levels were decreased immediately after thrombolytic therapy and continued to decrease significantly 24 hours after therapy. Decreased FXIII levels 24 hours after thrombolysis were significantly associated with mortality at 7 days, 16 days and 3 months after the event. FXIII levels in the lowest quartile 24 hours after thrombolysis increased the risk of unfavourable (mRS > 1) long-term outcome (OR 2.94; 95%CI: 1.22-7.04). Low levels of FXIII were not associated with bleeding complications. Among FXIII polymorphisms, FXIII-B intron K was associated with lower FXIII levels, but after adjusting to confounders, difference was not significant between carriers and wild type individuals. None of the investigated FXIII-A or FXIII-B polymorphisms were associated with stroke severity or with the outcome of thrombolysis.

YF.3

Increased expression of human epididymis protein 4 in cystic fibrosis

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Cystic fibrosis (CF) is an autosomal recessive disease causing abnormal mucous secretion with chronic inflammation in the lung. Although human epididymis protein 4 (HE4) is used as a tumor marker in epithelial ovarian cancer, abnormal HE4 expression was detected in CF lung biopsy samples with immunohistochemistry. No data were published if serum HE4 is also increased in CF. In this study, 77 patients were diagnosed with CF by sweat chloride test and molecular genetic analysis. Age- and sex-matched controls (n=77) without pulmonary diseases, 64 individuals with other non-CF lung disorders, and 12 parents of CF patients were also studied. Serum HE4 was determined by a chemiluminescent microparticle immunoassay (Architect, Abbott). In 29 CF subjects with exacerbation, C-reactive protein (CRP) was also measured by an electrochemiluminescent immunoassay (Cobas, Roche). Real-time PCR was used for quantifying HE4 gene expression in airway epithelial samples obtained with bronchoscope. Serum HE4 levels were significantly elevated in CF (99.5 [73.1-128.9] pmol/L) compared to controls (36.3
Increased HE4 concentrations were measured in severe bronchitis/asthma (63.7 [54.7-79.5] pmol/L) and pneumonia (57.8 [51.2-74.2] pmol/L), but these data were only moderately higher than normal. In contrast, heterozygous carriers of CF mutations had normal serum HE4 levels (33.9 [30.1-43.9] pmol/L). HE4 showed a significant positive correlation with the severity of the disease and sweat chloride values. There was a similar relationship between HE4 and CRP in CF, but not in other lung diseases. The relative HE4 gene expression in CF was upregulated with 2 orders of magnitude compared to that of controls suggesting that high HE4 may be due to its increased production in the airways. In conclusion, serum HE4 is increased in CF reflecting the clinical status, and may be used as a novel biomarker for detecting pulmonary inflammation in this disease.

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YF.4

Incomplete harmonisation of INR between five methods applied in Hungarian laboratories. Can these differences influence dosing vitamin K antagonists?

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The International Normalized Ratio (INR) is an official unit of the most used haemostasis screening test, the Prothrombin time (PT). The original aim was to make the results of different laboratories, different measurement systems, and different reagents comparable. As far as possible, the INR corresponds to this aim, but on the other hand we have to know the limitations and differences of the system. In our laboratory, various commercial measurement systems (reagent and instrument combinations recommended by the manufacturers) are compared to each other, matching PT result on 80 fresh human samples as the World Health Organization (WHO) standard describes. INR of normal samples and samples with oral anticoagulant therapy are compared by regression. We followed the prescription of reagent and instrument to the applied measurement system. Sample comparisons were performed in five measurement systems (A-E). The results showed various pictures. The slope of regression between A, B and C systems were acceptable: 0.85-1.15 while the slopes of regression of D and E to previous systems were outside this range. We still found individual pathological samples in the well-fit measurement system groups where deviation between the INR values was 22%, while this individual, pathological sample INR deviation obtained 75% in less well-fit measurement systems. R values of all applied fits were > 0.95.

It is important to know about divergences between INR results; the differences are not due to the fault of one or the other measurement system, but due to the difference characteristics of the measurement systems and variations of different INR traceability methods. Furthermore it is important to minimize the number of different results obtained from different methods, which can cause distinct clinical conclusions.

YF.5

Quantitative determination of psychotropic drugs in the serum of intoxicated patients by HPLC

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The clinical toxicology laboratory is continuously challenged; new medicines and drugs cause dangerous intoxications from almost month-to-month. The task of the clinician is helped significantly by knowing the cause of the poisoning material, however unfortunately we hardly rely on the case history in this aspect. Psychotropic benzodiazepines, antidepressants and antipsychotics are analyzed most extensively in the samples of the patients. Which is the most suitable biological matrix for toxicological analysis? We analyzed intoxicated patients’ urine and serum samples with Shimadzu Prominance TOX.I.S II. HPLC DAD system. We examined which sample is more appropriate for the determination of several drugs. For qualitative analysis the urine sample is the most suitable as it is in the clinical practice. For quantitative analysis the serum sample is the best. We can measure in the serum the correct concentrations of the toxic drugs or the rate of metabolism of them that can be even more important and turning into everyday method also in the practice of clinical toxicology. In the forensic practice we always have to determine exact quantities to define the rate of affection. The forensic institute and clinical laboratory works together in cooperation analysing qualitatively and quantitatively the psychotropics causing poisoning.

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Laboratory diagnosis of hemoglobinopathies in Hungary

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The genetic disorders of hemoglobin (Hb) affect about 7% of the world population; hemoglobinopathies are the most common monogenic diseases. Originally these disorders were found in the Mediterranean, African and Asian population, but today the increase of international migration has spread them all over the world. In many parts of Europe today Hb defects are regarded as endemic diseases. In Hungary the most common form of hemoglobinopathies is the beta thalassemia, usually with little or no clinical symptoms. Since in a lot of cases the exact clinical diagnosis is not established for a long time, the role of the laboratory is very important in the diagnostic process and test recommendations. In the last three years in our Laboratories 475 tests of Hb variants (HbA, HbA2 and HbF) were performed from peripheral blood samples with HPLC (Variant II, BioRad) and in 182 cases we found elevated levels of HbA2 or HbF. Of the 475 patient tested, the beta globin gene sequencing was carried out in 147 cases. We found genetic alterations in 107 cases. Most of these patients had beta thalassemia minor with HBB:c.118C>T (n=21), HBB:c.93-21G>A (n=19) and HBB:c.92+6T>C (n=14) mutations being the most common. In 4 cases hemoglobins with altered O2 binding affinity were diagnosed. Here we recommend an algorithm for investigation of patients with microcytic hypochromic anemia (the most common sign of thalassemia in Hungary), after iron deficiency has been excluded. Beside these cases Hb testing is also important in other rare clinical situations (like chronic hemolytic anemia, vascular obliteration crisis of unclear etiology, drug-induced anemia or hydrops foetalis of unclear etiology), where hemoglobinopathies might be suspected.

Flow cytometric analysis of naive and memory B cells on peripheral blood of patients with systemic sclerosis

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Several lines of evidence indicate that in addition to autoantibody production other B-cell functions play a key role in the development of systemic autoimmune diseases, such as systemic sclerosis (SSc). The anti-CD20 monoclonal antibody therapy seems to show some clinical efficacy in SSc further emphasizing the importance of B cells in the pathomechanism of the disease. The B-cell compartment in peripheral blood of SSc patients contains a high number of naive and a low number of memory B cells. The aim of the present research was to set up an algorithm for the extended analysis of these B-cell subsets and to evaluate the clinical significance of the defined subpopulations. Peripheral blood samples were obtained from SSc patients and healthy controls. PBMCs were isolated using Ficoll gradient centrifugation, followed by magnetic bead separation of CD19+ B cells. Multiparametric flow cytometry was performed with antibodies specific for CD27, IgD, CD80 and CD95 molecules. CD27 positivity was used to distinguish between naive (CD27-) and memory (CD27+) B cells. Non-switched (CD27+IgD+) and switched (CD27+IgD-) memory subsets were distinguished according their IgD expression. In addition to expression of CD80, which provides a co-stimulatory signal necessary for T cell activation and survival, expression of CD95 – FAS receptor – was also examined to investigate the activation state of the previously identified B cell subpopulations. According to our results a detailed flow cytometric analysis of naive and memory B-cell subsets could contribute to a better distinction between the two SSc subtypes, the assessment of disease activity, the evaluation of disease severity. It may be a useful new tool in routine immunological diagnostics.

Monitoring cell-cycle properties in malignant diseases could improve the effectiveness of chemotherapy

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Many of the chemotherapeutic agents used in the therapy of malignant diseases are cytotoxic due to the inhibition of cell-cycle progress. The concept of the therapy is that malignant cells are usually proliferate more frequently than normal cells thus they are more susceptible to cell-cycle inhibitors. However there are a number of normal cell types in the human body that are also highly proliferating (bone marrow, epithelial cells, fibroblast, etc.) thus chemotherapy has severe side-effects.
In this study we explored whether the therapeutic and side-effects of chemotherapeutic drugs could be better separated only by a careful design of the timing of the therapy. Since inhibitors of cell proliferation tend to synchronize cells (road-blocking the cell cycle at a given phase), if we know the exact schedule of the complete cell cycle for a given type, we could choose the best time-window to administer the drug.

We used a flow-cytometric method to detect cell-cycle changes in a malignant cell line. After determining S, G0, G2/M phase time, total cell-cycle time and population doubling time, we used a 2-step paclitaxel treatment protocol to block (and eventually kill) the cells in G2/M phase. Based on cell-cycle characteristics, we were able to determine the optimal length of the first treatment, and the optimal length and timing of the second treatment.

We believe that learning the proliferative properties of malignant cells and following-up on these properties during therapy (a.k.a. “therapeutic drug monitoring”) would help to evaluate and improve the effectiveness of chemotherapy.

YF.9

Detection of rare events in the bone marrow of patients with myelodysplastic syndromes

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Myelodysplastic syndromes are characterized by variable clinical outcome, therefore precise diagnosis and classification of cases to the appropriate prognostic category are indispensable. In addition to morphology and cytogenetic examination, flow cytometry also has a substantial role in the objective evaluation of these cases. We aimed to exploit this potential in our daily practice by testing the four-parameter flow cytometry score system (FCSM) elaborated by Ogata et al.

We assigned the patients to control (n=14) and MDS (n=57) groups on the basis of morphological, cytogenetic, and flow cytometric examination of their bone marrow samples. In addition to looking at the four FCSM parameters, we also investigated the percentage and surface antigen expression patterns of erythrocytes and rare events (plasma cells, normoblasts, mast cells (MC)).

When we compared MDS cases to normal bone marrows we detected significant differences in the myeloblast ratio (p=0.0037), in granulocytes SSC/lymphocytes SSC (p=0.0162) and in the proportion of lymphoblasts among CD34 positive cells (p=0.04). In addition, we found significant differences in the proportion of erythroids (p<0.0001) and – in terms of rare events – that of MC (p=0.01). The ratio of plasma cells and normoblasts did not differ significantly. In the morphological and immunohistochemical tests the MC were scattered individually and their atypical morphology was also observed in MDS patients. An association between MC and fibrosis has been found in various disorders, therefore we investigated the fibrosis grade of crista biopsy samples retrospectively. The results showed a higher mean MC ratio in myelofibrosis grade 2 compared to grade 0/1 cases.

Besides identifying the usual flow cytometric alterations previously described for MDS, we detected an increased MC ratio, which might have a role in the development of fibrosis in some MDS cases.

YF.10

Multi-platform whole blood hematology control

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Quality control is indispensable and a routine procedure in clinical hematology for a long time. Accuracy in the counting of different types of blood cells depends, in part, on the use of suitable control materials and their proper application. As several types of hematology analyzers are available on the market, quality control by the use of the suitable control products is necessary, because the risk of an instrument malfunctioning exists constantly. The manufacturer provides all of the critical values of a control such as cell count, cell size, and cell type, and also must guarantee an extended shelf life of control material to be used for months.

Since different instruments employ different methods to measure the five major populations of white blood cells, it became necessary to use a different type of control with each instrument.

To our best knowledge, control blood with long stability which works well in the variety of systems is not commercially available. In the Diagon Ltd. a new type of control blood has been developed to be used in a variety of hematology instruments.

The new control material for 5-part differential hematology instruments was tested in 5 different systems (Sysmex XE 2100, Abbott Cell Dyn 3700, Siemens Advia 120, Diagon D-Cell 5D and Beckman Coulter HMX) and it worked properly in each system and it has an extended stability up to 4 months and 21 days before and after opening, respectively.

The fact that the Diagon’s multi-platform control blood is suitable for most hematology systems gives a good chance to use it for not merely quantitative but qualitative blood cell count evaluation in external quality assessment programs.
YF.11

Chromosome analysis of amniotic fluid cells

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Cytogenetic studies of cultured fetal cells are the standard method for prenatal diagnosis of chromosomal abnormalities. Our objective was to investigate retrospectively the amniocentesis indications and karyotypes of amniotic fluid cells. A total of 2375 amniotic fluid specimens obtained via amniocentesis from January 2007 to December 2012 were successfully cultured and received a karyotype diagnosis. The results were grouped according to different indications: advanced maternal age (61.67%), abnormal ultrasound signs (9.56%), high risk serum screening (10.78%), advanced maternal age and ultrasound signs (5.05%), advanced maternal age and high risk serum screening (11.16%), ultrasound signs and high risk serum screening (0.59%), parents with chromosome abnormalities (0.25%) and other factors (0.85%). A total of 104 abnormal karyotypes were found and the rate of them was 4.37%. The numerical abnormalities (82/104, 78.85%) were trisomy 21 (52/104, 50%), trisomy 18 (15/104, 14.42%), trisomy 16 (1/104, 0.97%), trisomy 13 (1/104, 0.97%), supernumeric X/Y (6/104, 5.76%), X monosomy (4/104, 3.85%) and triploidy (3/104, 2.88%). The structural anomalies were translocation (13/104, 12.5%), inversion (5/104, 4.8%), deletion (2/104, 1.93%), Xq isochromosome (1/104, 0.96%) and +marker chromosome (1/104, 0.96%). Aneuploidy was the most common in most groups except the parents with abnormal chromosome. Karyotype abnormalities were prevalent in the following groups: advanced maternal age (27.89%), advanced maternal age and ultrasound signs (22.12%), ultrasound signs (16.34%), advanced maternal age and high risk serum screening (13.46%) and high risk serum screening (13.46%). The identification of these chromosome disorders was essential in the genetic prenatal screening introduced in our institute. Results are in line with literature findings.

YF.12

Cost-benefit analysis of Down syndrome screening, when life is appraisable

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Current laboratory techniques provide several approaches to screen Down's syndrome. The effectiveness and costs of different approaches vary.

Data and method: Using the Hungarian database of the 2008 birth statistics and maternal age distribution, I compared the performance and cost effectiveness of the available screening tests for Down's syndrome screening.

Outcome: The ultrasound (US) screening approach is the cheapest way to screen Down's syndrome, but misses one third of cases. Integrated test would increase the screening costs by 5%. Moreover, integrated screening test in comparison with the ultrasound screening would give us 91% less fetus with Down syndrome (9 vs. 102).

Conclusion: There are several alternatives of screening for the risk of Down syndrome before birth. It would be worth analyzing and modelling cost-effectiveness of screening methods to optimize national resources used for Down’s syndrome screening.

YF.13

Comparison of antithrombin III levels obtained by different measurement methods

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Antithrombin III (AT III) deficiency is one of the most important causes of thrombophilia. Hereditary AT III deficiency was the first recognized inherited thrombophilia. Since then various mutations have been identified and hereditary AT III deficiencies have been classified into two main classes. In type I AT III deficiency the synthesis of AT III is reduced resulting in a simultaneously decreased level of antigen and activity. Type II AT III deficiency is characterized by normal or moderately decreased antigen level and reduced activity. In type II deficiency the mutation can affect the heparin binding site, the reactive site or the a1C-a4B region in case of pleiotropic defects. The reduced level of AT III can also be due to acquired causes (e.g. liver disease, nephrotic syndrome, DIC, drug-induced). The latter is much more common than hereditary AT III deficiency. Commercially available AT III activity assays are chromogenic amidolytic assays. These tests can be FIIa- or FXa-based assays. The sensitivity of these tests towards different types of AT III deficiencies is different, thus in case of certain patients a clinically significant alteration in the AT III level can be observed. In the present work AT III result obtained by FIIa- and FXa-based chromogenic assays were compared at various patient groups. For testing both acquired and hereditary AT III deficiency, samples from 40 patients with liver dysfunction and 5 samples from patients with verified hereditary AT III deficiency were measured. However, the two methods showed an acceptable correlation, at certain cases the measured AT III levels differed significantly. In the presentation we will focus on these individual cases and analyze the biochemical reasons of the clinically important alterations.
YF.14

*Clostridium difficile* infections and their common risk factors in Uzsoki Hospital

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Background: *Clostridium difficile* is a common cause of infectious diarrhoea among hospitalized patients. This spore-forming, Gram positive bacillus can lead to an infection with symptoms ranging from mild diarrhoea to pseudomembranous colitis, sepsis and death.

Method: This retrospective study includes discharged patients between 01/01/2012 and 31/12/2013 from Uzsoki Hospital. *Clostridium difficile* infection (CDI) cases were identified by Central Laboratory-Microbiology Department of Uzsoki Hospital using enzyme-immunoassay (EIA) testing. The test detects glutamate dehydrogenase (GDH) and *C. difficile* toxins A and B. An incident case was defined as the first CDI episode for patients (with one or more events during the hospitalization).

Results: Our Study includes 501 cases. This number corresponds approximately to 45% of the nosocomial infections in 2012 and 2013. Number of nursing days was 4% higher in 2013 than in 2012, however the rate of CDI didn’t increase in 2013 (2012: 0.1023 and 2013: 0.1033).

The use of common antibiotics (quinolones and cephalosporins) was significantly associated with CDI.

Conclusion: The prevalence of CDI can be controlled and monitored by strict surveillance, recognizing the major risk factors and the appropriate use of antibiotics.

YF.15

Molecular characterization of new Antithrombin mutations

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Antithrombin (AT) deficiency is a rare but major risk factor in venous thrombosis. It is classified as type I (quantitative) and type II (qualitative) deficiency. More than 230 mutations have been described in the gene encoding AT.

Our aim was to characterize three novel (p.Leu205Pro, p.Asn450Ile, p.Gly456delins-Ala_Thr) mutations causing type I AT deficiency at molecular level.

Wild type and mutant plasmids were transfected to HEK293 cells and the expressed AT proteins were investigated in the cell media and cell lysates by ELISA and Western blotting (WB) techniques. Intracellular localization of the different mutants was examined by immunofluorescent staining detected by confocal laser scanning microscopy. Structural alterations of the mutant AT proteins were investigated by molecular modeling.

AT with p.Leu205Pro mutation was detected intracellularly in the same level as wild type, however only a tiny amount of mutant AT was secreted into the medium. This mutant showed significant co-localization with the 26S proteasome. *In silico* experiments using 6 μs molecular dynamics simulation suggested a major structural alteration. Based on these observations it can be concluded that p.Leu205Pro mutation leads to impaired folding and secretion defect; the mutant AT retains in the 26S proteasome and subsequently suffers intracellular degradation.

The level of p.Asn450Ile and p.Gly456delins mutants were strongly reduced in the cell lysates and no AT was detected in the cell media suggesting reduced protein synthesis.

YF.16

What is to know about HbA1c measurement 3 years after the introduction of IFCC standardization

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Background and aims: The IFCC Standardization of HbA1c was introduced in Hungarian laboratories in April 1st, 2011. We use only IFCC standardized tests, we release the results in mmol/mol and to help in interpretation we also provide it in DCCT%. Since the diabetologists in Hungary could see different HbA1c results in different laboratories for the same patients, we decided to compare the most frequently used HbA1c methods looking for the source of errors.

Patients and methods: We measured 102 patient samples using 2 HPLC, 1 MEIA, 3 immunoturbidimetry methods within 72 hours, where the patients visited diabetological consultations. Frozen samples were also measured by using 1 immunoturbidimetry and 1 POCT method. For statistical evaluation Microsoft Excel program and Anova tests were used.
Results: All evaluated tests had IFCC certificate. Since both the deviation and the CV% were the smallest in case of the BioRad Variant II. Turbo method and the result of the EQA control was also excellent, we used this method as basis for the comparison of the other arrays. The regression values are as follows:

- Abbott - Architect \( y = 1.04x - 1.39 \);
- Beckman - Oly AU 640 \( y = 0.91x - 1.33 \);
- Siemens - Dimension \( y = 0.91x + 5.52 \);
- Roche - Cobas \( y = 1.01x - 1.35 \);
- Diagon - Diasys \( y = 1.10x - 5.56 \);
- Siemens - DCA Vantage \( y = 0.98x + 0.71 \).

Every test correlated with each other strongly (r values were between the limits of 0.96 -0.99). The CV% values of the intra-assay were between the limits of 0.3-3.4%.

Conclusion: Although we identified strong correlation between the methods, the results differed significantly from each other in many cases. Differences of the methods, the bias of tests calibrators compared to IFCC reference material, the different application of the assays, the general condition of the instruments, the%SD set-up could contribute to these results. We should not ignore these differences, because they can influence the treatment of diabetic patients.

SE5.1

Protein C and Protein S in critically ill surgical patients and in overweight hypertriglyceridemic patients

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The protein C system is one of the main physiological anticoagulant mechanisms. We studied changes of protein C and S antigen levels (PC:Ag, PS:Ag) in clinical situations with increased prothrombotic risk. Therefore PC:Ag and PS:Ag levels were investigated in critically ill patients undergoing surgical interventions then in patients with metabolic syndrome (overweight, hypertrygliceridemic) in comparison with control subjects (normal weight, normolipidemic).

In critically ill surgical patients, PC:Ag and PS:Ag were significantly decreased \((63.3 \pm 4.2, \ p<0.001\) and \(59.2 \pm 4.96,\ p<0.001\) respectively) comparing to control subjects. Plasma PC:Ag and PS:Ag were significantly increased in overweight hypertriglyceridemic patients \((164 \pm 0.85\%, \ p<0.001\) and \(113 \pm 3.10\%, \ p<0.001\) respectively) when compared to the normal weight normolipidemic control subjects.

Decreased levels of PC and PS:Ag could be explained by the switch of the hepatic protein synthesis during the acute phase reaction developing in critically ill surgical patients towards the increased production of acute phase proteins, while reducing the secretion of PC and PS, cholinesterase and albumin. These observations highlight the risk for thrombosis in postoperative states and point the importance of a thorough investigation of hemostasis in surgical patients.

The increased values of PC:Ag and PS:Ag and serum cholinesterase activity reported in obese hyperlipidemic patients might be subsequent to the overweight related hyperinsulinism and to the accelerated turnover of free fatty acids and VLDL, characteristic for android obese and hypertriglyceridemic patients.

The relevance of these changes for the hemostatic balance should be considered with caution. In vivo effects of the protein C system are dependent not only on the amount of protein C but also on available endothelial thrombomodulin and endothelial receptor of protein C, while anticoagulant function of protein S depends on the amount of its free form. On the other hand, even if the levels of these anticoagulant mechanisms are increased, obese hypertriglyceridemic patients also have elevated levels of factors VII, X and XIII and an enhanced antifibrinolytic potential which contribute to a more fragile hemostatic balance.

SE5.2

Interaction of complement, neutrophils and endothelial cells in the pathogenesis of thrombotic thrombocytopenic purpura

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The protease catalyzing the maturation of von-Willebrand factor (ADAMTS13) plays critical role in the pathogenesis of thrombotic thrombocytopenic purpura (TTP). Genetic (mutations of ADAMTS13) and autoimmune (inhibitory autoantibodies against ADAMTS13) risk factors contribute to the development of TTP but direct triggers are needed to exacerbate acute disease.
Vascular calcification and chronic kidney disease

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Vascular calcification, especially in patients diagnosed with chronic kidney disease, is the major pathophysiologic process in cardiovascular diseases. Calcification develops in two main distinct sites within arteries. While in atherosclerosis the intima is affected focally at predisposed areas of vessels, in media sclerosis the media layers of the large- and medium-sized arterial wall are mineralized in a diffuse fashion. In calciphylaxis the small cutaneous arterioles are affected and the mineralization of vessels almost exclusively occurs in patients with advanced chronic kidney disease. Abnormalities in mineral metabolism are important regulators of vascular calcification. It is an actively regulated multistep process in which the trans-differentiation of smooth muscle cells (SMC) into osteoblast-like cells is induced by change in inorganic phosphate (Pi) level. After osteoblastic differentiation these cells lack characteristics of SMC, and develop osteoblast features. At physiological environment, cells express smooth muscle lineage markers, SM 22α and SM α-actin. After exposure to elevated phosphate, high glucose level, activated vitamin D, reactive oxygen, a dramatic loss of markers for SMC lineage occurs and simultaneously a gain of osteochondrogenic markers such as alkaline phosphatase, osteocalcin and core-binding factor-1 (cbfa-1) develops. Phosphate uptake through a sodium-dependent phosphate co-transporter, Pit-1, is implicated in vascular SMC calcification and phenotypic modulation. While types I and II transporters are restricted to the kidney and intestine, type III transporters are ubiquitously present in tissues including kidney, heart, lung, and bone. Pit-1 and Pit-2 represent the type III transporter. Of these known transporters, Pit-1 was found to be expressed in human SMC as well as in human aorta that facilitates entry of Pi into vascular cells. Vascular calcification being a delicately regulated cellular process where SMC gain an osteoblastic phenotype is also indicated by the fact that increased expression of cbfa-1 is observed in cells exposed to high phosphate or platelet-derived growth factor. Cbfa-1 is an essential regulator of osteoblast differentiation and fulfills a dominant function for other gene products. Osteocalcin is the major non-collagenous protein in bone matrix. The calcium binding properties of osteocalcin and its pattern of expression in bone suggests an important function in bone mineralization. Osteocalcin, one of the gamma-carboxyglutamic acid containing proteins is present in calcified atherosclerotic lesions and mineralized heart valves at high concentration. Other important factors acting directly (fetuin, BMP, MGP), or indirectly (FGF23, klotho, leptin, cytokines, LDL) on mineralization will be reviewed as well.

Diagnostic considerations based on the experience of genetic analysis in Protein C deficiency and molecular characterization of different mutations

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Protein C (PC) serves as a major anticoagulant and numerous distinct mutations in its coding gene result in type I (quantitative) or type II (qualitative) PC deficiency with high thrombosis risk. Screening for PC deficiency is executed by clotting or chromogenic functional tests. Both methods have several disadvantages. FV Leiden mutation (FVL) interferes with the clotting assay leading to low PC activity and FVL positive patients seem to have type II PC deficiency. Chromogenic tests do not suffer from this problem, however they may not detect some type II cases caused by certain mutations.
We evaluated the functional clotting and chromogenic PC assays from the point of view of FVL interfering effect and determined the mutation spectrum of PC deficiency in the Hungarian population. Moreover, the molecular consequences of certain novel or uncharacterized mutations were also investigated.

Non-related individuals having 70% or lower PC activity measured by the clotting test were recruited (n=109). PC activity by the chromogenic method and PC antigen were also determined. The gene encoding PC (PROC) was analyzed by direct DNA sequencing and by MLPA method in those cases if larger genetic abnormality was assumed. The fate of mutant proteins (p.Asp77Gly, p.Ala163Glu and p.Ala163Val) expressed in HEK cells was monitored by ELISA and Western blotting. Their intracellular localization was examined by immunostaining and confocal laser scanning microscopy. Structural consequences of the mutations were investigated by molecular modeling and dynamics simulations.

Most of the patients with low PC clotting activity were carriers of the FVL (n=72) and only 12.5% of them were positive for PROC mutations. As opposite, 78.4% of FVL negative patients had causative mutations in the PROC gene. Altogether 28 different mutations were detected including 15 novel ones (two of them were identified by MLPA). The 163Val and 163Glu mutant PC had undetectable levels in the culture media of HEK cells and showed intracellular co-localization with the 26S proteasome. The secretion of Gly77 mutant into the media was slightly decreased. The 163Val and 163Glu mutations caused significant changes in the relative positions of the EGF2 domains suggesting misfolding, while no major structural alteration was observed in case of the Gly77 mutant.

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**SE5.5**

**Progressive chromogenic anti-factor Xa assay and its use in the classification of antithrombin deficiencies**


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Antithrombin (AT) is a slow-acting progressive inhibitor of activated clotting factors. It is particularly effective inhibitor of thrombin and activated factor X (FXa). The presence of heparin or heparan sulfate accelerates its effect by several magnitudes. AT deficiency is a severe thrombophilia, which is classified as type I (quantitative) and type II (qualitative) deficiency. Mutations causing type II deficiencies may influence the reactive site, the heparin binding-site (HBS) and exert pleiotropic effect. Heterozygous type II-HBS deficiency is a less severe thrombophilia than other heterozygous subtypes. However, as opposed to other subtypes, it also exists in homozygous form representing a very high risk of venous thromboembolism. There are no commercially available tests for the differential diagnosis of type II-HBS deficiency.

We developed a modified anti-FXa chromogenic AT assay for this purpose. The assay determines both the progressive (p) and the heparin cofactor (hc) AT activities. The assays showed excellent reproducibility and were not influenced by high concentrations of triglyceride, bilirubin and hemoglobin. Reference intervals for p-anti-FXa and hc-anti-FXa AT activities were 84-117% and 81-117%, respectively. The usefulness of the assay in detecting type II-HBS AT deficiency was tested on 78 AT deficient patients including 51 type II-HBS heterozygotes and 18 type II-HBS homozygotes. Heterozygous type II-HBS AT deficient patients demonstrated low hc-anti-FXa activity with normal p-anti-FXa activity. Homozygotes had very low hc-anti-FXa activity and only slightly decreased p-anti-FXa activity. The hc/p ratio clearly distinguished wild type controls, type II-HBS heterozygotes and homozygotes.

Parallel determination of p-anti-FXa and hc-anti-FXa activities provides a reliable tool for the clinically important diagnosis of type II-HBS AT deficiency and distinguishes between homozygotes and heterozygotes.

**SE5.6**

**Changes of coagulation parameters in patients with end-stage renal disease**

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There is a significant risk of both thrombotic and hemorrhagic disorders in patients with end-stage renal disease (ESRD). Changes in the hemostatic system might contribute to the pathogenesis of these disorders. Our aim was to test the level of hemostatic factors, which might associate with the risk of thrombosis in patients with ESRD.

In the study 30 patients being on hemodiafiltration (HDF) treatment for at least 3 months were included. Treatment of the same patients was switched to conventional hemodialysis (HD) for 2 weeks and hemostasis parameters were also investigated after this period. Blood samples were taken just before and 1 and 4 hours after the initiation of HDF or HD treatment. Factor VIII (FVIII) activity, antithrombin (AT) activity, factor XIII (FXIII) activity and FXIII antigen levels were determined. The levels of the coagulation parameters were adjusted for serum albumin concentration.
In 30% of the patients on HDF treatment FVIII activity was above the reference interval at the start of an actual treatment. FVIII activity did not change significantly during the course of diafiltration. 20% of HDF treated patients and 26% HD patients treated patients had decreased AT activity at the start of an actual treatment. During HDF and HD treatment there was only marginal change in the AT activity adjusted for albumin. Before starting the treatment 26% and 43% of HDF and HD patients showed increased FXIII activity, respectively. Both HDF and HD treatments resulted in a further slight, but significant increase of FXIII activity. The increase of FXIII antigen levels during the treatment was evident only during HD.

Elevated FVIII and FXIII levels and the decreased AT activity in patients with ESRD might contribute to the increased risk of thrombosis. The identification of changes in specified hemostatic parameters during HDF and HD treatment could support a more effective prevention of thrombotic events.

SE6.1

Endocrine basis of in vitro fertilisation: treatment, markers of stimulation and success rate

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By definition, infertility is the inability to conceive after 1 year of unprotected intercourse. Specific causes of infertility are identified as female factors alone (one third), male factors alone (one third) or a combination of male and female infertility. The birth of the first baby Louise Brown in 1978 opened a new era in fertility medicine: the in vitro fertilization (IVF) moved from vision to reality. Nowadays the assisted reproductive techniques (ART) are wide-spread used and provide valuable help for infertile couples. However, the success of the treatment is strongly influenced by the age of the female and her ovarian reserve capacity. By determination of serum FSH or anti-müllerian hormone the number of available follicular pool can be estimated which is a key for the successful IVF treatment. The protocols for controlled ovarian stimulation consist of chlomiphen citrate or GnRH agonists/antagonists treatment together with or followed by FSH stimulation. folliculometry and sequential estradiol measurement are important tools for the evaluation of the success of stimulation and to avoid hyperstimulation of the ovaries. When the leading follicle reaches >17 mm in diameter, HCG is administered for the final maturation of oocytes and triggering ovulation. An alternative to HCG, bolus injection of GnRH agonist has been tested recently to mimic the spontaneous mid-cycle LH surge. Thirty-six hours after induction of ovulation, an ultrasound-guided needle biopsy is applied to aspirate the oocytes. The fine tuning of stimulation protocols resulted in change in concepts from “the more eggs, the more chance for successful IVF” to “the quality of eggs are more important than the number of oocytes” point of view. Obesity (BMI), endometrial thickness, estradiol/follicle ratio, estradiol level per mature follicle, progesterone level on day of oocyte retrieval, number of viable oocytes collected, embryo culture media, embryos transferred are examples of factors associated with the outcome of IVF treatment.

SE6.2

Vitamin D and allergy

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The aim of this study was to investigate vitamin D (VD) levels in allergic and non-allergic groups as well as to determine the association between VD, IgE and specific IgE levels. 209 samples of children (110 girls, 99 boys, mean age: 9 years) from “Magyar Imre” Hospital were tested for VD levels, total and specific IgE. Eighty-nine children suffered in allergy (asthma 55%, food allergy 30%, rhinitis 12%, atopic dermatitis 2%). The other group consisted of 120 patients with different disorders including coeliac disease (8%), resorptive dysfunction (48%), bronchitis (8%) and others (35%). VD and specific IgE were determined by chemiluminescence immunoassay. Total IgE was tested by immunoassay. Statistics were performed by Med Calc software. Mean VD levels in allergic patients were 59.8 nmol/l, and in the non-allergic group 59.4 nmol/l (95% CI:55.9-63.7 and 58.1-62.9). The mean total IgE levels were 365.9 kU/l (95% CI: 215.1-516.9) in allergics and in the non-allergic patients 105.3 kU/l (95% CI:40.5-170.2). Within the allergic groups children treated by VD, the average levels of VD were 50.9 nmol/l (SD:16.8) in contrast to non-treated ones, who had higher average levels, 63.3 nmol/l (SD:18.1). p=0.038. The mean IgE of the VD treated group was 534 kU/l against those of not-treated with 292 kU/l (95% CI:166.4-901.9 and 139.9-445.9, p=0.1212). Among asthmatic children with low VD levels the prevalence of house dust mite positivities was higher. Within the non-allergic groups IgE levels were significantly different in supplemented against non- supplemented children. (53 kU/l vs.134 kU/l, p<0.001). The VD levels were similar to those allergic groups, supplemented 51.6 nmol/l (SD:14.4) against non- supplemented 63.5 nmol/l (SD:18.0). In case of VD supplementation the allergic children showed higher mean IgE levels in contrast with non-allergic substituted groups. In spite of VD supplementation none of the groups has reached the ideal VD level.
Conn syndrome in a 12-year-old child, caused by adrenal adenoma

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Primary hyperaldosteronism is caused by autonomous production of aldosterone by the adrenal cortex (due to hyperplasia, adenoma, or carcinoma). Symptoms and signs include episodic weakness, elevated blood pressure, and hypokalemia. Diagnosis includes measurement of plasma aldosterone levels and renin activity. Treatment depends on causality. A tumor is removed if possible; in hyperplasia, spironolactone or related drugs may normalize blood pressure and eliminate other clinical features. Primary aldosteronism is caused by an adenoma, usually unilateral, of the glomerulosa cells of the adrenal cortex or, more rarely, by adrenal carcinoma or hyperplasia. Adenomas are extremely rare in children, but the syndrome sometimes occurs in childhood. Hyperaldosteronism owing to aldosterone-producing adenoma (Conn syndrome) is a very rare but potentially curable form of pediatric hypertension. The authors present a case-report of a 12-year-old girl who had symptoms of hypokalaemia (2.8 mmol/L), hypertension, and fatigue, and hyperaldosteronemia was implicated as a possible etiology. The diagnosis was confirmed by findings of increased levels of plasma aldosterone (390 pmol/l = 14 ng/dl) and decreased levels of plasma renin activity (< 0.15 μg/l x h). The aldosterone to renin ratio (ARR) was > 93. The 9 mm cystic tumor mass was localized in the right adrenal gland with ultrasonography and confirmed by magnetic resonance imaging. The right adrenal gland was subsequently excised by laparoscopy, and its pathological assessment revealed an adenoma. Following surgical intervention the patient is normotensive without antihypertensive drugs.

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TSH reference range in the second trimester of pregnancy

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Hypothyroidism during pregnancy could be a serious risk for the fetus. Therefore, it is important to know the TSH level at pregnancy to prevent this. Our study is focused on TSH values in pregnancy and to relate them to healthy non-pregnant range.

We used samples of 186 pregnant women in the second trimester on the day of the sampling at Flór Ferenc Hospital, Kistarcsa. We performed TSH supersensitive measurements with an electro-chemiluminescent immunoassay technique on a Cobas e 411 analyzer.

The pregnant women’s TSH levels ranged between 0.37-5.08 mIU/L. Of note, healthy (non-pregnant) reference range recommended by Roche and used by us are 0.35-4.1 mIU/L and 0.27-4.2 mIU/L). Then we reviewed the results of the 186 pregnant women back for years, and excluded those who were diagnosed with thyroid disorder. This case the reference range in 2nd trimester of pregnancy became 0.37-4.65 mIU/L.

In summary: TSH reference ranges alter at each trimester during pregnancy. Therefore, specific reference ranges (and not healthy non-pregnant reference ranges) are recommended to be used when pregnant persons are assessed.

The impact of age-specific reference ranges on the interpretation of laboratory results

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Background: Blood concentration of several hormones are influenced by physiological factors including age, pregnancy, menstrual cycles, nutritional status, diurnal rhythm and stress. Hormones are routinely measured by immunoassays on high throughput, automatized instruments. Usually age-dependent reference ranges of gonadal steroids and gonadotropins are presented on laboratory reports. However, for testosterone levels in adults many commercial suppliers provide one reference range for women and another for men with wide ranges. For prostate specific antigen (PSA) typically one value for negative and another one for positive and for concentrations between these two levels a grey zone is indicated.

Objective: to compare the clinical interpretation of serum testosterone and PSA measurements when method- and/or age-specific reference ranges were used.
Patients and methods: 727 serum samples (from patients between 15 and 94 years of age) and 5684 serum samples (from patients between 29 and 94 years of age) presented consecutively at a single regional laboratory centre were evaluated for testosterone and PSA levels, respectively. Both testosterone and PSA measurements were performed on a BD Access machine with competitive chemiluminescent enzyme immunoassay, and sandwich chemiluminescent enzyme immunoassay, respectively.

Results: For testosterone, using the method-specific reference ranges (two categories) 254 (35%) patients, while using the age-specific reference ranges (18 categories) 204 (28.7%) patients were diagnosed with hypogonadism. For PSA, using the method-specific reference range (one category) 4354 (76.6%) patients, while using age-specific reference ranges (4 categories) 4681 (82.3%) men had negative results. However, the number of patients in the grey zone was higher when a one-level reference range and not age-specific values were used (711 (12.51%) vs 384 (6.76%)).

Conclusions: Using age-specific reference values for PSA and testosterone that are influenced by age may contribute to better diagnostic performance of these assays and will provide a more accurate diagnosis.

**SE7.1**

**Distinct clinical characteristics in myeloproliferative neoplasms with calreticulin mutations**

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With the recent discovery of somatic insertions/deletions in the calreticulin (CALR) gene in myeloproliferative neoplasms (MPN), the definite molecular diagnostics has become available in >90% of cases. We aimed to apply a complex array of molecular techniques to identify driver mutations in a large MPN-cohort allowing diverse phenotypic comparisons. A combination of allele-specific PCR (JAK2V617F), quantitative TaqMan assay (JAK2V617F quantity), high resolution melting (MPL mutations), fragment analyzes by capillary electrophoresis and Sanger-mutations in a large MPN-cohort allowing diverse phenotypic comparisons. A combination of allele-specific PCR (JAK2V617F), quantitative molecular diagnostics has become available in

We conclude that similarly to previous studies, CALR mut MPN is associated with distinct clinical characteristics. The recent discovery of the somatic CALR mutations improves the precision of non-invasive diagnostics of patients with clonal disorder.

**SE7.2**

**Age-related reference ranges for serum thymidine kinase activity and validation in chronic lymphocytic leukemia**


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To date no age-related reference ranges have been defined for serum thymidine kinase (TKI) activity. Being a proliferation marker, it may be used as a prognostic factor in malignant diseases, including chronic lymphocytic leukemia (CLL). Our aim was to establish age-related reference ranges for TKI and examine its utility as a screening marker in CLL, a disease of the elderly.

Serum TKI activity was measured by a competitive chemiluminescent immunoassay in 369 healthy adults and 115 de novo CLL patients. We observed a statistically significant decline in TKI activity from young (18-35 years) to middle-aged (36-60 years) and further on to elderly (60-86 years) healthy individuals. Age-related reference range was: <30 U/L for young, <25 U/L for middle-aged and <19 U/L for elderly. There was no difference in TKI activity between the studied healthy men and women. In CLL patients, TKI activity was the highest in the advanced Rai stages. The area under the receiver operating characteristic curve (ROC-AUC) for TKI was 0.840 (95% CI: 0.787-0.892), for differentiating CLL patients from age and sex matched healthy controls, with a cut-off value of 10.5 U/L (sensitivity: 80.9%, specificity: 73.4%). TKI was significantly elevated in CD38+/Zap70+ CLL patients, and showed significant correlation with WBC and absolute B-cell count.

In healthy individuals serum TKI activity is not influenced by gender, but declines significantly with age. Therefore, the use of age-related reference ranges is recommended, especially when evaluating CLL patients who generally belong to the elderly age group.
SE7.3

Our experience with the qualitative and quantitative analysis of body fluids using CellaVision DM96 analyzer

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Clinical data derived from proper body fluid procedures and accurate test results are essential to make the appropriate diagnosis and to provide the best possible therapy for the patients.

In the course of the daily laboratory work different types of body fluid samples are investigated for white blood cell count and distribution. The aim of this study was to compare the classical microscopic evaluation of body fluid samples to the digital microscopic system DM96.

A total of 30 body fluid samples (ascites/peritoneal, pleural, pericardial fluid) were analysed according to Clinical and Laboratory Standards Institute guidelines. Haematology analyser (Sysmex XE-2100) was used for the determination of white blood cell count. From each sample cytopsin slides were prepared and stained (May-Grünwald-Giemsa). The qualitative evaluation of cells was performed by microscopy and also by Body Fluid Software of CellaVision DM96 analyzer. Our results clearly demonstrated that the results of standard and those of digitally processed microscopic cell-differentiation are comparable. This observation was confirmed on the basis of some interesting clinical cases.

Considering the high pre-classification accuracy, the software can be used to create a preliminary report in those cases when professional expertise is not available.

For laboratory professionals the cell-recognizing software is helpful in everyday practice and in professional training, too.

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SE7.4

Laboratory diagnosis of heparin induced thrombocytopenia

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Heparin induced thrombocytopenia (HIT) is a severe side effect of heparin treatment caused by IgG antibodies against platelet factor 4 (PF4)-heparin complex. Thrombosis and thrombocytopenia are the leading clinical symptoms of HIT. Clinicians can assess pretest probability of HIT by applying the 4T’s score system.

Among anti-PF4-heparin antibodies only platelet activating antibodies are pathogenic and cause thrombosis. Laboratory testing of HIT involves immunological detection of antibodies against PF4-heparin complex and functional assays. In our laboratory heparin-dependent activation of donor platelets by patient plasma is detected by flow cytometric assays measuring the increased binding of Annexin-V to platelets and the elevated number of platelet microvesicles/microparticula (MP).

ELISA test (IgG ELISA, Hyphen BioMed) was performed in case of 355 suspected HIT patients. Based on the results HIT was excluded in 86% of cases. The functional test was performed in 48 patients for whom the ELISA test result was positive (n=38) or weakly positive (n=10). Pathogenic antibodies were detected in 15 cases based on Annexin-V binding, with a single exception the platelet microvesicle assay produced identical results. The probability of a positive functional assay result was higher in patients with high antibody titer. The positive results of both functional assays (Annexin-V positivity and MP region analysis) were in good agreement with the presence of thrombosis. At low pretest probability of HIT only non-pathogenic antibodies are present even in samples with high antibody titer, while only pathogenic antibodies are present in patients with high pretest probability of HIT. At intermediate pretest probability the antibody titer was higher in case of patients having pathogenic antibodies.

The antibody titer is important, it predicts the presence of pathogenic HIT antibodies. HIT must be confirmed by a functional test.

SE7.5

Tests ordered by the laboratory when paraproteins are suspected

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In diseases with paraproteinaemia (heavy chain disease, Waldenström-macroglobulinaemia, myeloma multiplex) serum total protein/albumin ratio rises. Several cases of suspected paraproteinemias were identified during our daily routine work at the Central Laboratory in Petz Aladár County Hospital. In my lecture I will provide a short overview about them.
SE8.1

What guidance patients need from laboratories when undergo laboratory investigations?

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The most recent survey of the Hungarian Society of Laboratory Medicine that was run with a goal to learn external perception of laboratory medicine both within the population and medical community, revealed a general satisfaction with the analytical function of laboratories and expectations of patients for more clear laboratory reports and individualized interpretations. How specialists in laboratory medicine can communicate directly with the patients is an emerging topic worldwide. The Lab Tests Online (LTO) website (www.labtestsonline.hu) can be a useful channel in communication to the patients. LTO site was launched in Hungary with an aim to assist non-professional readers in understanding the clinical meaning of different laboratory tests. Visitors of the website beside finding information on laboratory tests can also ask laboratory professionals directly in e-mail about their laboratory-related problems.

We analyzed 89 questions, sent by Hungarian LTO-readers in the last two years, in order to learn what types of information patients look for when they undergo laboratory investigations. Vast majority of the questions targeted interpretations of laboratory results, which remained unexplained by the requesting physicians to the patients (58% of all questions). In this lecture the most challenging interpretative questions will be discussed. Information on preanalytical phase and general questions on the process of laboratory investigations (e.g. the time till receiving the results) were asked in 9% and 8% of questions, respectively. The remaining questions were either about finding a laboratory site providing rarely requested analytes (9% of the questions) or requested services out of the scope of the website (e.g. therapeutic advice).

Questions of the LTO website’s readers reflect significant gaps in communications to patients when laboratory service is requested. Our findings confirm a definitive need for direct communication between patients and laboratory professionals during the whole process of laboratory investigations. The development of effective channels for this communication can be an important future task of national laboratory societies.

SE8.2

The effect of integration on pre- and postanalytical phase

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In order to obtain an up-to-date, wide range clinical laboratory system, preanalytical and analytical processes were integrated with an efficient sample transport and storage system in our Department. For sample handling a centrifuging, aliquoting and sorting preanalytical line (MPA); for sample storage, an archiving post analytical robot (p501) were installed into the Cobas8000 system. Our aim was to evaluate test-specific interferences, throughput and turnaround time (TAT).

Methods: the types of sample containers and size of aliquots were adjusted to the preanalytical and pneumatic tube system. In the course of method harmonization identical IFCC reagents, multicontrols and calibrators were applied on each analyzer by daytime and night shifts. This reduced evaluation problems, but we had to change traditional reference ranges for amylase, alkaline phosphatase, LDH, lipase and sodium. Autovalidation parameters were also adjusted, preanalytical limits of hemolysis, icterus and lipemia (HIL) were checked for sensitive analytes. These steps resulted in transparent, lean sample handling and efficient analysis. The performance is now 2.4 million chemical and ∼300.000 special protein tests a year, with an appropriate reserve capacity. Median TAT for routine chemistry was about 1.1 hour in 2013, with 95 percent of tests completed in 2.3 hours. Although increased capacity of e-602 line reduced the median TAT of specific proteins from 4.2 hours to 3 hours, autovalidation and pneumatic tube transport also contributed considerably to the faster reporting of results. Objective sample assessment reduced analytical errors, HIL based test rejection is now ∼0.5%. Quality of samples is crucial for patients’ safety, therefore we generally do not report results biased significantly by preanalytical errors. Sample retrieving for add-on testing means a great leap forward, taking just 1–2 min due to the postanalytical robot linked to the laboratory software.
**SE8.3**

**Effect of local phlebotomy trainings on quality indicators**

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Quality indicators, used to control the quality of the laboratory processes are involved in producing test results. We use quality indicators in our laboratory since 2012.

In the first year we adopted the quality indicators that are approved by the IFCC working group on “Laboratory Errors and Patient Safety” (WG-LEPS) and added some additional indicators to the original list. The results were compared to the quality specifications mentioned in the literature.

In this study we selected five of the quality indicators of the pre-analytical phase. We quantified error rates and converted them into Sigma-metrics. The major factors contributing to pre-analytical errors were hemolysis (clinical chemistry) and clotted samples (hematology). The error rates exceeded 4 – 8-fold the limit of unacceptability.

Due to the above mentioned extremely high error rates general phlebotomy training was organized for all nurses who took blood in our hospital in February 2013. The rate of pre-analytical errors due to inappropriate blood collection technique decreased considerably. This effect persisted for only 6-8 months and later the error rates started to increase again. We decided to change phlebotomy training schedule: instead of casual education we set up a regular training program, which is obligatory for the new employees and is recommended once a year for the old ones.

Phlebotomy trainings contributed to reduction of the rate of pre-analytical errors considerably.

**SE8.4**

**Clinical interpretation of a new laboratory diagnostic test panel for the evaluation of stomach function**

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In connection with dispeptic complaints the detection of pathologic diversgency has got an important role. With the help of the GastroPanel® examination four parameters can be examined from blood samples (pepsinogen I, II, gastrin-17 and Helicobacter IgG). These inform us on the production of gastric acid, the conditions of the mucosa and the presence of H. pylori infection. The pepsinogen I level indicates the function of the corpus and the pepsinogen II level indicates the functionality of antrum and it refers to the structural deviations; the fasting gastrin level indicates the amount of the acid secretion. The laboratory findings are evaluated and assessed by a computer program concerning the age and other clinical data of the patient. The measures were carried out from the samples of 122 patients. In the samples the most frequent abnormal finding (n=32) was reduced gastrin level, which may be attributed to intensified acid secretion. In case of 25 patients Helicobacter IgG positivity was detected, and among them in 20 cases the infection was also accompanied by gastritis. In six cases the parameters suggested atrophic gastritis, which is a risk factor for gastric cancer.

In the presentation we demonstrate the diagnostic significance of each parameter, the possible interpretations of the alteration of the four parameters, and the association between gastroscopy and laboratory findings.

**SE8.5**

**Statistical analysis of all reimbursed laboratory test data between 2011-2013**

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Aim: to monitor changes and trends within the reimbursed Hungarian laboratory tests over a 3-year-period.

Methods: Patient and case numbers, German points, and reimbursed spending (in HUF) were custom collected for each of 780 coded (OENO) laboratory tests from the data bank of the National Health Insurance Fund (OEP). Data were separately provided for years 2011, 2012 and 2013 and given for each Hungarian county, as well as for Budapest. Other official data (e.g. population) were extracted from official data banks. Data were evaluated by conventional data and statistical analysis methods.
Results: Very large differences in many analyzed parameters were observed between the different regions of the country, among the various laboratory technologies, as well as between characteristic indices of the study.

Conclusion: It seems to be worthwhile to make comparative in-depth analysis of these data as it may provide a useful set of arguments to claim higher and/or better structured reimbursement from the law makers.

SY2.1

Undergraduate and postgraduate education in Laboratory Medicine in Europe

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Professionals in clinical chemistry and laboratory medicine (CCLM) should have the ability to apply biological knowledge to clinical requirements; should be able to cooperate with clinical colleagues; and, also, manage and direct laboratory work. Under- and postgraduate education serves to provide the skills required to these goals.

Undergraduate education provides the basis of general medical view essential for the work of CCLM professionals. It is organised in different ways in different types of schools across. The knowledge required for the appropriate request and interpretation of laboratory tests is provided by different types of courses.

Postgraduate including PhD programmes offer specialization and research skills. As the mobility between countries is increasing, there is a demand for standards and requirements for these programmes in the EU as it is reflected in ORPHEUS (Organisation for PhD Education in Biomedicine and Health Sciences in the European System) initiative.

The EC4 Register of Specialists in Laboratory Medicine was established in 1997. The minimum standard for registration as a European Clinical Chemist is a total of nine years university (undergraduate and postgraduate) study. The EC4 European Syllabus for Post-Graduate Training in Clinical Chemistry and Laboratory Medicine (2012) identifies the subjects required to maintain a high level of competence in CCLM. Training could be multidisciplinary, followed by specialisation that will provide the basic training in clinical chemistry in accordance with the Syllabus.

A better understanding of the organization and practice of laboratory medicine will help in harmonization of CCLM across Europe. The EFLM and UEMS Section of Laboratory Medicine/Medical Bio-pathology in 2009 decided to join several projects. Both the EFLM (EC4 Syllabus-curriculum) and UEMS (“Blue Book”) work at harmonizing the training of professionals with medical or scientific background. This process could be accelerated through more intensive exchanges of teachers and students.

Studies were supported by research project RVO-VFN 64165 and PRVOUK P25/LF1/2.

SY2.2

The role of laboratories in clinical research; education in clinical laboratory science

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Medical research is divided into three major well-defined categories: basic research, clinical research and applied research. The National Institutes of Health defines “clinical research” as research conducted with human subjects or on material of human origin including:

1/ Patient-oriented research that includes mechanisms of human disease, therapeutic interventions, clinical trials, and development of new technologies.

2/ Epidemiological and behavioral studies.

3/ Outcomes and health services research.

The scientific product of clinical research exceeds 50% of all medical research and clinical diagnostic and research laboratories play an essential role in such studies. Realizing that scientists with special expertise in clinical research are needed to carry out high quality clinical studies and diagnostic procedures corresponding to research standards, the Universities of Debrecen and Pecs introduced an educational program producing such specialists. First, in the education of medical laboratory analysts a research-oriented subprogram was created in which emphasis was put on research oriented laboratory technologies. Completing the diagnostic or the research oriented 4-year program results in a BSc degree. As a further step a three-semester MSc program in Clinical Research has also been started. This program aims to produce experts who are able to actively participate in the planning, execution and evaluation of clinical research, possess the knowledge of introducing new diagnostic and therapeutic measures and are fit to enter postgraduate education leading to PhD degree and/or clinical biochemist board exam. The MSc curriculum consists of a basic module (10-12 credits), a core module (45-50 credits), elective courses (18-30 credits) and the production of dissertation (12 credits). The basic module includes: pathobiochemistry, biostatistics and
scientific publications. Methods required for clinical studies in molecular genetics/genomics, protein biochemistry/proteomics, clinical immunology are parts of the core module. The other major part of the core module targets special knowledge related to clinical research: designing and executing clinical studies, evidence based medicine, clinical pharmacology, product oriented pharmacological and diagnostic research.

SY2.3

Quo vadis Hungarian laboratory medicine?

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The Hungarian Society of Laboratory Medicine (HSLM) was founded in 1946 (albeit the name of the society was different at that time). Although the society is one of the oldest in Europe, due to ongoing budgetary restrictions, professional, educational and regional reorganizations, the whole field of medicine – including laboratory medicine-is in transition in Hungary. Members of HSLM come from various backgrounds (medicine, chemistry, biology, molecular biology, clinical laboratory science). Now the specialist residency program for pharmacists and medics in clinical laboratory diagnostics is 5 years long, and has a polyvalent character. Further specialist qualifications can be gained in the fields of hematology and immunology, and molecular diagnostics. Since 2006, non-medical graduates (biologists, chemists, molecular biologists, clinical laboratory scientists) can take part in a 4-year-long, complex, disease-oriented training, followed by a specialist exam in clinical biochemistry. This exam certifies their status as specialists in clinical biochemistry. In 2013 HSLM decided to perform a survey with the professional assistance of a public relation company (RTB Medical Intelligence, Ltd). The ultimate goal of the survey was to assist strategic planning of the society with hard core data related to the corporate identity and external perception of laboratory medicine. Corporate identity was assessed by personal in-depth interviews with elected officials of the society and by an online questionnaire to all members of the society. External perception was measured by in-depth interviews with related medical professions and with hospital directors. An online self-completing questionnaire was asked to be filled by 500 registered members of “netpanel.hu”, a representative sample for age 18-60 nationwide. The survey was focused on the following problems:

- Number of laboratories in the country, utilization and availability of equipment.
- Reputation of laboratory medicine in the medical community.
- Financing of Hungarian laboratories.
- Satisfaction of the population with laboratory services.

Results of the survey will be discussed in the lecture in detail and will be used by the society for strategic planning for the period of 2014-2020.

SY2.4

Quo vadis laboratory medicine?

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Most human diseases can be investigated by pathobiochemical alterations. The tremendous amount of work that was carried out in optimizing these assays by researchers as well as diagnostic companies, resulted in an effective and automated handling of these parameters in large laboratories. Most of the requested tests are part of the ‘chemistry-hematology-coagulation’ triad and these Units build up nearly the complete palette of smaller laboratories. Larger laboratories complement these tests with immunochemistry, separation techniques, special immunology and hematology including cytometry and cytogenetics. All these studies are designed for providing diagnostic aid and many of them are suitable for disease monitoring. Some of them like cytogenetics, flow-cytometry, tumor markers etc. are also useful for predicting prognosis in an already diagnosed disorder. Nearly none of these are, however useful for predicting disease in a presently healthy person. This advent or curse is only possible by the ever-increasing use of genomic technologies. Questions raised for laboratories in a 10-year view: (i). What will be the optimal balance in the consolidation of smaller laboratories into large ‘mega-laboratories’ and can these 15-20 million test/year labs further increase without the introduction of nanotechnology into the routine clinical laboratory? (ii). Who will cover the expenses of the many newly introduced and expensive laboratory tests, that were already introduced into several clinical guidelines and protocols? (iii) Who will take the ethical responsibility of handling those genomic data that e.g. strongly predict colon or breast cancer in a teenager and how these pieces of information will reshape the life of people!

Today the clinical pathologist cannot answer all these questions, but there are two achievements, however that can definitely be done. First, establish an efficient ‘factory-like’ lab with outreach to clinical wards by pneumatic test-tubes lines and online connected POCT-system and improve turnaround time by utilizing autovalidation for these tests. Second, create an interpretative reporting for special laboratory tests and build up teams who are experts in the appropriate fields and establish strong connections with fellow colleagues particularly with pathologists.
Quo vadis clinical microbiology?

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In the diagnostics and successful therapy of infectious diseases routine microbiological laboratories have distinguished role all over the world. The special knowledge of the staff, the methods they use and the speed of their isolation and identification work may not only influence the outcome of the patients care, but can prevent the clonal spread of some resistant, multi-resistant, pan-resistant pathogens in the hospital or the community. Emerging and reemerging pathogens are first detected by the clinical microbiologists and the antibiotic stewardship can also not be practiced properly without their activities. Hospital hygiene cannot be practiced without a close cooperation between the clinical/medical microbiologists and the hygienists. In all fields listed here the timely report about the possible pathogens and their antibiotic susceptibility are determining. During the past 20 years automation in clinical microbiology also changed the practice in the laboratory. Beside the automation of the infectious serology several other manual techniques were changed. New automated spreading devices as well as automated incubation systems were developed. The most laborious process, the identification of isolated bacteria or fungi could not be solved by the automation of the biochemical tests, the problem of the rarely isolated or difficult to identify pathogens made this techniques not fully accepted. The nucleic acid based methods were very helpful in many fields (such as the diagnosis of virus infections or infections caused by difficult to culture bacteria or fungi, typing and subtyping of bacteria, detection of resistance and virulence genes, etc.), but still they are time consuming and not always available to be used in routine settings. The protein based technique, the mass spectrometry provided a new way to work in clinical microbiological laboratories. MALDI-TOF MS can be used for rapid identification of isolated pathogens, for direct identification from positive blood cultures and from originally sterile body fluids within a very short time. It can be used for typing of bacteria and for antibiotic resistance determination. The development of this technique further will really revolutionize the routine practice, however will not be able to replace the well trained clinical/medical microbiologist to interpret the results.

Comparison of white blood cell parameters determined by four different hematology analyzers

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Results obtained with different types of hematological analyzers may often differ. We assessed and evaluated the extent of difference among white blood cell results performed with 4 different automated hematological systems (i.e. Advia 2120i, BD DxH 800, CD Sapphire and Sysmex XN-100 system). The measurements were performed in the Central Laboratory Department of Pest, Semmelweis University, Department of Laboratory Medicine, Budapest, Hungary.

We used EDTA anticoagulated blood samples taken from 300 patients and measured CBC parameters within 4 hours after sampling on each instrument. The obtained results were compared by Friedman test, and in case of significant differences, Dunn tests were performed as post-hoc comparisons. Box-plot and Bland-Altman plot graphs were used to visualize these results.

The results indicated that several WBC parameters measured on Advia differed significantly from those obtained on other systems. The median values of WBC were 7.11; 7.50; 7.56 and 7.58. G/L on the Advia and other three instruments, respectively. Neutrophil% median values were 65.5; 65.5; 63.6 and 66.6%, respectively. Lymphocyte% median values were 18.3; 18.9; 19.8 and 19.2%, respectively. Differences in other parameters were also observed. In spite of significant difference, however, these discrepancies are of no clinical relevance in the majority of patients. The few patients with a difference that may be of significance should be individually assessed further.

Our results are in line with those published in national and international literature supporting that the instruments of different types provide comparable results. However, the comparison of the analytical performance of hematological instruments and the adjustment of reference ranges are highly recommended. Any physician and user requesting a CBC should be notified about any change in methodology and instrumentation.

Abnormal Cell Flagging Evaluation of Sysmex XE-2100, Sysmex XN-1000, Cell-Dyn Sapphire and DxH-800 Hematological Analyzers: a Comparative Study

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Modern hematology analyzers are able to quantify, classify and describe cell populations using different technologies, as well as to generate suspect flags for abnormal cell detection. In our hematology unit two Sysmex XE-2100 hematology analyzers are used for the qualitative and quantitative analysis of patients’ samples as parts of an integrated hematology analyzer system.
The aim of our study was to compare three hematological analyzers – the XN-1000 (Sysmex, Kobe, Japan), Cell-Dyn Sapphire (Abbott, Santa Clara, CA, USA) and UniCell DxH-800 (Beckman-Coulter, Miami, FL, USA) – with the routinely used XE-2100 (Sysmex, Kobe, Japan) system. The study focused on the flagging of abnormal white blood cells by these instruments.

During one month interval, 100 patient samples from the daily workload, analyzed on XE-2100, with the presence of flags for blast, immature granulocytes, left shift, abnormal lymphocytes and atypical lymphocytes were measured parallel on the evaluated three instruments. According to CLSI guidelines, from each of the selected sample a peripheral blood smear was prepared and reviewed by microscopy to determine a positive smear finding in relation to flagging.

Our evaluation demonstrated that Sysmex XE-2100 had the highest slide review rate due to the false flagging, especially the blast flags. Abnormal lymphocytes and atypical lymphocytes flagged as immature granulocytes and left shift on Cell-Dyn Sapphire and DxH-800 in the majority of the samples. After the smear review these cases proved to be falsely flagged by those two instruments.

False flagging requires these samples to be visually inspected with a smear review for possible abnormalities which has an impact on laboratory turnaround time and costs.

This work was supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0035 project.

P.3

Interference of free fatty acids on complete blood count analysis

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There are Advia 2120i hematology systems in our laboratory since November of 2011. A strange phenomenon was observed on both the Perox- and Baso cytograms: a special band appeared between cell clusters that was separated definitely from white blood cells. However, based on the size of particles generating this band, the analyser counted them to white blood cells, so total white blood count and differentiation of leukocytes were false in this case. Due to the operation’s manual, these particles are lipid droplets, and this phenomenon appearing on cytograms is called lipid interference. This effect was observed in all samples of breastfeed newborns and patients receiving total parenteral nutrition. In addition lipid interference was found in the samples of other patients suffering from variety of diseases. On the basis of their case history we found that presence of lipid droplets in their samples does not related to the nutritional state of them. It seems that a sort of transient metabolic problem causes the observed phenomenon. Lipid parameters such as cholesterol, triglycerides, HDL- and LDL-cholesterol were measured in the sera of examined patients and lipid electrophoreses were also performed but normal lipid conditions were found. After that it was supposed that elevated level of free fatty acids is responsible for the lipid interference. To verify our hypothesis lipid interference was generated artificially: some drops of olive oils were added to normal plasmas and lipid interference was evaluated after that. It is reasonable to postulate that lipid interference is really caused by high dose of free fatty acids in plasma. To confirm this finding we are planning to measure the levels of free fatty acids in these kinds of samples and negative controls.

P.4

Novel peripheral CBC parameters may be predictive for 1 year survival after myocardial infarction

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Modern hematological analyzers provide several data on WBC, RBC and PLT characteristics in cell blood count (CBC) reports. These characteristics may be associated with cell functionalities and may reflect systemic immune activation. In this study we tested the hypothesis whether they help to predict outcome in patients with myocardial infarction (MI).

We enrolled 115 MI patients (46 women, 69 men, mean age 70 years) subjected to percutaneous coronaryography and stenting. Of those, 95 patients survived beyond 1 year after the intervention. CBC measurements from samples taken on day of admittance were done on 3 different analyzers (i.e. Advia, Beckman-Coulter & Sapphire). CBC parameters between surviving and non-surviving patients were compared using parametric statistical tests.

Surviving patients’ RBC, RDW, hemoglobin and hematocrit values were higher compared to those of non-surviving patients when measured with Advia and Beckman-Coulter analyzers (p<0.05), while the difference was not significant with Sapphire. Reticulocyte maturation characteristics measured on Advia were associated with survival. Of platelet parameters, MPC [Mean Platelet Component] value on Advia and %rP (reticulated platelets) on Sapphire effectively predicted 1-year survival after MI. WBC parameters were comparable between the two groups irrespectively of the instrument used.

These observations indicate that specific CBC parameters offered by novel hematological instruments may support survival prediction in MI. The predicting factors, however, may vary according to specific analyzers.
P.5

Significant increase of Mean Platelet Volume (MPV) in atherosclerosis

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Background: Cardiovascular diseases are one of the main reasons of mortality in Hungary, so it is important to notice this early and easily. The increased Mean Platelet Volume (MPV) together with other risk factors of cardiovascular diseases like hsCRP, HDL-cholesterol, LDL-cholesterol and fibrinogen can support the diagnosis of stable and instable angina and acute myocardial infarction (AMI). Platelet activation can be observed already in the early phase of atherosclerosis. An increase in their size (mean platelet volume, MPV) may indicate this activation.

Patients and methods: We collected the MPV results from CBCs of patients suffering from atherosclerosis (n=382) angina (n=763), AMI (n=144) and healthy controls (n=255) from the database of Laboratory of St Lázár Hospital in Salgótarján between 2010 and 2013. CBC was measured with CellDyn-3700. We used the R-statistical program, Mann-Whitney U and Kruskal-Wallis tests.

Results: MPV was significantly higher (p<0.05) in patients with atherosclerosis (8.8 fl), instable angina (8.6 fl), stable angina (8.6 fl) and AMI (8.5 fl) than that of in the control group (8.0 fl). Results of female and young male showed a significant difference compared to the control group when the 1st diagnosis was AMI. The other standard risk factors did not show such a great difference from those in control group.

Summary: MPV values of peripheral blood increased in case of atherosclerosis and cardiovascular diseases. Further studies are required to establish whether MPV increase can be used as a diagnostic tool to differentiate between patients with atherosclerosis or other cardiovascular diseases and the control population.

P.6

Recognition of eosinophil peroxidase deficiency

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Leukocyte differentiation on Advia 2120i hematological analyser is based on flow-cytometric measurement of myeloperoxidase content of different white blood cells. Deficiency of this enzyme in neutrophil granulocytes and monocytes can be detected by an irregular localization of the neutrophil and monocyte clusters in the Perox cytogram showing a typical figure. There are approximately 1100 total blood cell counts in our laboratory on three Advia 2120i hematological analysers per day; the prevalence of myeloperoxidase deficiency (partial or total) is about 40-50 cases per month. During the routine hematological analyses of some samples interesting cluster appearances were detected that differed from both the normal and the partial/total myeloperoxidase deficiency figures. In these cases the eosinophil cluster was completely absent in its usual location and an additional cluster of cells was found between the monocyte and neutrophil clusters. Blood smear evaluation of these blood samples was performed and some or more eosinophils were confirmed by microscopy, while the instrument displayed 0.0-0.1% prevalence of eosinophils. We concluded that these patients have eosinophil peroxidase deficiency. Although eosinophil peroxidase deficiency is reported as an extremely rare phenomenon, probably lots of cases are overlooked because of lacking eosinophilia. Normally, the eosinophil peroxidase deficiency is much more rare than the myeloperoxidase one, however we have found more patients with this deficiency in our routine hematology practice than one would have expected.

We demonstrate an interesting family case report, when two sisters (10 and 12 years old) have eosinophil peroxidase deficiency, but their parents have not. We believe that the sisters have total eosinophil peroxidase deficiency and their parents are only carriers of this abnormality, so they were not disclosed by this kind of routine laboratory method. The molecular basis of this defect is not known, but it is supposed to be inherited in an autosomal recessive manner.

P.7

Determination of erythrocyte sediment rate reference range by Ves-Matic Cube 80 analyser

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New erythrocyte sediment rate (ESR) analysers (Ves-Matic Cube 80 by Diesse) were introduced in the laboratories of Synlab Hungary Ltd. in 2013. Since the analytical approach of this equipment differs in several ways from our previously used method (Becton-Dickinson Sedisystem), it was necessary for us to determine new healthy ESR reference ranges for both genders. The project was performed according to the CLSI EP28-A3c guideline instructions (Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved
Guideline – Third Edition. 327 reference individuals (180 men and 147 women) were selected in our two centres (Budapest and Székesfehérvar). The age distribution of reference individuals were as follows: 20-60 years old (93.6%), under 20 years (1.2%), and over 60 years old (5.2%). Selection of the reference individuals was done using an “a priori” approach with direct, questionnaire technique. Measurements were performed at room temperature and in a next step they were repeated at 18°C, as well. Samples were also measured using standard ESR technique (Sedioplast). During interpretation of the results, we also considered the completed questionnaires; values were excluded in those cases where presence of disease with elevated ESR was assumed. Data analysis was performed by non-parametric statistical method, using Stasis Pro statistical software. Calculating the 95% reference interval, the following results were obtained: the lower limit and upper limit of healthy reference range for women were 3.0 mm/h (1-3 mm/h at 90% CI) and 21.0 mm/h (17-24 mm/h at 90% CI), respectively; the lower limit of healthy reference range for men was 1.0 mm/h (1-2 mm/h at 90% CI) and the upper limit was 10.0 mm/h (8-11 mm/h at 90% CI).

P.8

Switching between methodologies for free light chains measurement: the risk of uncomparable results

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Serum free light chain measurements are based on immunoassay techniques. Commercially available tests apply polyclonal antibodies and turbidimetry or use monoclonal antibodies and nephelometry. Recently we compared results obtained by these two methods.

We measured free light chain levels in 65 sera and in 35 urinary samples of hematological patients, with immunoturbidimetry, (Freelite, The Binding Site, UK), and with nephelometry (N-Latex, Siemens, Germany) assays on an automated chemical analyser (AU 5800, Beckman) and on a nephelometer (BNII, Siemens), respectively.

In 15% of serum kappa chain values and in 24% of serum lambda chain values we found magnitude differences comparing the results measured by nephelometry and by turbidimetry. These values could not be used interchangeably when monitoring disease response in patients with monoclonal light chain diseases. After excluding the outlier results the r value of linear regression was 0.807.

These results warn that clinicians should be aware when methods are switched and should monitor free light chains using the same manufacturer’s assay on the same instrument. During such a method change the laboratory has a great responsibility in interpreting of the results. We decided to have a lengthy overlap period when free light chains were measured by both assays particularly for monitoring those patients, whose disease was diagnosed on the base of the previously used immunturbidimetric assay.

P.9

Comparison of Magister and Wadiana Compact immune-hematological analyzers used for blood group serology

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Background: Magister (manufacturer: Sanquin) is a recently developed immune-hematological analyzer used for blood group serology in some European countries. We analyzed and compared its performance with Wadiana Compact (manufacturer: Grifols) in a university laboratory setting.

Methods: Patients’ EDTA anticoagulated blood samples referred to the laboratory for AB0/Rh, direct Coombs and indirect antibody testing were measured simultaneously with Magister and Wadiana analyzers. Magister analyzer used Cellbind and Pelikloon reagents. Wadiana analyzer used Grifols reagents.

Results: In the period of 1st of October and 7th of November, 2013, the number of samples processed by Magister and Wadiana were 397. The results were identical in 86.5% of the samples. In 13.5% of the cases discordant results were obtained with Magister due to technical settings. All of these results were referred for cannot be assessed, by the analyzer. The prevalence of discordant results steadily decreased during the period of comparison. The majority of samples with discordant results were originated from the Department of Haematology and from patients having subjected to several transfusions.

Discussion: Magister analyzer is appropriate for the blood group analysis of patients treated in general hospitals. Specific patient populations may require other approaches offered by more specific tests.

This work was supported by Medimpact.
P.10

Dilemmas of a laboratorian

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We present five cases where the laboratory provided a significant contribution to diagnosis based on blood smear analysis. The analysis of blood smear of one-month-old anemic child revealed red blood cells with bizarre morphology. Including other test results the congenital defect of red blood cell membrane was verified. It also turned out that even some members of the family are affected.

During the routine general medical laboratory testing we identified a 64-year-old man with suspected hairy cellular leukaemia; this was confirmed by haematology testing.

A 4-month-old child with epilepsy on valproate therapy exhibited very low number of absolute neutrophil granulocyte. This was a side effect of medication, but we also detected an abnormality in bone marrow’s granulocyte development.

In a 30-year-old, 9-week-pregnant woman subjected to routine investigation we measured very low white blood cell count. As a cause, acute leukemia and related DIC were confirmed.

A 2-year-old child with bloody diarrhea and with invagination suspicion was admitted to the hospital. Our tests indicated that the decreasing platelet count is due to HUS.

These cases indicate that while the majority of tests are automated in nowadays’ lab, the human contribution to interpretation of test results is still essential.

P.11

Allergen specific IgE and total IgE levels of Hungarian adult patients

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Our goal was to overview the serum allergen specific IgE and total IgE levels of the past 5 years in our central laboratory, optimize the composition of the panels and determine the additional information gained with the total IgE value.

5 years ago we switched to ELISA based allergen specific IgE tests, which allowed us to create flexible panels. In our institute we carry out the laboratory tests of adult patients from the central region of Hungary. Based on these we obtained a large dataset that inclined us to check the suitability of specific IgE panels, to investigate the frequency of relevant allergens by statistical calculations, and, furthermore, to examine the diagnostic value of total IgE level.

4763 patients were tested for total and specific IgE. We offered an inhalative and a food allergy panel (both consist of 20 allergens) to the physicians. Specific IgE tests were evaluated by our ELISA based automatic system giving quantitative and RUST class results. Total IgE value was determined by an ELISA test with a cut off value of 100 IU/ml.

The most common inhalative allergen was the ragweed (28.1%) in accordance with other Hungarian studies. The prevalence of the grass, mugwort, hazelnut, and house dust mite d1 allergens were 17.8, 13.4, 10.6, and 8.5%, respectively. Among the food allergens, the most common was peanut (5.7%), its prevalence was followed by that of tomato, celery, soya and wheat allergens (4.1, 3.2, 3.1 and 2.7%, respectively). Comparing specific IgE and total IgE results we found the following: co-positive cases: 5.1%; total IgE negative, but we found positive specific IgE: 3.3%; total IgE positive although no specific IgE was found: 4.7%.

Conclusion: although the composition of the panels is mostly appropriate, minor modifications would be useful (e.g. omitting less frequent allergens). The added value of total IgE level is limited. It can be helpful in those cases, when no specific IgE is positive, but the diagnosis of allergy needs reinforcing.

P.12

Impaired modulation of T-cell function by pulmonary mesenchymal stem cells in hypersensitive pneumonitis

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Based on former reports, pulmonary mesenchymal stem cells (MSCs) may play an important role in the maintenance of lung integrity. These tissue resident cells could prevent the graft rejection after lung transplantation via altering the function of T-cells. In addition, MSCs downregulated inflammation in asthma, and their normal function resulted in a better survival ratio in sepsis. In this study, we analyzed the direct
effect of MSCs isolated from bronchoalveolar lavage (BAL) on T-cell function in severe hypersensitive pneumonitis (HP). First, we detected MSCs in the BAL samples of two HP patients by using flow cytometry and confocal laser scanning microscopy showing CD105, CD73, CD90 positivities but without CD34 staining. These cells were then maintained in cultures for further experiments. Normal MSCs were similarly obtained from BAL samples of two patients with psychogenic cough. Phytohaemagglutinin-stimulated (PHA; 1-10 μg/mL) normal lymphocytes were simultaneously incubated with the healthy and patient MSCs for 5 days. T-cell activation was determined by measuring CD25 positivity with the CD4/CD8 ratio, while their proliferation was followed by the mean fluorescence intensity (MFI) of carboxyfluorescein-diacetate-succinimidyl-ester (CFSE). We found that normal MSCs significantly suppressed the activation of T-cells (p<0.05) especially that of CD4+ cells causing decreased CD25 expression at 5 μg/mL PHA (from 13±2% in the activated control sample without MSCs to 6.5±1.5%) compared to patient MSCs (9.5±0.5%). However, no significant difference in CD4/CD8 ratio was observed. Furthermore, normal MSCs abrogated T-cell proliferation sustaining high CFSE intensity in contrast to patient MSCs having less impact on T-cells with lower MFI values. In conclusion, MSCs possess an abnormal immunomodulatory effect in chronic respiratory inflammatory diseases such as HP. These results may provide additional explanation for the pathomechanism of recurrent inflammatory events.

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P.13

Immunoassay for the determination of ferryl-hemoglobin

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Oxidative stress has been implicated in a wide range of diseases. Oxidation of cell-free hemoglobin produces (Fe(III)) methemoglobin. More extensive oxidation produces (Fe(III)/Fe(IV)=O) ferryl hemoglobin (FHb) and covalent heme to protein and protein to protein crosslinking. Cross-linked FHb thought to contribute to the pathogenesis of hemolytic disorders. For the study of in vivo FHb formation, electron paramagnetic resonance spectroscopy, HPLC and Western blotting methods have been used.

The aim of our work was to develop a new immunoassay for the determination of FHb by using monoclonal antibodies specific to neoantigenic determinants that formed in cross-linked hemoglobin. A pair of antibodies were selected from several monoclonal antibodies that were raised against FHb. The one which recognises only the oxidized form of hemoglobin was used as capture antibody and the other which recognises both normal and oxidized hemoglobin was labelled with horseradish peroxidase and used as tag antibody. The measuring range of the developed sandwich ELISA is 1-100 ng/ml. The low limit of detection (1 ng/ml) allows highly sensitive determination. The reproducibility of the method is appropriate, using citrated plasma and CSF samples; the within run variability (CV%) was 6.7% and 6.2%, and the between run variability (CV%) was 10.1% and 10.9%, respectively, and the total laboratory variability did not exceed 12.5%. Using this method we were able to detect the presence of ferryl-hemoglobin in liquor samples obtained at different times after intracranial hemorrhage.

In conclusion, we have developed a highly sensitive and well reproducible sandwich ELISA method for the determination of ferryl-hemoglobin in human samples. The new method can provide further help to clarify the pathobiochemical role of ferryl-hemoglobin.

P.14

Reactive and clonal eosinophilia – a case report

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Various diseases may increase blood eosinophil count. Some of them are reactive states like allergic disease, parasitic infection or malignant tumours. The other type of eosinophilia is of clonal origin that is associated with hematological disorders such as myeloproliferative syndrome, myelodysplastic syndrome, acute myeloid leukemia or acute and chronic eosinophilic leukemia.

We have detected two patients (a 65-year-old woman and a 60-year-old man) with 40% and 60% relative and 0.7 and 22.7 G/L absolute peripheral eosinophil count. While the qualitative blood test results were comparable, the diagnoses were reactive and clonal eosinophilia, respectively.

Diagnostic laboratory workup of extreme eosinophilia may require a complex process that includes clinical chemistry, immunological and microbiological laboratory tests, molecular pathology analysis and medical imaging technique.
P.15

Non-haemopoietic tumor cell detection by flow cytometry

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We aimed to evaluate the role of multicolor flow cytometry in the detection of bone marrow involvement in patients with solid tumors and in pediatric neuroblastoma cases. We investigated 55 bone marrow aspirates, 6 cerebrospinal fluid samples and 1 bronchoalveolar lavage fluid with multicolor flow cytometry (MFC) and correlated the results to morphology/immunohistochemistry (IHC) results of bone marrow biopsies if it was performed. Thirty-two samples of 30 patients with a clinical suspicion of solid tumor or transfusion-dependent anemia investigated by MFC revealed 8 cases of solid tumor with CD45/EpCam+ carcinoma cells (n=7) or CD45/CD99+ Ewing sarcoma cells (n=1) and 2 cases of histiocytosis with CD45/CD1a+ cells. In 17 cases results were concordant (10 positive and 7 negative) while in 1 case MFC was negative while pathological histiocytes could be detected by IHC in the bone marrow biopsy specimen. Thirty samples of 14 children with suspected neuroblastoma or during its follow-up was analysed in a four-color setting (CD45/CD117/CD81/CD56) by MFC and 22 samples were investigated by IHC. In 13 cases results were concordant (8 positive and 5 negative) while in 8 cases discordant result were obtained (6 MFC+/IHC- and 2 MFC-/IHC+). In conclusion the two methods gave concordant results in 77% of cases and most of the discordant results were found in those neuroblastoma follow-up samples where pathological histiocytic ratio was below 1% in a hypocellular bone marrow environment. Here MFC proved more valuable in minimal residual disease detection.

P.16

Detecting leukemia stem cells in AML and ALL samples by the Aldefluor Kit

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Aldehyde dehydrogenase (ALDH) activity is a marker for normal and malignant stem cells. Normal hematopoietic stem cells tend to have high ALDH-expression, while leukemic stem cells have lower expression. After optimizing the ALDEFLUOR™ Kit and examining several cell lines, we examined bone marrow samples from 24 AML and 4 ALL patients altogether. Samples from 6 AML and 3 BCP-ALL patients were studied in 3 time points (de novo, 1st MRD, 2nd MRD). Conventional cytogenetic studies, nucleophosmin, FLT3-ITD and FLT3-TKD investigations were done on de novo samples. An additional staining with surface CD34 (PE) and CD38 (APC) antibodies was also done along with ALDH staining on all samples. 500 000 events were acquired on a BD FACSCanto II flow cytometer. For cell lines 2x10⁵ cells/ml and 30-minute incubation time was optimal for the Aldefluor staining. For bone marrow samples it was 10⁵ cells/ml and 60-minute incubation. K562 and THP1 cell lines were ALDH-positive (71% and 83%, respectively), while MOLM13, MV4-11, JIMT1 and SKBR3 cells were mostly negative. We focused on CD34+/CD38- cells of the bone marrow samples and found several different ALDH expression patterns. The average absolute count of ALDHlow/CD34+/CD38- cells was 0.03, 0.03 and 0.002 G/L in the de novo, 1st MRD and 2nd MRD AML samples, respectively. For BCP-ALL patients the CD34+/CD38- cells was 0.015, 0.005 and 0.009 G/L in the de novo, 1st MRD and 2nd MRD AML samples, respectively. We examined bone marrow aspirates, 6 cerebrospinal fluid samples and 1 bronchoalveolar lavage fluid with multicolor flow cytometry (MFC) and correlated the results to morphology/immunohistochemistry (IHC) results of bone marrow biopsies if it was performed. Thirty-two samples of 30 patients with a clinical suspicion of solid tumor or transfusion-dependent anemia investigated by MFC revealed 8 cases of solid tumor with CD45/EpCam+ carcinoma cells (n=7) or CD45/CD99+ Ewing sarcoma cells (n=1) and 2 cases of histiocytosis with CD45/CD1a+ cells. In 17 cases results were concordant (10 positive and 7 negative) while in 1 case MFC was negative while pathological histiocytes could be detected by IHC in the bone marrow biopsy specimen. Thirty samples of 14 children with suspected neuroblastoma or during its follow-up was analysed in a four-color setting (CD45/CD117/CD81/CD56) by MFC and 22 samples were investigated by IHC. In 13 cases results were concordant (8 positive and 5 negative) while in 8 cases discordant result were obtained (6 MFC+/IHC- and 2 MFC-/IHC+). In conclusion the two methods gave concordant results in 77% of cases and most of the discordant results were found in those neuroblastoma follow-up samples where pathological histiocytic ratio was below 1% in a hypocellular bone marrow environment. Here MFC proved more valuable in minimal residual disease detection.

P.17

Mutations of ABC transporters as the genetic basis of high frequency blood antigens Jun and Lan

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We aimed to identify the genetic background of low RBC ABCG2 (Junior blood group-Jun) and ABCB6 (Langereis blood group-Lan) protein expression levels in healthy individuals. 61 healthy volunteers (47 unrelated individuals and 14 family members) and 239 healthy volunteers and 2 Lan-negative sisters were enrolled in the ABCG2 and ABCB6 studies, respectively. RBC ABCG2 and ABCB6 protein expression was measured by flow cytometry (FACS) using anticoagulated peripheral blood samples, stained with monoclonal antibodies specifically recognizing the respective membrane proteins. ABCG2 and ABCB6 mutations were identified by direct sequencing of exons and flanking intronic regions. Common SNPs V12M (rs2231137) and Q141K (rs2231142) of the ABCG2 gene were screened by LightCycler allelic discrimination. ABCB6 SNPs R192Q (rs150221689) and G588S (rs145526996) were genotyped by PCR-RFLP. Heterozygotes for ABCG2 Q141K variant had lower ABCG2...
expression (5.27±1.19) on RBC membrane, as compared to wild-type individuals (6.13±0.61; *p*=0.011). Sequencing of 2/47 individuals with lower erythrocyte ABCG2 expression (2.65±0.29) revealed two heterozygous mutations resulting in premature termination (rs160207606 and L264HisX14). Sequencing of two Lan-negative sisters without detectable ABCB6 protein expression revealed a previously described mutation in homozygous form (ABCB6 R192W (rs149202834)). Sequencing of the ABCB6 gene in individuals with lower RBC ABCB6 protein expression revealed three heterozygous mutations: R192Q, IVS9+1G>A and G588S. Analysis of the family members revealed that low ABCB6 expression segregated with presence of R192Q mutation. Genotyping of 235 unrelated volunteers identified 3 additional heterozygous G588S individuals, resulting an allele frequency of 1.1±0.9% for the Lan-mutation G588S. We conclude that the ABCG2 and ABCB6 genetic variants linked to the absence of high frequency blood group antigens may be more common than previously thought.

P.18
Quantitative JAK2 V617F mutational analysis in myeloproliferative neoplasms

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Janus kinase 2 (JAK2) V617F plays a pathogenic role in myeloproliferative neoplasms (MPN), occurring in >95% of polycythaemia vera (PV), 50% of essential thrombocythaemia (ET) and primary myelofibrosis (PMF). We aimed to introduce real time quantitative allele-specific PCR (QPCR) for its detection. We investigated 603 patients [560 males/43 females; median age: 60 years; 215 PV, 289 ET, 99 PMF] for the presence of JAK2 V617F with qualitative allele-specific PCR. V617F positive MPN cases [215 PV (100%), 154 ET (53%), 56 MF (57%)], 56 V617F negative MPN patients and 28 healthy donors were tested by QPCR. JAK2V617Fmut/JAK2total ratio was calculated. In healthy donors, the median V617F burden was 0.003% (range: 0-0.04%). This value for V617F negative MPN patients was 0.002% (0-0.2%) while for V617F positive MPN patients it was 34% (0.4-100%). Only a single MPN patient displayed V617F load below 1%. Among V617F positive MPN patients, the JAK2 load increased gradually in parallel with the appearance of more advanced stages of MPN [ET median (range): 19% (10-31%), PV 44% (24-73%), PMF: 49% (34%-100%)]. Only a single MPN patient displayed V617F load below 1%. Among V617F positive MPN patients, the JAK2 load increased gradually in parallel with the appearance of more advanced stages of MPN [ET median (range): 19% (10-31%), PV 44% (24-73%), PMF: 49% (34%-100%)].

P.19
FBN1 mutation detection in Marfan syndrome patients using next generation and Sanger sequencing methods

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Marfan syndrome is an autosomal dominant connective tissue disorder with skeletal, ocular and cardiovascular system involvement. Sequencing of the fibrillin-1 (FBN1) gene has a 70-90% mutation detection rate in patients with Marfan syndrome. We introduced a combined molecular diagnostic workflow for rapid and reliable mutation detection in the FBN1 gene that is based on the combination of Sanger and next generation sequencing (NGS) methods.

Eleven families (15 patients) were tested with a clinical diagnosis of Marfan syndrome based on the revised Ghent nosology. Exons and flanking regions of the FBN1 gene were amplified in 65 amplicons. Our combined molecular testing was carried out as follows i) exons containing homopolymer regions (n=16) were tested by Sanger sequencing method because of the known high error rate of pyrosequencing-based NGS in homopolymer regions of >4 identical bases, ii) all other amplicons were sequenced using NGS (Roche GS Junior) with a coverage criterion of 40x. Pathogenic mutations detected by NGS method were confirmed by Sanger sequencing. Mutation detection rate of FBN1 gene analysis was 8/11 (unrelated patients). Seven missense mutations (6 of them affecting cystein residues) and one base pair deletion causing frameshift were found. Altogether 5 novel mutations were detected, 4 of them are missense mutations all but one affecting cystein residues (c.2585G>A; c.6032G>C; c.2288G>A) which are meant to be pathogenic according to the Ghent nosology. In one case neonatal Marfan syndrome was confirmed caused by a new mutation found at a minor mutational hot spot (c.3038G>T). Another unpublished mutation (c.5196delC) resulted in a premature stop codon.

Our results are in accordance with the literature, most of the mutations are reported to be unique to a family and are most frequently missense mutations affecting cystein residues. Combining NGS and Sanger sequencing methods a reliable diagnostic workflow for FBN1 genetic testing could be established.
P.20

Detection of genetic abnormalities on enriched plasma cells from bone marrow by fluorescence in situ hybridization (FISH)

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Multiple myeloma (MM) is a hematopoietic neoplasm characterized by monoclonal plasma cells (PC) accumulated in the bone marrow. A variety of chromosomal abnormalities contribute to pathogenesis and affect risk stratification and treatment of MM. Cytogenetic aberrations are not frequently detected by karyotyping due to low proliferative rate of PC. Interphase FISH overcomes this limitation but it has been suggested that it should be performed on specifically identified PC. In this study our aim was to evaluate the utility of plasma cell enrichment combined with FISH for the detection of prognostically significant cytogenetic abnormalities in patients with MM. Plasma cells were enriched in bone marrow aspirates by using CD138 immunomagnetic bead selection in eighteen cases with low level of monoclonal PC. The PC selection was followed by FISH. The MM FISH panel included the following probes to detect high-risk genetic abnormalities: t(4;14), t(14;16), -13/del(13q), del(17p)(TP53). Plasma cell content in non enriched specimens ranged from 0.9% to 38% compared with 35% to 71% in enriched samples according to flow cytometric analysis in five cases. The most frequent chromosome aberrations were monosomy 13/del(13q) (n=8) followed by del(16q) (n=6), >2 ATM signals (n=6), >2 TP53 signals (n=5), del(14q32) (n=4), >3 IGH (14q32) signals (n=2). Specific translocations, t(4;14) and t(14;16), were detected in one-one case. The percentage of PC harboring genetic aberrations detected by FISH was higher than monoclonal PC content detected by flow cytometry in enriched samples. Based on our results plasma cell enrichment of bone marrow samples with low percentages of monoclonal PC increases FISH sensitivity for detecting high-risk cytogenetic abnormalities and can be of value to improve risk stratification and management of MM patients.

P.21

Problems and Alternatives in Relation to Hemostasis Panels

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Test panels are typically composed of individual laboratory tests, which are interrelated in some ways: groups of tests simultaneously performed to diagnose and to follow-up patients. Both organ-specific and disease specific test panels are currently available.

In the hemostatic diagnosis the patient’s chief complaint and other anamnestic data plays a key role in obtaining the personal and medical history and performing physical examinations.

In practice the hemostasis test panels could offer various advantages to clinicians and to laboratories performing the tests (efficiency, more targeted diagnostics, reduced costs and so on). In Hungary, although there are several attempts to set unified panels, because of the different criterions; those are not used thoughtfully enough. In order to ensure the appropriate co-operation, the laboratory specialists and clinicians should overview the local clinical and laboratory options, economic conditions and find better alternatives together.

Based on the findings of our data from 2013 we tried to find alternative solutions for these problems.

P.22

Prevalence of antithrombin Budapest 3 mutation in the Hungarian thrombophilic population; investigation of a founder effect

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Antithrombin (AT) is a key regulator of the coagulation. Its main function is the inhibition of thrombin and activated factor X. AT deficiency can be classified as type I (quantitative) and type II (qualitative) deficiency. In type II deficiency, the defect may involve the reactive site (IIIRS), the heparin-binding site (IIHBS) or it can exert a pleiotropic effect (IIPE). All types cause severe thrombophilia in heterozygous form, with the exception of type IIHBS variant, which represents a lower thrombotic risk. The gene encoding human AT (SERPINC1) is located at 1q25.1 position and contains seven exons producing a 1.4 kb messenger RNA.

In the period between 2007-2013 128 consecutive AT deficient patients were recruited. The great majority of cases (n=93) carried the AT Budapest 3 (AT Bp3; p.Leu99Phe) mutation. To investigate the founder effect rs2227596, rs941989, rs2227612, rs5877 and rs5878 intragenic SNP’s were examined by real time PCR and melting point analysis on a LightCycler 480 instrument. 5’ length dimorphism (5’ LP) was
investigated by PCR-RFLP. Four microsatellite markers (STRs SERPINC1-Alu8, D1S196, D1S218 and as a negative control F13A01) were also analyzed. The polymorphisms and STRs were detected in 200 healthy persons representing the general Hungarian population.

AT Bp3 was associated with the same SNP haplotype in all carriers, while different haplotypes were observed in healthy controls. The STRs Alu8 and D1S218 (0.6 cM distance from SERPINC1) represented linked inheritance with AT Bp3. D1S196 (6.3 cM distance form SERPINC1) and the negative control marker, F13A1 were detected in different repeat numbers even in carriers of AT Bp3. Family tree analysis also suggested founder effect.

In conclusion, the most frequent genetic abnormality in AT deficiency is AT Bp3 in the Hungarian population. The high frequency of this mutation can be explained by a founder effect. GRANT: OTKA PD101120, TAMOP 4.2.2.A-11/1/KONV-2012-0045.

P.23

Investigation of the prevalence of Antithrombin Budapest 3 and Cambridge II mutations in patients with arterial thrombosis and in children with thrombophilia

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Thrombophilia is a complex disorder, in which both inherited and acquired factors play an important role. Among genetic factors, antithrombin (AT) deficiency represents a high risk for venous thromboembolism (VTE). It was demonstrated in our previous study that AT Budapest 3 (AT Bp3, p.Leu99Phe) mutation leading to heparin binding site deficiency (HBS) was the most frequent in AT deficient patients with VTE and functional amidolytic assays using thrombin were not able to detect all HBS deficient subjects. Assays using activated factor X (FXa) are suitable for diagnosis of this type of AT deficiency. Contrary to our findings, AT Cambridge II (AT CII, p.Ala384Ser) is the most frequent mutation in the British population, and is not detected by assays using FXa. Prevalence of AT CII in our population is not known. Incidence of VTE is less frequent in children and the occurrence of AT deficiency in this age group is uncertain. Moreover, it is also uncertain if AT Bp3 is associated with atherothrombosis.

Our aims were to determine the frequency of AT deficiency and the prevalence of AT Bp3 and CII in children investigated for thrombophilia, and in patients suffered myocardial infarction or stroke. For the detection of these mutations real-time PCR followed by melting curve analysis was established.

In the time period between 2007-2012 n=765 children under the age of 18 were examined in our center. AT deficiency was the most frequent below 1 year of age (8.6% of babies investigated for thrombophilia) and most of the children carried the AT Bp3 (75%). Three patients were diagnosed with AT HBS deficiency among young (below the age of 40) individuals with myocardial infarction (n=81). Two of them carried the AT Bp3, while one patient was a carrier of AT Basel (p.Pro41Leu) variant. AT Bp3 was detected in one individual with stroke among randomly selected stroke patients (n=119). AT CII variant was not detected in either group of patients.

In summary, AT deficiency and AT Bp3 mutation are relatively frequent in children suffering from thrombophilia, in patients suffered myocardial infarction or stroke. For the detection of these mutations real-time PCR followed by melting curve analysis was established.


P.24

The new oral anticoagulants and the routine, daily coagulation laboratory practice

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Laboratories till this time for decades have achieved many prothrombin time (PT) measurements to check – mainly for monitoring – the only-available oral anticoagulant, the vitamin K antagonist therapy.

However in the last years there are more so called New Oral Anti Coagulants (NOACs) in the clinical practice. According to approved leaflets, studies and reviewed articles these therapies do not require any monitoring of the coagulation system. It is true but it doesn’t mean that they do not affect the measured haemostasis parameters and that at these patients there are no changes in the main laboratory screening (and of course in some others) coagulation tests. Because of this in our daily routine work we can meet strange, never seen constellations of PT, aPTT and TT results.

The authors – by the help of case presentations – try to show the main coagulation test result constellations at different NOACs (rivaroxaban, dabigatran, apixaban, etc.); they also would like to emphasize – by the same reason – the importance and usefulness of the knowledge of this medication at the time of test requiring by the clinicians either in the hospital or in the general practice.
Diagnostic considerations based on the experience of genetic analysis in Protein S deficiency in the Hungarian population with high frequency of FV Leiden mutation

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Protein S (PS) is a cofactor for active protein C in the inactivation of active factor VIII and factor V (FV). PS deficiency (PSD) is classified as type I (quantitative) with proportionally low PS activity (PS Ac) and free (and total) antigen (PS Ag) and type II (qualitative) disorder with low PS Ac despite normal PS Ag levels. Although more than 200 distinct mutations causing PSD have been discovered so far, the molecular genetic alteration often remains undiscovered. The functional clotting assay, which is used for screening is influenced by the FV Leiden mutation (FVL) leading to low PS Ac. Since PS Ag determination is not influenced by FVL, these patients seem to have type II PSD. This problem is especially of great importance in populations with high frequency of FVL, like in Hungary. Our aims were to determine the mutation spectrum of PSD and the frequency of type II PSD, moreover to evaluate the functional clotting assay from the point of view of FVL interfering effect. Non-related individuals having 65% or lower PS Ac measured by the clotting test were recruited after excluding the presence of acquired PSD (n=132). Free PS Ag was measured by immunoturbidimetry. The gene encoding PS (PROS1) was analyzed by direct DNA sequencing and MLPA method. More than 50% of patients with low PS Ac were carriers of the FVL (FVL+, n=73) and type I PSD was suggested in half of them. In contrary among non-carriers (FVL-, n=59) the laboratory results suggested type I PSD in 83%. In FVL- 10 known causative and 14 novel mutations were identified. Known polymorphisms were identified in 30 patients. In FVL+ 8 known causative and 6 novel mutations were found. Known polymorphisms were identified in 45 patients. In FVL+ individuals the ratio of causative mutations was markedly lower than in FVL- subjects (19% vs. 41%). Among patients with causative mutations only 2 (FVL-) and 3 (FVL+) had “true” type II PSD and the majority of patients with FVL+ showing type II PSD laboratory phenotype were negative for PROS1 mutation. The high rate of mutation negative cases in FVL- suggests that beyond MLPA, as second line genetic diagnostic tool, larger gene/chromosome alterations or epistasis should be hunted for. GRANT: OTKA PD101120.

Local parameters of hemostasis activation in the fibrillating atrium

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Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia associated with a high risk of stroke. Our aim was to identify hemostasis abnormalities, which are associated with AF and increase the risk of thromboembolism. Patient group consisted of 10 patients with AF, control group included 3 individuals with other types of supraventricular tachycardias undergoing transcatheter radiofrequency ablation. Venous and arterial blood samples of both groups were drawn from the femoral vein, left atrium and left atrial appendage after the insertion of the catheter. All medications influencing coagulation were discontinued at least 5 days before the procedure. The following tests were carried out from all blood samples: blood count, coagulation screening tests, fibrinogen, factor VIII (FVIII) activity, von Willebrand factor (vWF) antigen levels, factor XIII (FXIII) activity, thrombin-antithrombin (TAT) complex, quantitative fibrin monomer (FM) test, activated factor VII-antithrombin (FVIIa-AT) complex, thrombin generation assay, D-dimer. C-reactive protein was measured from venous blood samples. Clinical data of patients and controls (BMI, smoking habit, previous cardiovascular events, medications) were registered.

Levels of FVIII and vWF were elevated in the samples obtained from femoral vein of AF patients as compared to controls (142.5±33.9% and 164.2±52.4% vs. 86.5±67.4% and 101.6±43%, respectively). Elevation of FVIII and vWF levels were not associated with elevated CRP levels. Levels of these markers were not significantly different when measured from samples of the left atrium and left appendage. FVIII and vWF levels showed good correlation (r²: 0.88, p<0.001) in all sample types, suggesting that they are present in a complexed form. TAT complex levels were elevated in all AF patient and control samples obtained from the femoral vein. As compared to femoral vein samples, levels of TAT complex and those of fibrin monomers were significantly elevated in the samples obtained from the left atrium (p<0.001) and from the left appendage (p<0.05) in case of both groups. No significant difference was found between venous and arterial blood samples and between
patient groups in case of FXIII, FVIIa-AT, fibrinogen levels and in case of the thrombin generation test. Conclusion: atrial fibrillation patients have elevated FVIII and vWF levels which might be attributed to endothelial damage. Local hemostasis activation in the left atrium and left appendage could be demonstrated by elevated TAT complex and FM levels. GRANT: OTKA K109712.

P.27

Comparison of two methods of HbA1c measurement

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Measurements of HbA1c in EDTA-anticoagulated blood of 45 patients were done parallely using two different methods; using the particle enhanced (direct) immunoturbidimetric assay in which HbA1c is determined directly without measurement of total hemoglobin; and using high performance liquid chromatography (HPLC) assay.

Instruments: for direct immunoturbidimetric and HPLC method we used an Olympus 640 analyzer and a Variant II instrument, respectively.

The principle of direct immunoturbidimetric method: total hemoglobin and HbA1c in hemolyzed blood are bound with the same affinity to particles in R1. The amount of bound substances is proportional to the relative concentration of both substances in the blood. Mouse anti-human HbA1c monoclonal antibody (R2) binds to particle-bound HbA1c. Goat anti-mouse IgG polyclonal antibody (R3) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination occurs. The measured absorbance is proportional to the HbA1c bound to particles, which is proportional to the percentage of HbA1c in the sample.

Results: the precision of the direct immunoturbidimetric method in this study based on within run precision (n=10) measured on Olympus 640, 2-component system. Our results were for sample 1 with 22.25 mmol/mol mean HbA1c level: SD 0.304 mmol/mol CV 1.30%; for sample 2 with 38.63 mmol/mol mean HbA1c level: SD 0.567 mmol/mol, CV 1.46%, and for sample 3 with 73.6 mmol/mol mean HbA1c level: SD 0.558 mmol/mol, CV 0.70%.

The comparison of direct immunoturbidimetric assay (y) and HPLC method (x) using 45 samples provided the following correlation results: Y=0.8779x-4.75; r=0.994, p<0.001.

Conclusions: the direct immunoturbidimetric method has a good precision. The concordance between results of the direct immunoturbidimetric assay and HPLC assay is good, however the direct immunoturbidimetric method has the disadvantage of requiring larger sample volume (1000 μl of EDTA anticoagulated blood) and additional sample preparation before the measurements (20 μl of hemolyzing solution should be added and 5 minutes is required until complete lysis).

P.28

Effects of sample storage on plasma renin and aldosterone levels

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Preanalytical guidelines on aldosterone and renin are widely variable according to the different specifications of reagents released by different manufacturers. The aim of this study was the evaluation of impact of storage conditions on the stability of plasma aldosterone and renin measured in our laboratory. Blood samples of healthy patients were examined. Fifteen ml venous blood was collected into EDTA tubes. Plasma was separated within 10 minutes after sampling. Each sample was aliquoted. Two tubes contained 1.5 ml, while six tubes contained 350 μl plasma. The first aliquot was measured immediately, then stored at room temperature and was retested 1, 6 and 24 hours later. The second aliquot was stored at 4°C and was measured 2, 6, 24 and 48 hours later. Six aliquot tubes were stored at –20°C and were measured 24, 48 hours and one week later. One aliquot from the "one week" sample was frozen-thawed twice. Aldosterone and renin levels were determined by chemiluminescent immunometric method (Liaison, DiaSorin). The level of renin in the samples stored for 24°C at room temperature reduced by 20.51% in average. In samples stored at 4°C the difference was less than 3% after 6 hours, but the value increased 24 hours later. In frozen samples the reduction was 14% after 24 hours of storage. Refreezing had no significant impact on the result. The level of aldosterone in the sample stored at room temperature for 6 hours decreased by 7% reduction after. In samples stored at 4°C levels were stable for 6 hours, decreased by 6% and 9% after 24 and 48 hours. In samples stored at -20°C the levels decreased by 10% after one week. Refreezing had no significant influence on the level of aldosterone.

Different storage conditions influence aldosterone and renin test results. Therefore, it is a must to clearly define preanalytical conditions when these hormones are tested.
P.29

Investigation of vitamin D status in hospitalized patients

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Introduction: Vitamin-D has important extra skeletal effects. The total 25-hydroxy-vitamin-D (t-25OHD) level reflects vitamin D homeostasis which is also influenced by the levels of vitamin-D-binding-protein (DBP) and albumin. AIM: Investigation of vitamin D status of hospitalized patients and its adjustment for albumin and DBP levels. METHODS: Altogether 401 hospitalized patients (68 treated at the department of internal medicine (IMP), 72 with end stage renal disease on dialysis (ESRD), 203 with hip fracture (HFP), 58 treated at the intensive care unit (ICUP) were tested. 127 age- and gender-matched persons with active lifestyle served as control. We determined the serum t-25OHD (protein binding assay, Cobas, Roche) and albumin and DBP (colorimetric, immunoturbidimetry Dako, Modular, Roche)-bioavailable (b-25OHD), free- (f-25OHD) and free-index (fi-25OHD) were calculated also-in all patients. Results: Less than 20 nmol/l of t-25OHD levels were more frequent in hospitalized patients (ESRD: 60%, IMP: 54%, ICUP: 52%, HFP: 39%) compared to control group (4.7%). Considering b-25OHD (<18 nmol/l) the prevalence of extremely low levels of vitamin D increased in IMP (59%) and ICUP (69%) patients only, but according to f25OHD (<4.4 pmol/l) and fi-25OHD (<3.3) levels this prevalence decreased (IMP: 44% and ICUP 42%). The other groups showed no significant differences regarding various vitamin D fractions. In summary, hospitalized patients’ groups showed significantly lower 25OHD levels for each fraction, but lowest levels of calculated fractions of 25OHD were obtained in ICUP and IMP groups compared to the control (b-25OHD: 1.5±1.2 and 1.9±1.4 vs. 6.6±3.9 nmol/l; f25OHD: 6.0±5.2 and 6.9±5.2 vs. 15.6±8.7 pmol/l; fi-25OHD: 4.3±2.7 and 5.3±4.0 vs.12.1±7.1), (p<0.001 for each) respectively. The lowest DBP levels were measured in IMP (26±7 vs. control: 35±68 mg/l, p<0.001). Of patients, 88 died in the hospital. Their t-25OHD and b-25OHD levels were significantly lower than those of 24/4 surviving patients (24.5±21.9 vs. 35.7±26.7 nmol/l and 2.2±2.4 vs. 4.0±5.8 nmol/l).

Conclusions: The majority of hospitalized patients presented with severe vitamin D deficiency. In most cases t-25(OH)D levels are insufficient to assess vitamin D status. Higher levels of total and bioavailable 25OHD are associated with better survival.

P.30

Rare bacterial strains isolated from a patient with infective endocarditis in the Central Laboratory of Jósa András Teaching Hospital, Nyíregyháza: Case Report

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Infective endocarditis is a severe and often life-threatening disease. Main etiological agents are Streptococcus, Enterococcus and Staphylococcus species. Other bacteria are responsible for less than 5% of cases. We present a case of a 48-year-old slaughterhouse worker with endocarditis affecting the aortic valve. His medical history included cough, fever, epigastric pain, loss of appetite, weakness and weight loss during recent months. The echocardiographic examination indicated infective endocarditis. Two extreme vegetations were identified on the aortic valve. Physical exam revealed aortic, mitral and tricuspidal regurgitations. From blood culture a Gram-positive bacterium similar to alfa-hemolytic Streptococcus was identified, but in microscope non-sporing rods were visible. The culture was susceptible to penicillin, ampicillin, cefotaxim, erythromycin and clindamycin and resistant to tetracycline trimethoprim-sulfamethoxazole, aminoglycosides and vancomycin. The bacterium was identified as Erysipelothrix rhusiopathiae on a VITEK2 analyser and api Coryne kit (BioMerieux, France). Endocarditis caused by Erysipelothrix rhusiopathiae is uncommon in the human. This bacterium is present worldwide as a commensal or a veterinary pathogen. Infection in humans is usually due to occupational exposure. After the positive result the clinician took a focused medical history. The patient said, he had cut his foot during slaughtering of pork meal four months before admission. The patient was given 3x600 mg Dalacin C. After the positive result the clinician took a focused medical history. The patient said, he had cut his foot during slaughtering of pork meal four months before admission.

P.31

Streptococcus agalactiae infections in nonpregnant adults treated in the teaching hospital in Nyíregyháza: incidence and clinical characteristics

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Group B Streptococcus (GBS), or Streptococcus agalactiae, is a common cause of sepsis and meningitis in newborns. However, during the past three decades, an increasing incidence of invasive GBS disease in adults, especially in elderly, has been reported worldwide. In this retrospective study of a 10-year period (2004-2013) we reviewed the incidence and clinical features of nonpregnant adults with group B streptococcal diseases. The
incidence increased between 2004 and 2009 from 0.363 to 0.961 cases per 1,000, then in 2013 it decreased to 0.630 cases per 1,000 admissions. More than half of patients admitted during the last 5 years were at least 60 years of age. In different age groups up to 70 years there was a significant male predominance. The most frequent clinical manifestations were skin and soft-tissue infections, but bone and joint, urinary tract, peritoneal and catheter infections were also common. *Streptococcus agalactiae* were isolated from blood culture of 17 patients. The resistance rates to erythromycin and clindamycin among *Streptococcus agalactiae* isolates were both around 20% in 2004; by 2013 this ratio increased to 53% and to 39%, respectively. Although *Streptococcus agalactiae* is not necessarily pathogen in each sample, the importance of this bacterium in infections is likely increasing.

**P.32**

**Imipenem Resistant *Haemophilus influenzae* Isolates in the Bethesda Children's Hospital of Hungarian Reformed Church**

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In our hospital we perform bacterial examinations of different kinds of samples, such as blood, pleural fluid, paracentral secretions, upper airway’s secretions etc. We also investigate the resistance of the growing strains by disk diffusion methods and, as needed, MIC determination by E-test. The goal of this retrospective study was to investigate the frequency of the occurrence the imipenem resistant *H. influenzae* strains in our laboratory samples of in- and outpatients. *H. influenzae* is a Gram-negative rod that can cause severe invasive and non-invasive diseases, such as meningitis, pneumonia, otitis media, sinusitis, bronchitis, and can also colonize the upper airways.

We reviewed the data of a 2-years period. During this period we identified 189 *H. influenzae* strains: 1 from a blood culture (0.53%), 19 from paracentral fluids and spontaneous ear flows (10.05%), 1 from sinus maxillar punctatum, (0.53%), 1 from mastoid secretions (0.53%), 3 from eye secretions (1.59%), 2 from abscesses (1.06%), 1 from spittle of mucoviscidosis (0.53%). No *H. influenzae* isolate was detected in liquor or pleuric fluid specimens. Of the 189 isolates imipenem resistant was 11 (5.82%). Of these 11 isolates, 5 were from paracentral fluids and spontaneous ear flows (2.65% of all, but 26.32% of the ear secretions), the remains were colonizer flora of the upper airways. When *H. influenzae* grew up from a sample, we generally performed the β-lactamase test. When we identified an imipenem resistant strain, we sent it to the reference laboratory according to the Eucast recommendations. We also found, that all imipenem resistant strains were β-lactamase negative. Resistance to meropenem was not detected. In the future we plan to determine, whether different strains are epidemiologically connected. According to experience we recommend that an invasive *H. influenzae* strain should be simultaneously examined for imipenem and for meropenem resistance.

**P.33**

**Hepatitis C virus genotypes distribution among Hungarian patients during the period 2000-2006 and 2009-2014**

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Background and Aims: HCV is classified into 6 different genotypes (1-6) based on the underlying genome structure. The corresponding difference in nucleotide sequence is 31-34%. Within each genotype one or more subtypes are differentiated (e.g. a, b, c), among these the nucleotide sequence difference is 20-23%. Since 2002, the guidelines of the National Institutes of Health and the American and European Association for Study of the Liver recommend compulsory genotype determination before the initiation of antiviral therapy. In our laboratory, the viral type distribution of Hungarian HCV infected patients was first carried out in 2000. Since then the importance of this test has further increased as the efficacy of new antiviral drugs and the length of the therapy depend on the genotype and the subtype of the virus. In the current work we present the theoretical background to the tests, the genotype dependent application of current and forthcoming antiviral therapies and analyse the 2000-2006 and 2009-2014 test results of Hungarian patients.

Methods: 2000-2006: INNO-LiPA HCV II line probe assay (INNOGENETICS) test determination of 6 HCV genotypes and their subtypes. The assay is based on variations found in the 5’ untranslated regions (5’UTR) of different HCV genotypes. 2009-2014: VERSANT HCV Genotype 2.0 Kit LiPA (SIEMENS) is designed for use of 5’UTR and core region of HCV genomes.

Results: We tested 2146 patients between 2000-2006 and 1458 patients between 2009-2014. The relevant genotype distributions were: 4.5%/7.5% (1a); 87%/79% (1b); 4.5%/6.3% (1a+1b); 1.0%/0.2% (1a+1b+2); 0.8%/1.0% (1a+1b+4) 1.0%/1.5% (3); 1.2%/1.0% (mixed). After implementing the HCV genotype 2.0 test, the HCV genotype 1 appeared as a new group (3.5%) with no further subtypes to be differentiated.

Conclusions: Based on these results (n=3604), we can conclude that the genotype distribution of HCV infections in Hungary modified just for a minor extent over the past 5 years. Genotype 1 remained the dominant genotype. 12% of patients had mixed subtypes; the detection of these subtypes has a special importance because of the newly emerging therapies. HCV with genotype 3 with resistance to medicinal therapy is not prevalent in Hungary.
P.34

Comparison between the Hepatitis B surface antibody titres and different routes of Hepatitis B Virus vaccination

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Background: Intramuscular Hepatitis B virus (HBV) vaccination (0-, 1-, 6-month schedule) has excellent immunogenicity in healthy neonates and infants, children, adolescents and adults, with seroprotection rates of 85-100% (international data). The aim of this study was to compare sero-conversion and -protection rates after the completion of three doses and two doses of vaccine.

Methods: We measured anti-HBs titres of 1888 individuals whose samples were sent to the laboratory for variable medical reasons from the Central Hungarian region. Anti-HBs antibodies were measured with ECLIA method using Roche anti-HBs test. Group I (956 subjects) were: children who had completed their Hepatitis B vaccination program and were administered with one of the HBV vaccines at months 0, 1, and 6 according to protocol. Group II (932 subjects): children who were administered with one of the HBV vaccines at month 0 and 6 according to prescribing information.

Results: Seroprotection rates were different between the vaccination groups after administration of the final dose. Seropositivity rates were 75% (>11 mIU/ml) in group I, 68% (>11 mIU/ml) in group II. The mean titres of seropositive subjects were similar in both groups. Sero-positive rates were only 26% in vaccinated children (46) within five years. There was no significant gender-dependent difference in the anti-HBs titres.

Conclusions: based on a comparison of the anti-HBs titres the use of recombinant HBV vaccines in this study was found less effective than the international data. According to international data the final third dose induces protective anti-HBs levels in more than 95% of adolescents. The high number of seronegative samples can be statistically significant in the second group, although the sample size of our study was limited.

P.35

The Human Papilloma virus: molecular biological detection possibilities in the laboratory

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The topic of our poster is a presentation of a well-known high risk factor of the cervical cancer and the interpretation of the Human Papilloma virus’s oncogene attributes, prognostic significance and to show two molecular biological demonstration possibilities. Our laboratory worked with two molecular diagnostic methods and we had the opportunity to make a comparative examination between two tests. One of the tests is Roche Linear Array HPV test, which can detect 37 anogenital HPV genotypes of the virus. The other test is DiagCor GenoFlow (GF) HPV Array test, by means of which 33 genotypes of HPV can be detected. During our work we have examined 343 female patients’ cervix samples with the Linear Array (LA) HPV test. Moreover we have compared the results obtained by the LA test to that of the DiagCor GF HPV test in samples of 43 patients. Of all the analyzed 343 female cervix samples, after 341 DNA isolations were successful and we could obtain results. This is a 99 per cent (%) success rate. Just in two samples we couldn’t obtain any results. From the 341 successfully genotyped samples 163 (47.8%) and 178 (52.2%) were HPV positive and HPV negative, respectively, when Linear Array (LA) HPV test was used. Using the DiagCor GF HPV test in 43 cases we received 25 positive and 18 negative results. The results of the two tests were discordant in 6 samples. According to the genotyping results, we calculated sensitivity, specificity, negative and positive predictive values in the light of both tests. We have taken the gynecological cytology results as basis. Both tests had about the same specificity (around 70%) and sensitivity (70-80%). During our examinations we not only determined the genotype of the virus successfully, but also tried to find association between the genotype and the cytological and histopathological results as well.

P.36

Molecular Diagnosis of Human Cytomegalovirus (CMV) in our laboratory

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Human cytomegalovirus (HHV-5) infections are prevalent in human population. Clinical manifestations following CMV infection may range from asymptomatic to mild disease in immunocompetent hosts and to severe and sometimes fatal illness in immunocompromised patients. Correct and quick diagnosis is essential since CMV infections may rather be successfully treated if diagnosed in time.
Several methods exist for diagnosis of CMV in the laboratory practice. The most widespread technique in routine diagnostics is serology, but the measurement of CMV specific antibodies is of limited value in immunocompromised individuals.

Molecular biological methods as Polymerase Chain Reaction (PCR) and Antigenemia are useful tools for early diagnosis even in cases such as congenital and newborn infections, breast milk donation, in patients with transplant, monitoring viral reactivation etc.

The large number of similar cases treated in our hospital, beside serology carried out in the Microbiological Department of the Central Laboratory, the nucleic acid based PCR detection of the microorganism was also introduced by the Molecular Genetic Department of the Central Laboratory.

In this study we would present the results of our method when is used to test various types of samples (blood, plasma, dried blood, urine, biopsy tissue).

Quantitative PCR assay (GeneProof CMV PCR assay, specificity 100%, sensitivity 95%) helps to monitor the progress of the infection and therapy in individuals. The advantage of our method is that it allows to process clinical samples other than plasma.

A total of 220 clinical samples (169 patients) were sent to our laboratory for CMV real-time PCR between January, 2012 and April, 2014. 23.2% of samples were positive for CMV DNA. Thus we had a chance to monitor the therapy of some patients.

Based on our experience we plan to prepare protocols in order to support early detection of the infection and to initiate a cost effective therapy. We also plan -cooperating with the clinicians- to optimize sampling mode, sampling time and monitoring of viral load and to establish the “cut off” value in different patient populations.

P.37

Patient Rights Monitoring implementation in laboratory practice

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The European Patient Rights Chart which came into force in November 2002 defines the protection of patients’ rights at a European level. In the present work we assessed whether laboratories adhere to patients’ rights.

We asked patients to fill out a 12-item questionnaire. During six months 300 questionnaires were distributed and completed at the laboratory sampling site in Dunahíváros of Synlab Hungary GmbH.

Respondents’ age distribution: 18-30 years old 40% 30-60 years old 50% and aged over 60 years: 10%. Respondents’ educational level: 67% of secondary school, 27% in higher education, 6% elementary school. Regarding information on sampling procedure 39% and 50% received information from primary physicians and laboratory staff, respectively. Regarding information on nutritional status 60% and 31% received information from primary physicians and laboratory staff, respectively. Eighty five percentage of patients received complete information about the accessibility of their results. Only 15% of respondents felt nuisance concerning venipuncture. The results of this study were applied to monitor the pre-analytic factors and to increase patient safety in the Synlab Hungary GmbH. For executing the monitoring process the data were collected in the current year and in last year. In order to reduce laboratory errors risk management elements as routine methods were introduced to the laboratory operational processes. Risks concerning patient safety were estimated by the FMEA method (Failure Mode and Effects Analysis). The FMEA method identified two major causes of failure, which were analyzed in details. Ishikawa diagram was used to analyze the cause of presenting inadequate laboratory results due to laboratory errors in the analytic phase and the cause of delayed release of results due to laboratory mistake in the post-analytical process.

P.38

Quality improvement by Six Sigma

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Statistical quality control (QC) provides an independent assessment of process performance. QC must be carefully planned and implemented to assure the necessary error detection, minimal false rejections, minimal costs, and operational simplicity.

Quality becomes measurable and manageable in a quantitative and objective way, Six Sigma. Following this idea the sigma-metric was calculated for our clinical chemistry tests. In a few cases where we could eliminate the bias or changing QC material we could achieve better variation of coefficient (CV). Both of them resulted in higher sigma-metric.

In spite of our efforts the analytical quality of several laboratory tests was not appropriate. At lower sigma methods better QC is needed. The strategies were to increase the number of control measurements, change from single-rule to multi-rule QC procedures, implement multi-stage QC designs, and add patient data algorithms, such as average of normals.
Improvements in quality lead to reductions in costs: fewer tests are being repeated, reordered and misinterpreted, establishment of diagnosis and therapeutic decision making are quicker, and patients spend less time in hospital.

There is no system to track these savings and to feedback their effect to the laboratory. But it is our task in the laboratory to ensure the methods to perform acceptably.

P.39

**Evaluation of hemolysis index with six sigma, and the effect of hemolysis on clinical chemistry parameters**

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Hemolysis is a critical issue in laboratory diagnostics, which may produce unreliable results with a number of tests. The aim of this study was to monitor the degree of hemolysis in samples which were sent to the laboratory and it was used as a quality indicator for measure of preanalytical variability. 5 ml aliquot of blood-cell lysate was prepared from K-EDTA blood according to the 2005. CLSI EP7-A2 document. The concentration of hemoglobin was determined on Siemens Advia 120 analyzer. There was collected serum pool with 0 hemolysis index (HI), and this sera pool was spiked with varying amounts of lysate. The HI was tested in triplicate on the Roche Modular P System. The following analytes were tested in serum according to this CLSI protocol: GPT, Fe, CK, Dbil., Tbil., GGT, total protein (TP), homocystein (HC), fructoseamine (FA), triglyceride (TG) and phosphates (P). Mean, SD, and accuracy were calculated. A bias of less than 10% is not considered a significant interference. 3895 serum samples were sent to the laboratory for analysis of the listed analytes, and from these samples HI was determined. Six Sigma (6S) value was calculated using previously determined HI limits. In case of GPT the HI:6, 6S:2.1; Fe HI:100, 6S:4.9; CK HI:28, 6S:3.7; Dbil HI:6, 6S:2; Tbil HI:5, 6S: 1.9; GGT HI:18, 6S:3.3; TP HI:160, 6S:6; HC HI:10, 6S:1.7; FA HI:40, 6S:3.9; TG HI:82, 6S:4.5; P HI:70, 6S:3.9. In 4 cases the HI results were similar to the HI of the manufacturer, in 6 cases the HI results were higher. The sample senders were reviewed by 6S. 5 of the 11 groups had 6S results between 2-3, 2 groups had between 3-4 and 4 had above 4. The low concentration of hemoglobin is not detectable visually, but interferes with the determined clinical chemistry analytes. The HI measured by us seems to be higher than measured by manufacturer. Preanalytical variability exceeds the value given by the literature.

Based on 6S results preanalytical trainings should be kept more frequently.

P.40

**Reagent carry over study in order to prevent analytical errors**

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Background: The majority of modern clinical chemistry analyzers can perform user-defined assays. Our laboratory used third party reagents on the Olympus AU600 analyzer, which was replaced by an Architect c8000. Even though the same reagents and calibrators were used unusual results were observed.

Methods: During the evaluation period it was noticed that the magnesium levels are above (1.25 mmol/L) the approved range (0.9-1.1 mmol/L) in the dialysis solution. The problem seemed to occur intermittently.

Results: Immediate re-measurement of magnesium levels indicated a lower value (1.04 mmol/L). Evaluation of reagent carryover revealed a significant increase ($p<0.0001$) in magnesium after analysis of alkaline phosphatase, amylase, iron, total protein, triglyceride, cholesterol, sodium and potassium. The difference was clinically significant incase of amylase (18%), iron (20.2%), triglyceride (19.1%), cholesterol (15.7%), sodium and potassium (20.2%) based on reference change value (RCV) calculation ($p>0.95$ RCV $= 12.5$%; $p>0.99$ RVC $=16.5$%). Carryover significantly decreased ($p<0.0001$) when an additional reagent probe washing step was applied.

Reagent carryover was checked for all analytes measured on Architect c8000 and probe washing was optimized to eliminate reagent carryover.

Conclusions: Laboratory evaluation of a new clinical chemistry analyzer should be done carefully using third party reagents. Fortunately, Abbott Architect c8000 clinical chemistry analyzer allows the user to optimize probe washing to eliminate or control reagent carryover, hereby reducing laboratory analytical error and improving patient safety.
Preanalytical aspects in case of laboratory samples transported by pneumatic tube system

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We studied preanalytical errors in case of routine and emergency laboratory diagnostics in a large regional clinical laboratory. Furthermore, we evaluated the effect of the pneumatic tube system (PTS) on turnaround time and on the laboratory results.

The ratio of preanalytical errors was below 1%, the main cause of test rejection was haemolysis (0.51% of laboratory tests) in case of serum samples. In citrate anticoagulated blood samples the number of samples with clots and those with citrate excess were comparable. The pneumatic tube transport resulted in a significantly faster sample transport, more equalized sample arrival and processing. Hence, the turnaround time as experienced by the clinicians became shorter both for routine and emergency samples. Our PTS (Sumetzberger, Austria) utilizing automatic capsules does not affect sample quality significantly. Compared to manual transport, in case of PTS transport a non significant elevation of lactate dehydrogenase activity ($p = 0.399$) was detected. Regarding platelet function, no significant difference was found according to the two transport methods, although in several cases of PTS transport a pronounced platelet shape change and a slightly increased aggregation were observed. PTS transport resulted in elevated expression of platelet surface P-selectin receptors ($1.44 \pm 0.64\%$ vs. $0.66 \pm 0.32\%$) in normal controls. Because of this mild platelet activation the impact of PTS transport on platelet aggregation and secretion tests should be considered. PTS is not recommended when markers of direct platelet activation and coagulation activation are examined or red blood cell membrane defects are investigated.

In large hospitals the autovalidation and the control of preanalytical errors are essential to provide a rapid and reliable service. The pneumatic tube system is an important element in this system.

Verification of reference intervals of routine laboratory tests using retrospective analysis of patient results in the laboratory information system

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The majority of laboratories apply adapted reference limits, which should be approved by CLSI guideline C28-A3. Alternatively, a method for calculating new reference ranges from patient data is available, based on the assumption that the results of “healthy” subjects and “sick” patients in the laboratory information database (LIS) represent different statistical distributions and these can be separated when plotted in a normal probability graph. A modification of this LIS-based method has just been proposed by Bolann to use for verification of adapted reference ranges. In this approach instead of defining new reference limits from the probability plot received on LIS values, the present reference limits of the laboratory are entered into the graphs additionally to the collected values from LIS for visual comparison.

The aim of this study was to verify reference ranges of fourteen clinical chemistry parameters and haemoglobin concentration that are applied routinely in our laboratory by the CLSI method and Bolann’s approach in parallel. Samples of apparently healthy volunteers (24 females and 23 males, between 22-58 years of age) were examined for all 15 investigated laboratory parameters in the CLSI verification process and the results were analysed using StatisPro software (Analyse-it Ltd., free trial). Results of outpatients’ from primary care services, between 18-60 years of age, visiting our laboratory in January 2014, were collected anonymously from the LIS (LabWorkS, GlobeNet) and analysed by Bolann’s approach.

Both verification methods indicated that reference limits used routinely for haemoglobin, glucose, sodium, potassium, calcium, magnesium, creatinine, LDH in our laboratory were correct, while those of phosphate, uric acid, cholesterol, triglycerides were inappropriate. Despite the fact that the applied reference ranges of the remaining three parameters (chloride, urea, alkaline phosphatase) could be successfully verified by CLSI principles, the Bolann’s evaluation showed that present reference intervals of our laboratory did not match the corresponding healthy population suggesting the need to establish local reference limits. Bolann’s approach can be used with limitations. Still it can be a fast screening method in reviewing adapted reference limits before applying CLSI verification for many commonly used laboratory parameters.

Was the implementation of the Cobas p312 in the Central Clinical Laboratory at Kecskemét County Hospital successful?

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Laboratories, that are increasingly pressed to automate their operations due to the continuing increase in workload are also required to reduce their costs. Process improvement is an important management goal for laboratory managers. Workflow analysis helps to make the correct decision and to meet the desirable outcome.

Our work is to perform the subjective and objective evaluation of task-oriented automation. One of the evaluations was based on the working satisfaction survey. We also analysed the evolution of the work processes with the new Cobas p312 automatic sorter(s) (Roche Diagnostics, Basel, Switzerland) before and after its introduction. Workflow diagrams were created and we examined whether the use of the Cobas p312 increased efficiency and sample processing safety. We also analyzed the daily distribution of samples to the laboratory. The laboratory turn around time (TAT) was analyzed by representative sampling of the laboratory specimens from the arrival of the sample, across spending time on the laboratory machines to the time of the last result released automatically.

In order to improve the laboratory service, our aim was to reduce the turn around time in case of routine samples. We examined both the clinician’s and the laboratory staff’s perspective, regarding whether the usage of the Cobas p312 shortens this time interval.

The objective analysis of our results indicate that TAT is reduced. Employee’s satisfaction surveys also indicated that the introduction of the Cobas p312 increased efficiency and safety. Our analysis showed that workflow analysis improved the efficacy of laboratory processes. Laboratory automation improved the quality in the clinical laboratory, however preanalytical conditions have significant effect on the analytical phase and influence the whole laboratory process.

Analytical and short term biological variation of a commercially available automated thrombin generation assay in healthy individuals

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Thrombin generation (TG) is a global test of haemostasis that is used for ex vivo monitoring of the formation and inhibition of thrombin. Since an increasing number of studies suggests usefulness of TG method in the assessment alterations of haemostasis, automatized assays have become commercially available. However, the information on the analytical (AV) and biological variation (BV) of these tests is limited. In the absence of data regarding AV and BV these assays cannot be interpreted.

We investigated the AV and BV of three parameters (Lag time, Peak, AUC) of a commercially available TG assay using low phospholipid concentration (TGA Reagent C Low) and the coagulometer Ceveron alpha (Technoclone, Austria). The analytical imprecision was tested (1) in within-run measurements (n=10) of a normal platelet-poor-plasma pool; (2) as repeated measurements of value-assigned commercial control plasmas in high and low ranges on 5 consecutive days; (3) and were estimated as average variance of replicate analyses of the specimens from the patients samples. Short-term BVs of the investigated parameters were calculated on five apparently healthy individuals, on 5 specimens each with 3-4 days of intervals between samplings. Biological within-subject (WS) variance was estimated from the total WS variance minus AV. Biological between-subject (BS) variance was estimated from the total variance of the set of duplicate data from the assay performed on each subject minus AV and BS components.

AUC (total thrombin amount generated in the assay) showed the lowest CV⁸, CV⁹, CV¹⁰ values, as < 3%, 4%, 11%, respectively. Analytical imprecisions of Lag time and Peak thrombin parameters of the TG assay were in range of 9-14% in all tested conditions. Short term CV⁸ and CV⁹ of the Peak thrombin were found 12 and 15%, respectively. Calculations resulted in CV⁸: 20% and CV⁹: 40% of Lag time parameter.

Short term BV of Peak thrombin and Lag time parameters of Technoclone TG assay could be fitted in the broad range of variance that have been published by other TG methods. CV⁸ and CV⁹ received in our study on AUC parameter of Technoclone TG assay, corresponded well with the previously published values using other methods, while our CV⁹ was found smaller. Although calculations of CV⁹ principally require low number of individuals, it is worth confirming calculations with more individuals involved in testing.
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Calculation of a reference change value with application of locally estimated biological variation of Hemoglobin A1c

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Background: Measurement of glycated haemoglobin A1c (HbA1c) is essential for the monitoring the metabolic control of diabetic patients. HbA1c is an excellent index since it reflects the mean glycemia of the previous two-three months. To determine the probability that a change between two glycated haemoglobin (HbA1c) results is significant and that clinical actions are required, the biological variation of HbA1c must be known. We, therefore, determined the short-term (twenty weeks) biological variation of HbA1c in a non-diabetic and in a type 2 diabetic population.

Methods: Analyses included 10 patients with type 2 diabetes and 10 healthy patients. We took ten times EDTA whole blood specimens from all patients every two weeks for twenty weeks and measured HbA1c levels using ion exchange high-performance liquid chromatography-based device. We have used the Fraser and Harris method to calculate within-subject biological variation (CVw), which allowed the determination of the probability whether a change is significant between results.

Results: The within-subject variations of HbA1c were CVw = 4.2 ± 0.8% in healthy individuals, and CVw = 3.9 ± 1.4% in diabetes patients. The difference was not significant. The Reference Change Value for HbA1c was 14% in healthy, and 13.4% in diabetes individuals.

Conclusions: In conclusion, the short-term within-subject biological variation of HbA1c did not differ significantly between type 2 diabetes patients and healthy individuals. Moreover, a change of 14% between two consecutive HbA1c results corresponds to a 95% probability that this change is highly significant, which indicates that clinical action is required.

P.46

Examination of Makro-CK through Inactivation and Precipitation

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Background: Macro enzymes are enzymes present in the serum that form immune complexes with high molecular weight or compose polymers with other components of the plasma. Macro enzymes are of great clinical interest as they have an impact on enzyme activity measurements; therefore, can have an impact on the interpretation of results.

There are two common types of Macro-CKs [Macro-CK1 and Macro-CK2]. Our present research was directed towards serum samples, which were suspected to having Macro-CK, with a special aim of being able to differentiate between Macro-CK1 and Macro-CK2.

Methodology: Serum samples with high CK activity [CK-MB] were analysed by means of inactivation and PEG precipitation. Inactivation was performed at 45°C and at 50°C afterwards, while PEG precipitation was made with a 25% solution of PEG 6000. We used an ADVIA 1650 analyzer for all the examinations.

Results: We calculated a re-establishment percentage during both procedures. The data of re-establishment percentages made it possible to indicate the presence of the Macro-CKs. The data of re-establishment percentages varied between 16.5 and 87.5%. The activity of inactivated serum samples were then shown in a function according to their dependence on the temperature, and afterwards activation energy was calculated. We measured activation energy between 38 and 170kJ/mol. As a result of the PEG precipitation experiment we could obtain the figures of re-establishment calculated in percentages, and these data were shown in a function in their relation to the full activity. Then an average percentage of re-establishment was calculated from the data.

Conclusion: The measurements of macro enzyme data should be considered in order to avoid diagnostic failures. Further experiments should be conducted to be able to refine our technique and methodology. Both examinations can be performed under average laboratory circumstances. The procedure enables us to make supplementary examinations to the analysis of the CK enzyme. In this way, it is possible to explain the falsely high levels of CK-MB.


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High ascorbic acid levels are recommended to be tested with urinary strips

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Urinary strip analysis is one of the tests routinely performed in clinical laboratories. The simultaneous measurement of at least 8 analytes present in urine provide an option for quick assessment of patients even in offices without laboratory background.
While urinary strip assessment is a simple test to be performed, inappropriate use of test strips and methodological issues may interfere with results. The latter include the presence of reducing agents that may lead to false-negative results in assays based on oxidation of the chromogenic substrate. Ascorbic acid (ASA) is one of the most common substance preventing the exact determination of blood, glucose and nitrite levels in urine. Therefore manufacturers of urinary strips increasingly apply a separate test pad to detect high ASA levels in urine.

The Department of Laboratory Medicine recently switched to IRIS Velocity strips that indicate the presence of ASA above 20 mg/L. The number of patients presenting with ASA levels were quite high: it was more than 6 percent from 3000 urinary samples. While the majority of samples were normal upon microscopic examination, some still presented with erythrocytes in spite of negative RBC test.

Our experience support the routine assessment of ASA on urinary strip. High ASA levels as possible source of interference should be commented on lab reports.

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Chemistry in the everyday practice – a simple case of a pseudo-hypolipoproteinemia

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Hypolipoproteinemia is defined as serum total cholesterol <3.1 mmol/L or low-density lipoprotein cholesterol <1.3 mmol/L. The low levels of lipoproteins in the blood may be a result of primary (genetic) or secondary (acquired) factors. Secondary causes (e.g. hyperthyroidism, anemia, malabsorption, malnutrition, infections) are far more common than primary ones. Aggressive treatment of the hyperlipoproteinemia with lipid lowering drugs may lead to clinically significant drug-induced hypolipoproteinemia. Generally, oxidase-based assays by chromogenic oxidative reagents such as Trinder’s reagent have been applied for the detection of the clinically important lipoproteins and lipids in the serum. Exogenous materials (e.g. hemoglobin, bilirubin) present in body fluids may interfere with accurate quantification of the lipid components. Exogenous substances such as ascorbic acid and other reducing compounds from the medications and the dietaries may also cause negative interference with the enzyme-based oxidative reactions. Abnormally low levels of lipoproteins and lipids in the blood are quite rare and it is usually diagnosed by chance in case of routine lipid profiling. Unexplained hypolipoproteinemia should always be investigated. We present a case of an 83-year-old white male with known hypertension and diabetes mellitus. The patient fell at home and he was admitted to the emergency department with syncope. Initial laboratory tests revealed low total serum cholesterol level (1.63 mmol/L). In this case we attempted to determine the possible cause of the unexpected hypolipoproteinemia. A chemical interference with negative errors has been found; it was caused by a non-specific reaction in the determination of the lipoproteins and lipids based on oxidase-peroxidase principles. Unfortunately, we had only indirect evidence to explain this phenomenon.

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Examination of the biocompatibility of magnetite nanoparticles designed for medical use

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Aqueous dispersions of magnetite nanoparticles (MNPs) designed for diagnostic purposes or therapeutic applications have been in the focus of scientific interest since the last decades. A protective layer is needed to prevent the aggregation of uncoated particles, to stabilize the dispersion and to avoid chemical and biological degradation of nanomagnets. Recently the pegylation (covering nanoparticles by polyethylene glycol), is the most preferred way to ensure their biocompatibility.

Our aim was to establish a cost effective method on the basis of routinely used techniques for the evaluation of biocompatibility of magnetite nanoparticles designed by the Aqueous Colloids Research Group of the Department of Physical Chemistry and Material Science. This method seems to be suitable to replace the more expensive animal experiments. On the basis of our previous haemocompatibility examinations, the erythrocyte sedimentation rate was used to examine the predisposing factor of magnetite nanoparticles for aggregation. In addition, the effects of magnetic fluids on platelet aggregation from peripheral blood smear have been tested. The protein adsorption on the surface of magnetic fluids was verified by serum total protein and albumin determinations.

During sedimentation tests supplemented with peripheral blood smear test a remarkable colour-change has been observed when the blood plasma was mixed with magnetic fluid. In the presence of certain magnetic fluids, haemolysis has been observed shortly after direct contact with red blood cells and magnetite nanoparticles. The degree of haemolysis was different. Magnetite nanoparticles with other type of coverage caused platelet aggregation in anticoagulated blood.

The magnetic fluids examined in this study did not change the composition of serum protein content, thus the degree of surface adsorption was negligible.

Our results demonstrate that this newly developed method can be appropriate for the quick assessment of biocompatibility of magnetic fluids. This work was supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0035 project.
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Veterinary euthanasia drug in human urine

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On the field of forensic and clinical toxicology sometimes unexpected results can be found. Surprisingly we proved the presence of embutramide in the urine sample of a suspected offender of a violent homicide. Embutramide is the active component of T-61 or Tanax, an euthanasic drug used often in the veterinary practice. It is a narcotic agent developed for general anesthesia, but because of its dangerously narrow therapeutic range it was never used in human medicine. In T-61 it is combined with tetracaine and mebenzonium-iodine. All veterinary doctors can access to this formula easily as the offender who was a non-practicing veterinary doctor. We use Shimadzu Prominence TOX.I.S II: HPLC DAD system for the toxicological analysis. Embutramide UV-VIS spectrum was stored in the library of the system without retention time so we needed reference material to confirm the presence of this substance in the suspected person. We used diluted formula as standard. We also found alprazolam and drotaverine in the urine sample of the offender. Alprazolam concentration in the serum was in toxic concentration. These two samples were collected two days after the crime. A few weeks after this finding we could detect the same substance in the urine of a sixteen-year-old boy with serious dizziness and nausea. According to his story he abused the T-61 formula by dripping it on cigarettes. These cases showed us that beside human medical- and usual abused drugs also veterinary medication can be important compounds on the palette of abused drugs.

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Determination of benzodiazepines in plasma and urine

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Nowadays the overwhelming majority of the suicide cases are committed by benzodiazepines. In the medical practice benzodiazepines are widely used since these drugs have a wider therapeutic range. The overdosed patients may tolerate relatively high concentration of drug without dramatically impairing the respiratory and circulating systems. On the other hand high drug concentrations or by co-administration other drugs can cause severe, often life-threatening symptoms. The synergists can be alcohol or psychotropic drugs of different pharmacodynamic groups.

We usually perform semiquantitative test (Abbott Axsym FPIA) in the urine of every poisoned patient to detect the potential toxic level of benzodiazepines. Then we analyse the sample by HPLC (Shimadzu TOX.I.S II) to identify the benzodiazepine and synergic pharmaceuticals. Besides we have developed an SFC-MS method for the quantitative analysis of benzodiazepines in plasma and recently, we have tried to install a new HPLC method, too. The latter two methods are sensitive enough and appropriate also for therapeutic drug monitoring. While in the year of 2008 35 seriously intoxicated patients by benzodiazepines were found in our toxicological laboratory the annual number of cases has risen to 187 till 2013. The cause of the intoxications is mostly suicide but it can also be abuse. The severity of the poisoning not always correlates with the concentration of the drugs; it depends strongly on the tolerance of the patient and on the presence of the synergic drugs. There are some cases when the mode of the drug administration or the extreme high concentration claims our attention. Usage of medical drugs for suicide is a widespread mode because of the easy accessibility of everyday medicaments e.g. benzodiazepines. Our set of analytical methods is suitable for identifying and determining the potentially toxic medicines.
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