XII Baltic Congress in Laboratory Medicine

Abstracts book
It is my pleasure to invite you to participate in the 12th BALM 2014 Congress in Riga, a city of great beauty, on 18-20 September, 2014, hosted by the Latvian Society of Laboratory specialists.

Distinguished colleagues will come together in what promises to be a very interesting, fruitful event, very relevant to today’s ever changing global reality.

BALM congresses occur only once in every two years. As the name suggests it is the Baltic’s, showpiece international congress for clinical chemistry and laboratory. The scientific programme contains high quality science coupled with a strong focus on how important laboratory medicine services are to clinical medicine and the wellbeing of patients and the public. Our profession is in a growing partnership with the clinical diagnostic industry. The exhibition of equipment, diagnostics, software and the industrial seminars will be at the sharp end of discovery and delivery and it will encourage participants to consider new and exciting ways to improve the laboratory medicine services that they provide.

Radisson Blu Hotel Latvija will be hosting us for this Congress. It is located within the heart of the city and conveniently in walking distance to Old Riga, all major hotels as well as shopping centers, restaurants and night life district. A vibrant social program will provide an opportunity to explore the fascinating city of Riga and other highlights of modern and historic Latvia. Riga keeps a distinguished place among the North European cities, assigned to keep the Baltic’s states together with its cultural heritage spanning almost one thousand of years.

My very best wishes, and we are looking forward to meeting you in Riga in September 2014.

Dzintars Ozolins, MD, PhD
President, BALM 2014 Congress
Vaginal flora types in pregnant women in their first trimester

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Introduction. One of the most often reasons why females attend doctor are vaginal infections vaginal flora is dynamic environment where a great variety of microorganisms exist in homeostasis. The main normal flora inhabitant is *Lactobacillus* species who protect from pathogens. A lot of things still remain unclear about this genteel environment and its interaction.

Aim of the study. To analyze vaginal *Lactobacilli* species in pregnant women who were at their first trimester using polimerase chain reaction methods.

Materials and methods. From 06.08.2012 till 31.01.2013 in study participated 65 pregnant women before 12th week of pregnancy participated in this study. All participants were divided in to 2 groups, group A (n=45) normal pH and group B (n=20) pH (≥4,7). Their vaginal fluid were analysed polimerase chain reaction (PCR) method.

Results. *Lactabacillus* genus were found in all pregnant women with PCR method, most common species were *L. crispatus*, *L. jensenii*, and *L. inners*, less common were *L. gasseri*, *L. plantaris*, *L. rhamnosus* and *L. reuteri*. *L. inners* were present in both patient groups and there was no difference in bacterial vaginosis and intermediate flora group detected by Nugent score and group B.

*G. vaginalis* was present in both patient groups but it was significantly higher in bacterial vaginosis and intermediate flora group by Nugent score and group B. *Megasphaera*, *Leptotrichia/Sneathia* were detected more common in pathogenic flora than normal flora. *A. vaginae* finding was associated with pathologic flora.

Conclusion. Most common isolated species in pregnant women vaginal flora were *L. crispatus*, *L. jensenii*, and *L. inners*. *L. gasseri* and *L. plantaris* second most often found species. *L. jensenii* was more often found in pregnant with normal vaginal flora. *Megasphaera* and *Leptotrichia/Sneathia* are more common for patients with pathologic flora. PCR method is most precise to identify microorganisms in vaginal flora but is rather expensive and time consuming than vaginal fluid examination by *Nugent* score.
Iron deficiency as predictor of reproductive disturbances

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Relevance of research. Problem of reproductive health of women - one of the most important problems of reproductive health around the world. It is known that the frequency of premature interruption of pregnancy fluctuates ranging from 10 - 30% from all pregnancies. Besides, it is established that if interruption of pregnancy happens two and more times of times, it is classified as abortus habitualis. Its frequency in population makes from 2 to 5%.

Iron-deficient Status (IDS) can be conveniently divided into express Iron-deficient Anemia (IDA) and Latent Iron Deficiency (LID), which is diagnosed at the normal level of red blood cells and hemoglobin (Hb), a reduced (<15-20 mcg/l) ferritin content in blood serum and an increased (vs. the norm) soluble receptor-to-transferrin blood level (sTfR> 5.6 mg/l). The medico-social problems of the last years probably couldn't on affect objective parameters of health of inhabitants of Latvia. At the same time, the presence of a ‘negative gain’ among the population of Latvia (preponderance of the death rate over the birth rate) makes the aim of the present study, which is to investigation of links between IDS and reproductive disturbances in women of fertile age, very pressing and topical.

Material and methods. 50 women of fertile age (26-43 y.) diagnosed with (a) missed abortion (MA), and (b) spontaneous abortion (SA), were followed up at the Riga Reproduction Centre Ltd. To confirm the IDA or LID data of clinical and laboratory investigations were used: common blood analysis and assay of the ferritin level by means of the conventional immunoassay method.

Results and discussion. IDS were diagnosed in 41 (82%) out of 50 female patients of the RRC: 17 (34%) – IDA and 24 (48%) – LID. Hematological parameters (hemoglobin and serum ferritin) were close to the lower limit of normal in 9 (18%) out of 50 female patients. 20 women (48.8%) diagnosed with IDS had MA and 7 women (17.1%) – SA. 2 (22.2%) women with normal hematologic parameters had MA, and 2 (22.2%) – SA. The MA rate among women with IDS, when calculated per person, was 1.6 times higher than in the absence of IDS. Similar parameters for SA did not differ significantly

Conclusion. The percentage of missed miscarriages in the setting of IDS is twice higher than in the absence of iron deficiency. Therefore medicament correction of IDS is necessary in all women with IDS for prophylaxis of reproductive disturbances.
Human immunodeficiency virus type 1 (HIV-1) drug resistance tendencies in Latvia

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Human immunodeficiency virus type 1 drug resistance may limit the benefit of antiretroviral therapy (ART). Our previous study showed that prevalence of HIV with drug resistance mutations (DRMs) in Latvia in 2006-2008 was 5.3% in treatment-naïve and 41% - in treatment-experienced patients.

The objective of this study was to analyze pattern of HIV-1 DRMs and predicted viral susceptibility in treatment-naïve and treatment-experienced patients in 2009-2011 y. and to estimate trends in HIV-1 DRMs prevalence over time.

Methods. The study included 141 treatment-experienced and 54 treatment-naïve HIV-1 infected patients. HIV-1 genotyping was performed in case of treatment failure or in newly diagnosed ART-naïve HIV-1 patients, using commercial assay or in-house protocol. DRMs prevalence in naïve patient group was defined by the presence of at least one resistance mutations from the WHO-recommended Surveillance DRM list (2009). For the estimating of DRMs prevalence in treatment-experienced patients group, IAS-USA mutation list (2010) was used. HIV-1 drug susceptibility to NRTI, NNRTI and PI was predicted using Stanford HIV-1 database algorithm, v.6.1.0.

Results. Among treatment-naïve patients, three patients (5.6%) displayed transmitted DRMs: K103N, G190S mutations that cause resistance to all NNRTI, PI mutation M46I and NRTI mutation K219E that not reduce drug susceptibility. In the group of treatment-experienced individuals DRMs (excluding “minor” PI mutations) prevalence was estimated as 53% (75/141). Most frequently DRMs were found for NNRTI (57/141; 40.4%), followed by NRTI (54/141; 38.3%) and PI (5/141; 3.5%). In the group of NNRTI mutations K103N (35/141; 24.8%), G190S (24/141; 17%), K101E (11/141; 7.8%), P225H (6/141; 4.3%), NRTI mutations M184V (46/141; 32.6%), L74V (10/141; 7.1%), K70R (8/141; 5.7%), K219Q (9/141), PI mutation I50V (3/141; 2.1%) occurred most frequently. Resistance to one drug class was predicted in 32/141(22.7%) patients: for the NRTI-in 13/141, for the NNRTI-in 19/141. In 38/141, 27% patients HIV-1 resistance was observed to two drug classes: NRTI+NNRTI-34/141, NRTI+PI-3/141, NNRTI+PI-1/141. Resistance to all three drug classes was predicted for one patient.

Conclusion. 1. The estimated prevalence of DRMs in treatment-naïve HIV-infected persons in Latvia in 2009-2011 was 5.6%. Thus, the prevalence of transmitted drug resistance remains stable over time. 2. Drug resistance was predicted in 50.4 % of treatment-experienced individuals. NNRTI RMs in treatment-experienced HIV-1 infected persons in 2009-2011 were detected more often, than in previous period (2006-2008), 40.4% and 18% respectively. The prevalence of PI RMs was declined, 3.5% and 10% respectively. The results can be explained by fact, that from 2009 y. NNRTI more often were used in first-line ART.
Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and HIV infected patients

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**Introduction and aim.** Tuberculosis (TB) is most often HIV opportunistic infection in Latvia. Main challenges in TB diagnosis in HIV infected persons are more often lack of sensitivity of sputum smear microscopy and long time for diagnosing TB with culture. Use of Xpert MTB/RIF assay has been endorsed by WHO as a rapid method for simultaneous detection of MTB and rifampicin resistance as a surrogate marker for MDR-TB. Aim of study was to evaluate the sensitivity of TB diagnostics by GeneXpert MTB/RIF test in HIV infected patients as experience in LCID.

**Materials and methods.** In the study were involved 48 (35 men) HIV infected patients from LCID hospital with first time diagnosed TB confirmed by positive TB culture. Primary laboratory testing for simultaneous detection of MTB DNA and rifampicin resistance was performed by GeneXpert MTB/RIF Realtime PCR (Cepheid) on one or more respiratory samples - sputum or bronchoalveolar lavage (BAL). HIV viral load detected by Realtime PCR Cobas Amplicor/Cobas Taqman HIV Test (Roche), CD4+ count performed by flow cytometry. Sputum smear microscopy and TB culturing were performed in laboratory of Tuberculosis and Lung Disease Centre.

**Results.** *M. tuberculosis* (MTB) DNA positive results were observed in 38/48 patients (28/38 in sputum, 10/38 in BAL samples), negative results in 10/38 patients (4/10-in sputum, 2/10-in BAL, 4/10-in both sputum and BAL samples). In patients group with DNA positive GeneXpert MTB/RIF tests results average CD4+ count was 144 cells/ml, average HIV viral load - 5.83 E5 RNA cop/ml. In patients group with negative MTB DNA results average CD4+ count was 212 cells/ml, average HIV viral load - 1.23 E5 RNA cop/ml. In 24 out of 38 patients with MTB DNA positive results smear microscopy was negative. Semi quantitative GeneXpert MTB DNA results were in this group as follows: very low - in 11/24, low - in 9/24, high - in 1/24 patients. Rifampicin resistance was detected in 5 out of 24 patients with negative smear microscopy. In group of 14/38 patients with positive smear microscopy semi quantitative GeneXpert MTB DNA results were as follows: low - in 1/14, medium - in 9/14, high - 4/14. Rifampicin resistance was detected in 2 out of 14 patients with positive smear microscopy.

**Conclusions.** 1. Sensitivity of TB diagnostics by GeneXpert MTB/RIF test in HIV infected patients in the study was 79.2% (38/48). 2. Sensitivity of smear microscopy comparing to GeneXpert MTB/RIF test in the study was 63.2% (24/38). 3. Rifampicin resistance was detected in 18.4% (7/38) of HIV infected patients diagnosed as MTB DNA positive, 5 of them - in patients with negative smear microscopy.
Genetic Approach to Coronary Artery Disease: The EGY-NO-MI Study

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Introduction & Aims. Cardiovascular disease (CVD) is one of the leading causes of death among Egyptians. Thus there is always an immense need for exploring the genetic and environmental factors contributing to the development of this disease. Among these factors emerge modulation of nitric oxide (NO) homeostasis and oxidative stress (OS) as central players according to recent reports. A range of biochemical disturbances including reduced availability of NO and OS has been shown to be associated with endothelial dysfunction (ED). Recent evidence indicates that ED may be genetically determined. Therefore, studies in our lab are running to identify the role of genetic polymorphisms in enzymes and regulatory proteins related to NO metabolism and OS in the predisposition of early-onset myocardial infarction (MI) in Egyptians. Among the major aims are to detect dimethylarginine dimethylaminohydrolase (DDAH) type 2 gene polymorphisms among Egyptian patients, to explore functional correlations of these profiles with serum asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-arginine, and C-reactive protein (hsCRP), and to assess their association with risk of CVD in young susceptible individuals.

Methods. 100 Egyptian male CVD patients (35-50 y) were recruited for the study from National Heart Institute, Cairo, Egypt. They were classified according to severity of coronary insufficiency, as verified by coronary angiography, into: patients under conservative medical treatment (Med, n=12), patients directed for PCI (PCI, n=41), patients directed for GABG operation (CABG, n=36), and patients suffering from acute myocardial infarction (AMI, n=11). Age and sex-matched controls (n=100) were included from general population.

Results. Data revealed a complete association between two polymorphisms (SNP1, -1151 C/A, rs805304 and SNP2, -449 C/G, rs805305) of the DDAH2 gene. A allele / AA genotype for SNP1 and G allele / GG genotype for SNP2 were significantly associated with CVD in the Egyptian patients and their frequency is correlated with severity of coronary insufficiency. No significant association between serum levels of biochemical parameters and carriage of specific DDAH2 genotype. Patients having AMI showed higher serum levels of ADMA, SDMA, and hsCRP; and lower serum L-arginine and L-arginine/ADMA than chronic patients.

Conclusion. DDAH2 gene polymorphisms are correlated with the early incidence of CVD in Egyptians. This study was supported by the Science and Technology Development Fund (STDF) grant No. 2951.
Prevalence of HIV-1 genotypes and antiretroviral drug resistance in Lithuania in 2011-2013

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Introduction. Although Lithuania HIV-1 drug resistance testing carried out since 2006 have not yet been carried out detailed HIV-1 subtypes and antiretroviral drug (ARD) resistance analysis. The aim of this study is to demonstrate a resistance prevalence in Lithuania and encourage primary HIV-1 drug resistance testing.

Materials and methods. During the period from 2011 to 2013 at National Public Health Surveillance Laboratory were analyzed 179 patients plasma samples for HIV-1 resistance to ARD. For testing were used plasma samples with viral load 2033-5130000 cop/ml (mean value - 307 851 cop/ml). Quantity testing was performed on automatic real-time PCR system m2000 (Abbott Laboratories, USA) using HIV-1 Amplification Reagent Kit (Abbott Laboratories, USA) and Sample Preparation System RNA (Promega, USA). Genotyping and resistance to ARD studies have been carried out with ViroSeq HIV-1 genotyping Systemv2.0 (Abbott Laboratories, USA) kit, the sequence of scanning performed by genetic analyzer ABI PRIZM 3100 - Avant (Applied Biosystems, USA), a consensus was collected by software Viroseq HIV-1 genotyping system software v2.8 (Abbott Laboratories, USA), final data (including genotypes, resistance profiles, a list of mutations and quality control) was obtained by Stanford University's HIVdb Program: Sequence Analysis (http://sierra2.stanford.edu/sierra/servlet/JSierra?action=sequenceInput).

Results. After data analysis it was observed domination of these subtypes: A - 24% B - 31%, and the different types of CRF - 33%. The large part of the CRF consisted of CRF01_AE variant in combination with subtype A (61 %), and pure CRF01_AE (25 %). The total number of patients with ARD resistance mutations were 84 (47 % of the observed cases), while patients with varying levels of resistance to ARDs was 37 (21 % of the observed cases). In 4 noteworthy cases from those 37 where detected resistance for PI NFV, without ARV resistance mutations: in 3 cases it was a potentially low resistance, and one - a low resistance. In 51 cases (29 % of the observed cases) was confirmed resistance with associated mutations in patients with no resistance to ARDs. Most of those was the PI resistance mutations (43 cases or 84 %), from which 24 (47 %) were A71V.
Celiac disease, an autoimmune disease of all ages

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Celiac disease is apparently a modern disease, but it has been described since old times, got her name in the Greek period, and the first efficient treatment comes from the 19th century. In the 20th century the biopsy became the best method of diagnosis, and only in the last 20 years, with the evolution of laboratory immunological tests, the clinicians improved the diagnose. Being defined as an autoimmune multi organ disease, we have to look for this disease in a wide variety of situations: anemia, diarrhea, malnutrition, weight loss, puberty and growth delay, miscarriage, Infertility, dental anomalies, depression, in children, in adults and more carefull in patients with other autoimmune diseases: thyroid and liver diseases, type I diabetes, Ig A deficiency, all the patients having first degree relatives with the disease.

What is the role of laboratory in the management of the disease? In order to follow the guidelines the clinicians has to relay in a good laboratory. The laboratory specialist knows what is the sensitivity and specificity of different diagnostic tests, proposes different panels of antibodies, different methods of work and different commercial kits, explains the time between the diet intake and the normalization of antibodies serological levels. We will discuss why we have different algorithms for symptomatic and asymptomatic but at risk patients and the conditions of the new guidelines that offer the option of omitting biopsies in selected cases with symptoms suggestive of Celiac disease without increasing the risk of misclassification.
Mobility of laboratory specialists in the European Union

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The strongest idea of the EU Constitution is the free movement of its citizens within its borders and consequently equal health care facilities in all the countries, a so called “euroharmonised health care system.” Are the laboratory professionals able to move from one country to the other to find a job? Due to the different individual historic, geographical and political conditions, each nation diverged on its own option to supply well educated and proficient specialized professional.

Once member of EU, each country has to harmonize the conditions needed for education and recognition of its specialists to the EU standards. Who is in charge with this harmonization process? The European Federation of Laboratory Medicine has settled a Syllabus for quality standards for laboratory professionals education and training. The requirements proposed are a minimum of 10 years education and training for the specialty, with a minimum of 4 years education and 4 years professional training and flexibility for the remaining years.

The harmonization process is not an easy work because there is a diversity of professions working in laboratory medicine, each country having its criteria for settle the laboratory medical specialty and the economic and social conditions differ a lot.

The job to do this harmonization goes to the professionals who have to be involved because is their interest to have a regulated profession, a high academic education and an up date training in the profession. The European Register of Laboratory Specialists EC4 has took the role of lobbying for this process and invites all the colleagues to join this idea because “as many we are as strong our voice is” and we need to built together the future of the profession in an harmonized Europe.
New challenges in the biomedical science: biobanking problems and solutions in Lithuania

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A huge progress has been recently made in the field of biomedical research and personalized medicine. Therefore, for the modern biomedical research the huge amount of best quality biological material is needed. Biobank – it is a type of biorepository that stores biological samples and associated data for scientific use. Despite the advances of biobanking, significant limitations remain that are restricting the impact of researches. The major issues include the need to increase the quality and standardization of biospecimens and to maintain public trust. In general it is named as harmonization of biobanking activities.

Today in Lithuania there is a great potential to perform the high technology biomedical research and to participate in the international research projects. In the new scientific valleys adequate and sufficient research infrastructures with new high technologies, equipment and human resources are concentrated.

In Lithuania currently only disease-based biorepositories or project-based biobanks exist in the few research institutions, university hospitals and clinics with insufficient number of samples stored. One of the examples is cancer tissue Biobank in the Institute of Oncology, Vilnius University (IOVU), established in 2012.

The majority of biobanking problems in Lithuania are influenced by the fact that regulation of collecting, storing and distribution of biological resources in Lithuania is administered according the Law of Bioethics created in 2000 year with some corrections in 2008. It means that Lithuanian legislation oriented to biobanking has not been fully created or adjusted according to the EU directives. The main challenges in this field of biobanking are related with bioethical problems, personal data protection and the collection, processing, storage and sharing of biological samples.

In conclusion, first steps for biobanking activities, SOPs creation and process harmonization were made in IOVU. Finally, after appreciation of general situation the main threats in Lithuanian could be proposed. The general working plan and strategy for biobanking in Lithuania, according international rules and national needs, must be applied.
Duplicate retesting of initially reactive anti-HBc samples: a useful practice?

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Introduction. The hepatitis B virus (HBV) consists of an external envelope and an inner core. Antibodies against the core (anti-HBc) appear shortly after the onset of infection and persist for life. They are an indicator of present or past HBV infection. Laboratories have historically rerun initial positive samples in duplicate after recentrifugation before deciding on the final result. This study was designed to examine the utility of this practice for the Roche Cobas Anti-HBc assay.

Methods. Anonymised records of all repeat testing of anti-HBc analysis performed on a Roche Diagnostics e601 immunoassay analyser between March 2012 and April 2014 were examined in Microsoft Excel and Access. Repeat testing in duplicate of initial positive samples (cutoff-index COI $\leq 1.0$) was performed as per the manufacturer’s instructions. Any changes in result classification due to duplicate retesting noted. Paired t-testing between initial COI1, repeat COI2 and repeat COI3 values was performed.

Results. 2570 requests were received of which 33% were non-reactive. Of the remaining 841 which underwent duplicate retesting after recentrifugation, only 1 was reclassified as non-reactive (COI1 1.000, COI2 1.001, COI3 1.010). There was no significant difference ($p<0.05$) between COI1, COI2 and COI3.

Discussion. A no-repeat testing strategy gives sensitivity of 100% and specificity of 99.88% with a 57% reduction in tests performed compared the triplicate testing practice. Singleton repeat testing with additional testing for samples with discordant results gives 100% sensitivity and specificity with 29% reduction in tests performed and balances cost with result reliability.

Conclusion. Duplicate retesting of initially reactive anti-HBc samples is not a useful practice. It is costly in staff resources and analytical reagents and delays result reporting unnecessarily. Alternatives include a no-repeat testing strategy or singleton repeat testing with additional testing of discordant results - either approach is preferable to the present practice of triplicate testing.
Ethnic differences in serum immunoglobulin concentrations.

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Introduction. Serum immunoglobulin concentrations can vary between groups based on differences in sex and ethnicity. This study determined whether differences in serum immunoglobulin G (IgG), A (IgA) and M (IgM) exist between Chinese, Indian, Malay and other race individuals in Singapore.

Methods. Anonymised records of serum IgG, IgA and IgM measurements performed during health screening between June 2012 and May 2013 were extracted from the laboratory database for analysis in Microsoft Excel and SPSS v16. To avoid inclusion of paraproteinaemic patients, the following exclusion criteria were used: haematology service patients, all inpatients, IgG/A/M > 2 times locally established upper reference limit. Repeat samples were excluded. All testing was performed on Beckman-Coulter LX20 and DxC-800 analysers using manufacturer supplied reagents and calibrators. Linear regression analysis was performed for IgG/A/M concentration as the outcome variables and age, sex and race as predictor variables.

Results. There were 1763 records in the final dataset, of which 78% were Chinese, 7% Indian and 9% Malay. Males comprised 39% and average age was 62y. For prediction of IgG (g/L), linear regression showed average effect sizes of: Indian vs. Chinese +0.324 (p 0.497), Malay vs. Chinese +1.097 (p <0.010). For IgA (g/L), average effect sizes were: Indian vs. Chinese +0.183 (p 0.207), Malay vs. Chinese +0.469 (p 0.001). For IgM (g/L), average effect sizes were: Indian vs. Chinese -0.047 (p 0.479), Malay vs. Chinese +0.101 (p 0.09).

Discussion. Laboratories in Singapore presently do not partition reference intervals based on race. The higher concentrations in Malays represent IgG, A and M elevations of 8%, 16% and 6% of the midpoints of the respective reference intervals and may justify use of different reference intervals for Malay individuals.

Conclusion. IgG and IgA concentrations in Malay outpatients are statistically higher than Chinese and Indians when corrected for age and sex. Further work is needed to establish the cause of these differences and the feasibility of partitioning immunoglobulin reference intervals by race.
The Nova Glucose Connectivity Meter Evaluation in Intensive Care Unit

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**Background.** The CLSI POCT12-A3 guideline states that a) 95% of meter measurement results should be within 0,67 mmol/L for glucose <5,55 mmol/L and within 12,5% for glucose ≥5,55 mmol/L and b) 99% of values should fall within 0,86 mmol/L for glucose <4,2 mmol/L and within 20% for glucose ≥4,2 mmol/L comparing with laboratory.

We assessed the performance of a point-of-care (POC) glucose meter by using the spreadsheet program is designed for estimating the bias between two methods using patient samples.

**Methods.** The study was performed over a three week period using samples obtained from the intensive care unit of Tartu University Hospital. Method correlation was performed by analyzing 120 whole blood specimens on the Stat Strip glucose connectivity meter (Nova Biomedical) compared to ABL blood gas analyzer (Radiometer). Sample collection was performed by arterial Disposable Pressure Transducer Kits and Safeset Closed Blood Sampling/Conservation System (Philips). Mean glucose concentration was 7,31 mmol/L (range 3,8-26 mmol/L).

**Results.** The imprecision for glucose meter of the 3 levels was 6,1/3,4/5,4 %(mean values: 3,3/6,4/16,1 mmol/L) and for ABL was 3,7/1,1/1,1% (mean values: 1,6/5,6/14,1 mmol/L).

The linear regression analysis demonstrated a slope 0,99, intercept -0,38 and R² -0,988. The glucose meter had the lowest mean biases (-0,151 mmol/L) compared with laboratory method (ABL) (p<0,001). Mean relative difference was 5,93 %.

114 (95%) of glucose meter results was within 12,5% and 11 of them (glucose value <5,55 mmol/L) was within ±0,67 mmol/L. Furthermore, 120 (100%) of glucose meter results was within 20%.

**Conclusion.** Stat Strip glucose connectivity meter met POCT 12-A3 performance criteria and demonstrated a close correlation to the laboratory method.
Transient myeloproliferative disorder in neonates without Down syndrome: two cases with distinct immunophenotypic features of blasts

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Introduction. Transient myeloproliferative disorder (TMD) is defined by clonal proliferation of blasts that occurs in neonates with Down syndrome and is distinguished by spontaneous resolution. In most cases blasts are attributed to megakaryocytic lineage and show distinct immunophenotypic features. We present two cases that meet the criteria for TMD disorder yet patients have normal karyotype.

Materials and methods. Patients were boys, born on week 40 and 36. Automated hematology analysis performed first day after birth showed marked leukocytosis (158,0 x 10^9/l and 110,6 x 10^9/l respectively). Analysis of blood smear revealed that 55% and 47% of white blood cells were blasts. Flow cytometric analysis of peripheral blood samples was carried out (FACS Canto flow cytometer, whole blood sample preparation technique, and six color acute leukemia marker panel).

Results. The following phenotype of blasts was established in the first case: CD45 dim, CD34 partial, CD38+, CD117+, CD4 dim, CD7+, CD33 partial, CD56+, cyto CD41 partial, cyto CD61 partial, CD13-, cyto MPO-, HLA-DR-. Blasts were identified as having the megakaryocytic origin.

Blasts of the second patient had similar phenotype: CD45 dim, CD34+, CD38+, CD117+, CD4 dim, CD7+, CD33+, CD13-, cyto MPO-, HLA-DR-. In this case megakaryocytic markers were found only in 10% of blast population and were considered to be negative. This phenotype was consistent with acute myeloid leukemia with minimal differentiation.

As other hematologic parameters were within normal range, and clinical condition was satisfactory, children did not receive any specific antileukaemic therapy. Flow cytometric analysis of blasts in bone marrow samples was repeated monthly. The amount of aberrant blasts was gradually decreasing (55% - 23% - 2% - 0% - 0% and 47% - 19% - 13% - 4,9% - 1,6% respectively) while amount of normal B-cell precursors, that dominate the niche of blasts in healthy infants, increased (0% - 8% - 41% - 45% and 0% - 21% - 23% - 29%). The second patient is still being followed.

Discussion. Our previous analysis of four cases of patients with TMD and Down syndrome showed that blasts had comparable phenotype to the cases of neonates without Down syndrome. This immunophenotype was distinct from acute myelogenous leukemia in general children population. This finding might indicate the similar biological origin of myeloid blasts in neonates with or without Down syndrome and might predict its favorable course.

Conclusion. Even in neonates without Down syndrome identifying specific blast phenotype (positive early, myeloid and aberrant T cell markers along with the negativity of HLA-DR) might be crucial for the diagnostic, therapeutic and prognostic decisions.
Vitamin D and immune state correlation

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Introduction. Vitamin D have been shown to be important regulator of immune system. It can modulate the innate and adaptive immune responses. Deficiency of D vitamin is associated with increased autoimmunity as well as with increased susceptibility to infection.

Materials and methods. We examed 272 patients and detect vitamin D level. Vitamin D level was detected with electrochemiluminescence method (ARCHITECT, ABBOTT), values 30-70 ng/ml were recomended as normal values.

Cellular immunity was examened usig laser flow cytofluorimeter (FACS Calibur BD), humoral immunity parameters determined by nephelometer (BN II SIEMENS).

Results. 63% (171 patients) of examined patients had decreased vitamin D level below 30 ng/ml., but 19% (51 patients from 171) had vitamin D below < 20 ng/ml, 36% (99 patients) had normal vitamin D levels.

Patients with decreased vitamin D level had lowest CD 4, CD 8 absolute counts and reduced average IgG level in comparison with patients who had normal vitamin D level.

Discussion. Literaturae data show vitamin D influence on immune state and own data confirm that.

Conclusion. Vitamin D level influenced to cellular and humoral immune parameters – decreases immune answer and therefore it is important to evaluate vitamin D level and immune status in patients with different diseases with immunological pathogenesis mechanisms.
Frequencies of Red Blood Cells Alloantibodies in Patients Hospitalized in Vilnius University Hospital Santariskiu Klinikos

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**Introduction.** The blood transfusions and pregnancies are the main reasons for Red blood cell (RBC) alloimmunization. Vilnius University Hospital Santariskiu Klinikos hospitalized patients dons most blood transfusion. The goal of this study is to find out how much their our clinics hospitalized patient have RBC alloantibody.

**Materials and methods.** In the Center of Laboratory Medicine done ABO/Rh typing and other clinically significant blood group typing, Red Blood Cell (RBC) antibody screening, RBC antibody identification and compatibility testing. The RBC Antibody Screening performed the indirect antiglobulin test (IAT), using 2 cell reagents ID-DiaCell I-II in the gel technique ID-Card LISS/Coombs (BIO-RAD, Switzerland). The RBC antibody identification using 11 cell panel- ID-DiaCell 1-11 (BIO-RAD, Switzerland).

**Results.** In the 2013 study there were tested adult patients who were hospitalized in our clinics. They were divided into 2 groups. One group consisted of patients who didn’t have transfusions or who had a single transfusion. The number of patients in this group was 3563 (1885 men and 1678 women). We found 15 (0,8%) immunized men and 41 (2,4%) women. The overall immunization level in this group is 1,6%.

The other group consisted of adult patients from the haematological department. During the year these patients received the re-transfusion. In this group of 461 patients there were 220 men and 241 women. We found 17 (5.0%) immunized men and 19 (5.8%) women. The overall immunization level in this group was 7.8%.

**Discussion.** We can see that the first group of immunized women is much bigger and it suggests that the part of women were immunized during the pregnancy. In the second group, the most important reason was transfusion immunization. We didn’t evidence any difference between the percentage of immunized men and women.

The similar study was carried out in 2007 where we did not separate the patients by sex. The first group consisted of 2712 patients and there were 26 patients (0.96%) with RBC antibodies. The second group consisted of 561 patients and 44 (7.8%) of them were immunized. We see a high correlation between 2007 and 2014 results. We also noticed that the proportion of immunized patients in our hospital remained stable over the years.

**Conclusion.** In the study (n=4024) the total number patients with irregular RBC antibodies was 2,3% (n=92). The percentage of immunized women was 3.1% (60 out of 1919) and the percentage of immunized men was 1.5% (32 out of 2105).
Experience of measles laboratory diagnostics in vaccinated and unvaccinated individuals

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Introduction. In 2014 outbreak of measles in Latvia occurred with 31 laboratory confirmed cases registered by 12 May 2014, 19.4% of reported cases were known previously vaccinated. Laboratory confirmation of measles infection in previously vaccinated individuals may represent difficulties. Objective of this study was to analyse differences in the testing results in vaccinated and unvaccinated patients.

Materials and methods. Clinical samples from 89 patients suspicious for measles were tested in NRL in time of measles outbreak. Urine and nasopharyngeal swabs were tested for measles virus (MeV) RNA by RT PCR, blood samples - by ELISA for MeV IgM and IgG antibodies as well IgG avidity. PCR positive samples were used for MeV isolation in Vero-Slam culture. Isolates viruses were genotyped by Sanger sequencing.

Results. In 31 patients measles diagnosis was confirmed. MeV was successfully isolated from 8 persons and genotype was detected as B3. Sequences were identical and confirmed transmission of infection from one source. In 11 patients measles was confirmed only by positive MeV RNA, in 14 patients – both by positive MeV RNA and MeV IgM, in 6 patients – only by positive serology: in 3 patients positive MeV IgM and in 3 patients – more than 4-fold increase of MeV IgG antibodies. In 2 vaccinated patients MeV IgM in blood samples collected on 4th day after the rash appearance were negative. In 1 vaccinated person with negative MeV PCR infection was confirmed only by 4-fold increase of IgG with high avidity. In 4 persons with unknown vaccination status and/or unclear previous measles infection history the same serological response was detected: IgM negative, increase of IgG and/or high IgG avidity (>60%).

Conclusion. Laboratory diagnostics of MeV infection in previously immune individuals requires more complicated approach and should include testing for MeV RNA and broad spectra of serological parameters like specific IgM, IgG in paired serum and IgG avidity detection because IgM response may be absent and molecular detection of MeV RNA may be limited.
The effect on lactation of postpartum back massage for women with premature babies

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Aim. It is known that mother’s milk is a nutrient which completely supports the development of the newborn child. Lactation, which begins after the women gives birth, along with the physiological changes associated with it, is thought to be affected by hormones. In order to investigate the effects of back massage on lactation in mothers who had given birth prematurely, we examined their levels of oxytocin, prolactin and noradrenalin.

Method. Our study group consisted of 30 cases, and another 30 cases formed the control group. Women were accepted into the study who had had a normal healthy birth, were in the 18-35 age group, had begun suckling within one hour of giving birth and had no breast problem that would obstruct suckling, had suckled at least twice in the first four-hour period and had eaten, got up, and emptied their bladders within 3-4 hours of giving birth, and had no chronic disease. Mothers in both groups had to abstain from suckling for 30 minutes before blood was taken. The control group were given no massage of any kind, and blood was taken within 3-4 hours after giving birth. Blood samples were stored at -80⁰ until they were analyzed. After the blood samples were collected, they were all analyzed by the same methods to recognized standards. Prolactin, oxytocin and noradrenalin levels were measured by the Elisa method.

Findings. No statistically significant difference was found between the massage group and the control group in terms of age and body mass index. In the massage group, noradrenalin levels were significantly reduced (p<0.05), and oxytocin and prolactin levels were distinctly increased in comparison with the control group.

Discussion. According to the data obtained, we consider that regular back massage raises the levels of oxytocin and prolactin by a statistically significant amount. Because this rise will increase the amount of the mother’s milk, no extra nutrition will be needed for the baby’s development. This will result in healthier babies and fewer economic losses.

Results. It was seen that back massage performed on mothers who had given birth prematurely increased hormonal secretion and lactation; therefore it is recommended that massage should be carried out in this period by other people who are care-givers.
Quick and donor-saving method for mesenchymal stem cell extraction for clinical use

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\textbf{Introduction.} Mesenchymal stem cells (MSCs) are currently exploited in numerous clinical trials to investigate their potential in immune regulation and tissue regeneration. The most common source of MSCs is human bone marrow (BM). To generate sufficient numbers of cells relevant to clinical use high volumes (20-50 ml) of BM aspirates are taken and MSCs are isolated by density gradient centrifugation (DGC). However, aspiration is invasive and painful and centrifugation is difficult to standardize. Application of easier standardized and less patient frustrating method is needed. The aim of our study was to investigate the feasibility to isolate MSCs from low BM volume (6 ml) using red blood cell (RBC) lysis method and expand the cells up to adequate therapeutical dose.

\textbf{Methods.} 8 healthy BM donors were included in this pilot study. 2 different MSC extraction methods were evaluated: DGC using 60 ml of BM and RBC lysis using 6 ml of BM.

\textbf{Results.} MSCs were efficiently isolated from 6 ml of BM. The adequate therapeutical dose of MSCs was achieved during 3-4 weeks.

\textbf{Discussion.} RBC lysis method is quick and efficient since MSCs can be expanded to relevant amounts during similar period of time as using DGC method. It is donor-saving method since MSCs are isolated from residual material after BM transplantation and there is no need for additional BM aspirations.

\textbf{Conclusion.} RBC lysis method for MSCs extraction is feasible and advantageous over common density-gradient centrifugation. It should be standardized and promoted for clinical use.
4-year-old boy with pure de novo 17q25.3 microduplication

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Pure duplication of 17q25.3 region is rarely reported, most of cases described are with associated monosomies due to familial translocations. The smallest pure duplication is reported by Lukusa et al.(2010) in a dysmorphic girl with growth retardation, developmental delay and arthrogryposis. We report an other small (2.8 Mb) pure duplication of 17q25.3 region in a boy with developmental delay and overweight.

Chromosomal microarray analysis (CMA) was performed as a routine first-trial test in a 4-year-old boy with developmental delay using HumanCytoSNP-12 BeadChip (Illumina Inc.). Analysis revealed a 2.8 Mb duplication in chromosomal region 17q25.3. For confirmation, metaphase-FISH analysis was done with Cytocell 17p and 17q subtelomere probes. The additional 17qter signal was located on a small additional marker chromosome. After G-banding the final karyotype is:

47,XY,+mar.ish der(17)(q25.3)(qter+).arr[hg19] 17q25.3(78,175,854-81,047,565)x3 dn.

Clinical evaluation at the age of 4y 4m: weight 22 kg (97 percentile), height 103 (25 percentile), OFC 51 cm (50 percentile). His bone age was delayed with Z score -3.49, centile<1. Some dysmorphic facial features, hepatosplenomegaly (both +2.5 SD) and central hypotonia with exaggerated deep tendon reflexes was noted. His intellectual and speech development was delayed and corresponded to the age of 2y 6m.

17q25.3 region duplicated in our patient harbors more than 30 genes, several of them are shown to be associated with nervous system development and function (NPTX1, BAIAP2). Still, molecases are needes to establish accurate genotype/phenotype associations.
Confirmatory diagnostics of HCV infections in Latvia

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**Introduction.** Since August 2013 laboratories in Latvia should test all HCV Ab reactive samples for presence of HCV core Ag or HCVRNA or should send reactive samples for confirmatory diagnostics to National reference laboratory (NRL).

**Objective.** To analyse the efficiency of HCV infection confirmatory diagnostics

**Materials and methods.** In the study 923 HCV Ab reactive serological specimens received from 25 laboratories in August – December 2013 were included. Samples were tested for HCV core Ag (Architect, Abbott). HCV core Ag negative samples were retested for confirmation of HCV Ab by second ELISA or immunoblot. In the same period NRL received 10 samples collected in ICL outpatient department from patients suspected for HCV infection but tested as negative by PCR in other laboratories. Samples were tested for HCV RNA by PCR with detection limit 50 IU/ml (Roche Diagnostics) or real time PCR with detection limit $\leq$15 IU/ml (Roche Diagnostics, Abbott Molecular) and HCV core Ag. Since the end of 2013 routine confirmation of HCV infection for ICL outpatient department patients with HCV Ab reactive results was performed by HCV RNA PCR or real time PCR tests: during four months 307 blood samples were tested.

**Results.** Confirmatory diagnostics results on specimens received from other laboratories were as follows: 592/923 (64.1%) samples were found positive for HCV core Ag; 248/923 (26.9%) samples negative for HCV core Ag were confirmed as HCV Ab positive either by second ELISA or immunoblot, collection of second sample for PCR testing was recommended; 83/923 (9%) samples were tested in NRL as negative both for HCV core Ag and HCV Ab. All 10/10 samples from patients tested as negative by PCR in other laboratories were positive for HCV Ag and/or for HCV RNA by PCR. In ICL outpatient department patients positive HCV RNA results were found in 208/307 (67.8%) cases.

**Conclusions.** To improve the diagnostics of acute and chronic HCV infection in Latvia, approach based on the detection of HCV RNA by sensitive molecular methods with lower limit of detection $\leq$15 IU/ml should be applied.
Prevalence of Norovirus, Rotavirus, Adenovirus and Astrovirus infections in Latvian hospitalized children.

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Introduction. On the base WHO data, acute gastroenteritis remains a major cause of morbidity and mortality in children around the world, accounting for 1.34 million deaths annually in children younger than 5 years, or roughly 15% of all child deaths. Viruses remain by far the most common cause of acute gastroenteritis in children, are spread worldwide and main of them are Norovirus, Rotavirus, Adenovirus and Astrovirus. The aim of our study was to detect the prevalence of different viruses in acute gastroenteritis etiology in infants and young children. The study population consisted of children up to 15 years of age with acute gastroenteritis who sought medical advice at the two mains children’s medical hospitals of Latvia in 2013 year.

Materials and methods. Stool samples were tested by enzyme immunoassay for qualitative determination for noro- rota- astro and adenoviruses antigen using r-biopharm (Germany) appropriate diagnostic kit in accordance with physician request.

Results. During the whole 2013 period totally 6952 children’s clinical materials were examined. The highest number of positive samples was found among 3233 specimens tested for noroviruses - 890 samples (27.5%). 202 samples (17.2%) from 1170 specimens tested for rotavirus were positive. Prevalence of astro – and adenovirus was lower, we have found 85 (4,7%) astrovirus positive samples from 1793 tested samples and 54 adenovirus positive samples (7,1%) from 756 tested ones. In 4,9% from all positive samples were found mixedinfections in different compositions: noroviruses+rotaviruses (44,2%); noroviruses+astoviruses (29,6%); Norovirus+rotaviruses+astoviruses (13,2%); rotaviruses+astoviruses(9,8%); noroviruses+adenoviruses(1,6%); noroviruses+rotaviruses+adenoviruses(1,6%).

Conclusions. This study shows the prevalence norovirus infection in Latvian children up to 15 years in 2013 year - 72,3% from all positive samples. Rotavirus was the second etiological agent of acute gastroenteritis -16,6%. Detection of high present mixed infections in investigated samples highlight of the necessary to complete diagnostic procedures for all spectraviruses cause of gastroenteritis.
Maternal UPD(14) in a patient with 47,XXX karyotype: a case report

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Uniparental disomy (UPD) occurs when both homologues chromosomes are inherited from one parent. UPD manifests as either heterodisomy (both copies of the same homologue from one parent) or isodisomy (two copies of one of the homologues from one parent). Uniparental disomy for chromosome 14 is a quite rare condition and most reported cases have been from maternal origin (matUPD14). Clinical symptoms of those patients are quite specific.

Triple X syndrome incidence is 1:1000 to 1:1200 female births and this syndrome may be diagnosed incidentally at amniocentesis/chorionic villus sampling.

We report clinical and laboratory data of 13 months old girl with developmental delay, growth retardation, facial and non-facial micro anomalies. She was born in week 38 with C-section due to retarded intrauterine growth and decreased amniotic fluid amount. Her birth weight was 2489g (-1 SD) and height was 44cm (-2 SD). While the proband qualified for chromosomal microarray testing due to her developmental delay and micro anomalies this was the first option of choice. Chromosomal microarray analysis showed that the proband has maternal uniparental isodisomy for chromosome 14 and triple X syndrome. Females with 47,XXX karyotype are rarely showing any physical abnormalities and slight developmental delay occurs only in some cases. Therefore we suggest that the chromosomal microarray analysis should be the method of choice analyzing patients who qualify to this test having developmental delay, dysmorphic features or congenital anomalies, when using only routine karyotyping these findings would not have been detected.
Comparison of routine microscopy and flow cytometry for the detection of childhood acute leukemia blast cells in peripheral blood

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Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy. Early recognition is essential for treatment, but could be complicated since only 50% childhood ALL present with leukocytosis. Cytologically, tumour population is often inconspicuous. Thus, morphology may be not sufficiently sensitive.

The study. The aim was to compare blood blast counts obtained by microscopy and flow cytometry. 127 blood samples of 82 pediatric ALL patients with proven diagnosis were simultaneously tested by microscopy and flow cytometry (77 primary ALL, 18 at relapse and 32 at day 8 of treatment; 110 samples of B-cell and 17 of T-cell ALL). Microscopy was performed by experienced cytologists with panel diagnosis in case of doubt. 4-colour flow cytometry was applied in 2007-2010 (Coulter Epics XL) and 8-colours since 2011 (FacsCanto II). Data were analysed by IBM SPSS v.21 (Spearman rho and Mann-Whitney U).

Results. Both relative and absolute blast counts by the two methods correlated significantly (p=6.8E-22 and 5.0E-22). Still, the results were significantly different (p=2.18E-15 for percentages and p=2.48E-13 for absolute counts); flow cytometry counts were higher in 57% cases, counts were equal in 39%, cytological count was higher in 4%. Blasts >5% were found in 82.1% primary patients by flow and in 53.7% by microscopy; diagnostic blastosis >20% was defined in 64.2% primary cases by flow and only in 35.8% by microscopy. Absolute count of morphologically unrecognized blasts in 17 samples was above 10x10E9/L, in 4 cases above 100x10E9/L.

Conclusions. Determination of blast counts by flow cytometry was significantly more sensitive than cytology; microscopy turned out to be ineffective in screening for blast cells in a third of primary cases with blastosis and missed ALL diagnosis in half of patients with diagnostic blast counts. Low leukocyte counts at presentation, specific “pediatric” morphology and other factors may be responsible.
Clinically significant myeloperoxidase deficiency in children: Screening algorithm and preliminary results

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Introduction. Myeloperoxidase (MPO) is linked to neutrophil antimicrobial activity and other functions. Data on clinical relevance and incidence of MPO deficiency are scarce and contradictory. Haematological analyser Advia 2120i (Siemens AG) routinely measures leukocyte MPO as myeloperoxidase index (MPXi, normal 0±10); the data could be used for identifying abnormally low neutrophil MPO. The aim of the study was to propose an algorithm and to retrospectively screen for cases of possible MPO deficiency in the Children’s Clinical University Hospital patients. The study was a part of the Latvian National Research Program „BIOMEDICINE”, project No. 8.

Methods. Blood tests performed from 02.2011 till 01.2014 and clinical data of selected patients were analysed. Hematological/oncological patients and neonates were excluded due to possible artefacts; the remaining database included 64002 tests of 33770 patients.

Results. MPXi below -10 was found in 4601 samples of 2731 patients. In 767 patients (28.1%) both decreased and normal values were detected. The finding excluded the majority of cases (1894) due to small number of measurements and/or admittances; finally, at least three episodes of illness without a single normal MPXi measurement were considered as the requirement. Of 70 remaining patients, 15 were excluded because of immunosuppression for asthma or autoimmune diseases. 55 patients were left; they were preliminary checked for common features. Median MPXi in the group was -17.0. The patients were 2 month - 18 years old (median 50 months), M:F =1.6:1. The patients had been repeatedly tested (mean 6.8 tests, mean for the whole database – 1.9 tests). Recurrent infections were the most common cause of admittance. 9 patients suffered from genetic abnormalities, 5 patients had coeliac disease.

Conclusions. The study identified 55 cases of probable MPO deficiency (incidence 1.6:1000 pediatric patients); by definition, the screening missed subclinical MPO deficiency. More objective methods like immunophenotyping or genetic testing are necessary for the final diagnosis. A deeper clinical study is necessary to understand the findings' significance and practical relevance.
CD4-/CD8- double negative T-cells in pediatric setting

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**Introduction.** CD4/CD8 double negative T cells (DNC) are a small (<10% lymphocytes) population with both helper (particularly in HIV infection) and regulatory/suppressive activity. Little is known about DNC in children. The aim of the study was to retrospectively check pediatric lymphocyte subset tests for presence of DNC and to evaluate their clinical relevance.

**Methods.** 2077 consecutive pediatric testshad been performed in 1012-2013 by BD FACSCanto II flow cytometer with 6-Color TBNKkit and FACSCantosoftware that provides automatic indication of DNC>10%. For the test accuracy evaluation, the selected files were manually analyzed byINFINICYTv.1.5 (Cytognos). IBM SPSS v.21 was used for statistical analysis (Spearman for correlations, Wilcoxon for differences).

**Results.** 92 samples (4.4%) of 79 patients contained >10% DNC (median 12%, absolute count 0.36x10E9/L). Only in 5 cases DNC were >20% and in 1 case >1x10E9/L. 6 patients had repeatedly increased DNC (2-8 tests). DNC levels in the group correlated with age (rho=0.25, p=0.016 for relative and rho=-0.34, p=0.001 for absolute counts). 81% patients had major clinical symptoms: 14 had severe acute or recurrent infections, 15 - severe allergy or autoimmune diseases, 12 - primary immune deficiencies (PID), 6 - celiac disease and 4 - nonspecific stigmata. HIV status was not assessed.

Median difference between automat and manual analysis was only 1.1%, in 95% cases manual counts were higher (p=6x10E-16). The automatic software failed in 2 cases of SCID, returning high counts of DNC due to false CD3 positivity.

The study revealed that DNC differ from other T cells by higher FS (in 99%) and stronger CD3 expression (92%). Median FS difference was 5.5% (p=8x10E-14), median difference in CD3 fluorescence 48.5% (p=1x10E-15).

**Conclusions.** The study demonstrated that routine 6-TBNK test is suitable for measuring DNC, with several exceptions. The selected group had disproportionally high rate of immune disorders, including PID, indicating a possible diagnostic value of increased DNC. Detailed analysis suggested that DNC represent a distinct population rather than a differentiation stage.
Activities of the Latvian society of laboratory specialists

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The Latvian Society of Laboratory specialists (LSB) was founded in 1993. The society has almost 300 individual members, including laboratory doctors, laboratory specialists, and trainees. LSB is a full-fledged member of International Federation of Clinical Chemistry and Laboratory Medicine, European Federation of Clinical chemistry and Laboratory Medicine, and Baltic Association for Laboratory Medicine.

LSB’s operating objective is to contribute to development of the laboratory diagnosis in Latvia. In order to reach the above objective, the Society develops its activities along the following lines:

* give propositions and proposals to the institutions responsible for health care in the Republic of Latvia,
* work out standard requirements draft for its specialty, take part in the licensing and certification of specialists,
* care out of the professional training of its Members, organize and implement the supplementary education arrangements,
* organize seminars, conferences, congresses, and exhibitions,
* organize the legal defense of its Members, as well as make claims in cases of the lack of professional competence, deontological infringements, and other events,
* cooperate with other professional medical associations and societies,
* facilitate contacts of its Members with their colleagues in other countries,
* generalize and popularize the traditions of the Latvian medical school.

The Society supreme executive body is General Meeting. LSB organises 3 to 4 general meetings annually. The meetings are free for the participants and usually attended by 100 to 150 colleagues. The Board manages activities of the Society during the periods between General Meetings, as well as decide on all issues other than within the sole competence of the General Meeting.

Every year starting from 2012 LSB gives grants in the total sum of 4300 EUR per year to the Members of LSB for attending in the scientifical or educational events such as seminars, conferences, congresses, and courses. Usually, 10-14 colleagues annually used this opportunity.
Widely distributed infectious agents after implantation of joint endoprostheses in Hospital of Traumatology and Orthopaedics, Riga, Latvia

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The implantation of joint endoprostheses (arthroplasty) is associated with an increased risk for infection. Infection must arise either by contamination at the time of operation or via the bloodstream. Individuals, particularly if they are moving around, contribute the largest proportion of pathogenic bacteria to the wound. Even if the patient escapes the hazard of infection during the operation, there is always the risk of haematogenous infection during the bacteraemia which is often associated with severe systemic illness. The primary foci included the respiratory tract, the skin, the urinary tract, the teeth, the gastrointestinal tract and the middle ear.

**Aim:** to detect widely distributed infection agents after implantation of joint endoprostheses in Hospital of Traumatology and Orthopaedics, Riga, Latvia

**Material and Methods.** All registered cases in the hospital of deep infections after the implantation of joint endoprostheses in 2013 have been investigated. The detection of aetiology has been made using automated microbiology growth and detection system BACTEC 9050, microbiology identification system BD Crystal and conventional microbiology methods.

**Results.** Totally, 37 patients had deep infections in the hospital in 2013 after the implantation of joint endoprostheses. Detected infectious agents were Coagulase-negative staphylococci (48.6% of all cases), *Staphylococcus aureus* (16.2%), *Pseudomonas aeruginosa* (5.4%), *Enterococcus faecalis* (5.4%), *Enterococcus faecium* (5.4%), *Escherichia coli* (5.4%), *Lactococcus lactis ss.lactis* (5.4%), *Aerococcus urinae* (2.7%), *Klebsiella pneumonia* (2.7%), *Streptococcus agalactiae* (2.7%). In the most of cases (64.9% of all patients) the development of deep infection occurs one year or later after the implantation of joint endoprostheses.

**Conclusion.** The species involved are often those thought usually to have negligible pathogenic potential.
**Neisseria gonorrhoeae** infection in Latvia: laboratory diagnostic facilities and antimicrobial susceptibility

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**Introduction.** According to European Centre for Disease Prevention and Control data in 2010 till 2013 one of the highest rates of a gonorrhoea were observed in Latvia. Involved in the Euro-GASP project, there were necessary to increase diagnostic values for a gonorrhoea and detect antimicrobial susceptibility of *Neisseria gonorrhoeae*. The aim of this work was to introduce of *Neisseria gonorrhoeae* laboratory diagnostic methods and antimicrobial susceptibility results of Latvia.

**Materials and methods.** Summarized the laboratory diagnostic possibilities of gonorrhoea, designed an algorithm for *Neisseria gonorrhoeae* and analysed antimicrobial susceptibility results from 2010 to the end of 2013 in the Latvian Centre of Infectious Diseases. An antimicrobial susceptibility was detected by E-test and agar dilution methods. Results were interpreted by EUCAST.

**Results.** Laboratory criteria for a gonorrhoea were an isolation of *Neisseria gonorrhoeae* from a clinical specimen (using selective and non-selective media, 5-10% CO₂, 36 ± 1°C), a detection of *Neisseria gonorrhoeae* nucleic acid in a clinical specimen (NAAT’s) and a microscopic detection of intracellular gram-negative diplococcic in an urethral specimen (men samples). For culture conformation was performed oxidase test (positive), Gram staining (gram-negative diplococi) and used API NH panel.

There were isolated 114 *Neisseria gonorrhoeae* cultures from 2010 till 2013. There were 88 (77.2%) men, 26 (22.8%) women samples. From all samples we observed that 33 (28.9%) samples were resistant to ciprofloxacin, 7 (6.1%) samples were resistant to azithromycin and 2 (1.8%) were resistant to ciprofloxacin and azithromycin. There were no resistance to ceftriaxone and spectinomycin. β – lactamase positive strains were 5 in 2013, previous years there were no β-lactamase positive strains.

**Conclusions.** Laboratory diagnostic of a gonorrhoea was performed according to EU case definition, ECDC technical reports and a laboratory diagnostic algorithm for detection of *Neisseria gonorrhoeae* infection. There were observed a resistance to ciprofloxacin (22.8%), azithromycin (6.1%), but no resistance to ceftriaxone and spectinomycin. Compared with other European countries a spread of *Neisseria gonorrhoeae* resistant strains was not increased in 2010 – 2013 (average rates of ciprofloxacin and azithromycin resistance across Europe were 63% and 13%, respectively). A successful diagnosis and treatment of gonorrhoea is based of all three testing methods (culture, NAAT’s, microscopy) and antimicrobial susceptibility results. For improvement of *Neisseria gonorrhoeae* resistance surveillance is necessary to increase number of culture isolation for detection of resistance. According to legislation of Latvia (Cabinet Regulation No. 7 Procedures for registration of Infectious Diseases) these tests are covered by state budget and it is possible to perform it in National Microbiology Reference Laboratory.
Modification of the ordinary Konelab hsCRP method for analyses of c-reactive protein at low concentrations in human plasma

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In the Research Institute we have used the ordinary method of Konelab for the determination of plasma high sensitivity c-reactive protein (Konelab CRP High Sensitivity, code 981798, hsCRP) with a Konelab 20XTi® analyzer (Thermo Electron Co., Vantaa, Finland). According to the manufacturer this hsCRP method has a measuring range from 0.25 to 10 mg/l of hsCRP, which in our hands gave linear response between low levels of CRP 0.34 to 3.00 mg/l (Konelab hsCRP control 981852/J436, r = 0.998). The total variations by the original method were 5.3 to 2.3 % at hsCRP levels of 0.80 to 7.02 mg/l and antigen excess problem exist over levels of 300 mg/l.

To enlarge the measuring range of the hsCRP method we have changed the sample volume from 10 to 25 µl and increased the number of standard points so that the measuring range is from 0.12 to 6.0 mg/l at which level the method is fully linear. Over 6.0 mg/l the calibration curve is nonlinear and over that sample volumes should be returned to the original 10 µl and samples appropriate diluted with the sample dilutor. The major antigen excess problem exists over hsCRP values of 200 mg/l.

The within run variations of the modified method (no. 10) were as follows: at hsCRP levels of 0.12 mg/l 12.1 %, 0.34 mg/l 5.9 %, 0.75 mg/l 4.8 %, 1.5 mg/l 2.6 % and 4.9 mg/l 1.39 %. The between run variations (no. 10) were at hsCRP levels of 0.75 mg/l 2.8 % and 1.4 mg/l 1.39 %. Total variations (no. 10) were at hsCRP levels of 0.76 mg/l 5.6 % and 1.47 mg/l 3.0 %. Thus the precision of the modified method quite well correspond to those of the manufacturer.

By comparing the modified hsCRP method to the high-sensitive CRP method of Roche Ltd (C-Reactive Protein High Sensitive Assay [CRPHS], measuring range 0.15 - 20 mg/l) the following results were obtained for 15 parallel plasma samples from 0.46 to 4.64 mg/l: mean values, Konelab 1.84 ±1.51 mg/l, Roche 1.74 mg/l ±1.26, and r = 0.983.

This modified hsCRP method can better be used than the original one for studies the effects of aerobic physical exercise on the influence of the exercise to the welfare of human individuals in diagnosis and treatment of cardiovascular diseases, diabetes and many other diseases at low plasma CRP levels.
Modern molecular biology tests for detection of urogenital tract pathogens

D. Perminovs
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Along with the fast progress of molecular biology, it is possible to develop new and to improve existing clinical methods for the detection of pathogenic organisms. Here we report our laboratory experience with GeneProbe Transcription Mediated Amplification (TMA) and Seegene Tagging Oligonucleotide Cleavage and Extension (TOCE) technology based methods.

TOCE technology is improved Multiplex Real-Time Polymerase Chain Reaction (PCR), which allows to detect up to 20 targets in a single tube in a single sample. The major components of this reaction - DPO primer pairs, pitchers and catchers make PCR reaction very specific. Using this technology we test 7 urogenital pathogens in approximately 300 patient samples per month.

TMA is modified PCR, where RNA molecules are used as a template for amplification. Amplicons in the next step of the reaction are hybridized with single-stranded chemiluminescent DNA probes allowing detection of the target. High quantity of RNA molecules in single cell makes TMA technology very sensitive. Using this technology we test Chlamydia trachomatis and Neisseria gonorrhoeae in approximately 900 patient samples per month.
Screening of beta-thalassemia trait in Estonia

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Introduction. Thalassemia is the most common type of hemoglobinopathy transmitted by heredity. Non-endemic countries such as Estonia, are also involved in thalassemia-related problems because migration from endemic to non-endemic areas is bound to increase. The differentiation between thalassemic and non-thalassemic microcytosis has important clinical implications. In non-endemic countries family physicians need to know how to diagnose thalassemias, how to distinguish them from other causes of a microcytic anemia, and the treatment options for severe forms of thalassemia.

Methods. We analyzed 64 blood samples received in the laboratory for hemoglobin electrophoresis. This study was designed to prospectively evaluate the reliability of RBC parameters, two RBC indices provided by the Sysmex-XE 5000 and the capillary hemoglobin electrophoresis (MiniCap, Sebia) in the differential diagnosis of microcytic anemia and β-thalassemia. Iron status studies, such as serum ferritin, (Cobas-6000-c501) was done.

Results. The main results are summarized as follows:

Patients with a HbA2 level >3.5%, MCV<80fL, MCH<27pg were classified as beta-thalassemia trait. The diagnosis of beta-thalassaemia was confirmed by molecular genetic methods using APEX – Arrayed Primer EXtension (Asper Biotech Ltd.) and Sanger sequencing in 6 cases with an elevated HbA2 level. The following heterozygous HBB gene mutations were found: +20C>T/IVSII-745C>G [c.-31C>T(;)c.316-106C>G] (1); Cd36/37delT c.112delT (3); IVSII-745C>G c.316-106C>G (2).

Conclusions. These results could be used in developing an algorithm that would be helpful in differentiating iron deficiency anemia from beta-thalassemia trait. The hemoglobin electrophoresis should be done on all patients with microcytic anemia who are not iron deficient and do not respond to iron replacement therapy. It should be remembered that a normal value of HbA2 does not rule out beta thalassemia trait, especially if there is coexistent iron deficiency, which can lower HbA2 levels into the normal range. DNA analysis will confirm the nature of any haemoglobin variant.
Biomaterial associated infections – causative agents and mechanisms

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In the last few years, the use of originally synthesised biomaterials in practical medicine has been extensively utilised in Latvia (maxillofacial and reconstructive surgery etc.). Research has been done to determine morphologic and chemical analysis for the utilised biomaterials; clinical results have been evaluated as well as analysis of various microorganism adhesion intensity and the resulting colonisation activity on various biomaterials has been done.

Reactogenicity is characteristic of biomaterials used in implants – they can cause counteraction of microorganisms of various intensity. Counteraction according to qualitative features is always the same – inflammation and restriction of foreign bodies with a conjunctive tissue capsule, if it cannot be degraded or pushed out. The biomaterial also possesses the characteristic to attract microorganisms.

There are two principal paths for the bacterial contamination of biomaterials – direct implant contamination during a surgery, which is the most common contamination form, or the contamination of implants due to haematogenous or lymphogenous bacterial dissemination. The source of bacterial contamination is the microbiota either of skin or of mucous.

Hence, the ability of microorganisms to infect and colonise implanted biomaterials or other appliances is still a common problem and serves as a risk factor in the development of infections at hospitals without regard to the use of aseptic and antiseptic methods.
Point-of-care testing (POCT) in Estonian family medicine centres

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Introduction. Point-of-care testing (POCT) is an expanding area of diagnostics where the testing is carried out by persons without lab-related education and without knowledge from quality of lab-studies. The evidential-based information on using POCT in family medicine in Estonia is incomplete. The aim of this study was to find out which POCT methods and means to ensure quality are used in Estonian family medicine centres. This is the first study of POCT devices in Estonia, providing facts concerning devices, their maintenance and quality assurance.

Materials and methods. The study was conducted as a joint effort by Tartu Health Care College and Estonian Association of Laboratory Medicine. A convenience sampling from family medicine centres was studied with the questionnaire-based survey in the summer 2012. The 173 family medicine centres provided information about 453 devices which are used.

Result. POCT are in use in 95% of family medicine centres. The most widely used POCT devices are different instruments which measure glucose levels (30%) and also urine analysers (19%). The staffs have completed user trainings provided by the sellers regarding 86% of the devices. The family medicine centres possess certificates related to only 14% of the devices. POCT device maintenance is done only on 53% of the instruments, and maintenance on only 13% of the instruments is documented. Internal quality control (IQ) procedure is carried out for 47% of the devices, comparative testing is conducted with 61% of the devices, whereas there are devices on which quality control is never performed.

Discussion. The study enables to initiate the unification of POCT quality and lab quality standards. Family doctors, nurses and also other POCT users have to understand and follow the rules of instrument maintaining and accept quality control necessity as the POCT expands in future. The remarkably low application of IQ is a current disadvantage.

Conclusion. POCT devices are in use nearly in all family medicine centres, the mean number of devices is 2–3 per centre. The maintenance of instruments is insufficient; also the maintenance and usage trainings are often not recorded in documents. The quality control implemented in POC testing is in many ways deficient. The laboratory staff assistance is needed for family doctors centres in the application of quality assurance and trainings.
Application of Immunoblot Method for Detection of Intrathecal Synthesis of Specific Antibodies in Lyme Neuroborreliosis

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Introduction. The analysis of cerebrospinal fluid (CSF) is of major importance for diagnosis of Lyme neuroborreliosis (LNB). According to European diagnostic guidelines (EFNS), investigation of CSF/serum pair for Borrelia specific antibodies, intrathecal antibody production and signs of CSF inflammation is obligatory for the diagnosis of LNB.

The aim of our study was the use of immunoblot method for visualization and confirmation of Borrelia-specific intrathecal antibody production.

Methods. We determined the Antibody Index (AI), as a marker for intrathecal antibody synthesis for 16 patients with neurological symptoms suspected neuroborreliosis and signs of CSF inflammation (lymphocytic pleocytosis, elevated total protein or blood/CSF barrier dysfunction), which had elevated values for Borrelia specific antibodies in paired CSF/serum samples drawn on the same day. AI was calculated, using the values from clinical chemistry and extinctions obtained, according the method described by Reiber.

16 CSF/serum samples were further investigated by Immunoblot method (recomLineBorrelia, Mikrogen), which detects antibodies against main immunopathogenic Borrelia genospecies (Borrelia afzelii, B. garinii, B. burgdorferi s.s., B. spielmani) on one single test.

Results. In 12 CSF/serum pairs was detected pathological elevated AI (from 2.82 till 26.58), in 4 CSF/serum pairs was detected normal/borderline AI values (AI<1.5).

Using immunoblot with identical concentrations of IgG in CSF and serum, 8 patients displayed intrathecal synthesis of specific antibodies to at least two Borrelia burgd. proteins.

In 8 CSF/serum pairs with pathological elevated AI we observed more intensive bands in CSF than in serum and additional positive antigen band in CSF, which is a clear indication for intrathecal antibody production. In 4 cases bands in liquor present, intensity of the bands was equal or weaker to the bands in serum, that means the detected Borrelia specific antibodies have passively passed from serum into CSF space (blood brain barrier failure).

The same results show 4CSF/serum pairs with normal/bordeline AI values.

Conclusion. Immunoblotting allows detection of antibodies to individual recombinant antigens of Borrelia burgdorferi sensu lato, is more specific and in some cases can provide further indication of stage of the infection. Immunoblot method can be useful additional tool for detection of intrathecal synthesis of Borrelia specific antibodies, when AI positive or borderline is, which allows visualize and confirm intrathecal antibody production in case of LNB.
Familial adenomatous polyposis – case report in Estonia

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Familial adenomatous polyposis (FAP) is an autosomal dominant colon cancer predisposition syndrome. FAP is characterized by the development of hundreds to thousands of adenomas in colorectum. Almost all affected patients will develop colorectal cancer (CRC) if left untreated and untreated in the early stage. The syndrome is caused by the mutations in tumor-suppressor gene APC. FAP is rare condition, represents approximately 1% of all CRC cases.

We have identified a family with classical FAP syndrome. The objective was to investigate the molecular characteristics, diagnosis and treatment of FAP.

According to family history of the index patient 36 year old female with severe colorectal polyposis, we surveyed the pedigree and prospectively performed genetic analysis to 17 family members in 2 generations (age 6 – 40 years).

Among all 17 cases, we identified 10 affected persons (CRC or adenomas). Eight patients carry pathogenic mutation APC:c.3184_3187delCAAA; p.Gln1062Valfs*63. One affected person died before testing and one was not available for genetic analysis. All mutation carriers underwent to colonoscopy. Colorectal polyps were found in 7 cases. Prophylactic colectomy was performed to one adult patient. Other patients are monitored regularly by gastroenterologist.

In conclusion, the early clinical manifestation of FAP is nonspecific. Thorough pedigree investigation together with appropriate genetic counselling, molecular screening and colonoscopy for high risk individuals is important to find asymptomatic FAP patients.
External quality assessment of urine strip tests

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Introduction. External quality assessment (EQA) involves distribution of identical samples to the participants and statistical analysis of the results. While the samples simulate real life cases, results from EQA provide an invaluable tool in evaluating laboratory performance. This study describes the design of Labquality’s EQA scheme for urine strip tests and summarizes the results of 2012-2014.

Materials and methods. Urine strip test A scheme involves assessment of 1 lyophilized urine sample for glucose, ketones, leukocytes, nitrite, pH, protein, erythrocytes and relative density. The participants analyze the sample and report back their results on a scale of 0-4 except for pH and density. Information on the method used is provided with the results enabling the grouping into visual tests and laboratory instruments.

In this study, the most frequent result category was regarded as the expected result for the analytes assessed; erythrocytes, glucose and proteins. Results diverging from the expected value by 1 category were considered acceptable. The expected result was determined separately for each manufacturer owing to scale variations between the manufacturers.

Results. During 2012-2014, altogether ~6000 EQA results were obtained for each analyte. About 20% of the results were obtained using visual tests while the rest using various laboratory instruments. To facilitate comparison between these test types, results obtained with a given manufacturer’s visual tests were compared to those obtained with instruments. The comparison was limited to manufacturers whose devices were used by >100 participants.

Overall the performance was good with about 99% of the results being acceptable for the analytes assessed. Visual tests however showed larger deviation from the expectable result (correct results 97.3% vs. 99.4% for visual tests and instruments, respectively).

Conclusions. EQA provides clinical laboratories a means to ensure competence and monitor trends in analytical performance. Results from the Urine strip test A scheme reveal that the performance is in general good. Visual reading tests however appear to show a slightly larger dispersion of the results.
Assessment of kidney graft function from deceased donor by investigation of acid-base composition of the preservation solution effluent

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**Introduction.** Delayed graft function after renal transplantation complicates kidney graft survival. In order to identify new predictors of renal graft dysfunction we investigated acid-base composition of the effluent of HTK preservation solution at different stages of organ retrieval from deceased brain death donors.

**Methods.** We have analysed effluent of HTK solution samples taken from left renal vein at the time of organ retrieval from 36 deceased donors. We compared acid-base balance of effluent taken at the time of kidney retrieval and at the time of the graft preparation on the «back table» surgery with the pure HTK samples. A comparison of acid-base and electrolytes indicators in donors groups was carried out in order to find relationship between the incidences of immediate graft function (1st group-8 recipients) and delayed graft function (2nd group-11 recipients). The investigation of acid-base balance was done on Radiometer ABL800 FLEX.

**Results.** The use of additional kidney transplant perfusion with the HTK preservation solution during the «back-table» surgery leads to significant decrease the levels of Са++, Cl-, Na+, Ph drop, Glu, pCO2, HCO3- and increase the levels of K+, pO2, BASE(B), BASE(E) compared with effluent samples taken during the time of kidney retrieval. Comparing effluent samples between 1st and 2nd groups of recipients revealed trend to differences of K+, Na+, Ca++, Cl- and lac levels, but there were no statistically significant difference (p>0,05)

**Conclusions.** The use of additional renal graft perfusion during kidney preparation for transplantation allows optimize acid-base balance in renal graft. K+, Na+, Ca++, Cl-, lac contain in HTK effluent could be used as predictor of kidney graft function in early postoperative period. Further studies of acid-base and electrolytes balance and it’s reliable target reference indicators during kidney transplant preservation are needed.
Streptococcus pneumoniae isolates serotyping and antimicrobial susceptibility in 2011-2014

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Introduction. Streptococcus pneumoniae (S. pneumoniae) is a serious human pathogen. Several types of vaccine against S. pneumoniae are available: PCV7, PCV13 and PPSV23, so it is very important to detect S. pneumoniae serotypes. With every year more and more resistant S. pneumoniae species are identified and the treatment is becoming more complicated.

Methods and materials. For the detection of S. pneumoniae in blood and cerebrospinal fluid (CSF) the conventional microbiology methods were used and all the typical colonies were taken for further identification. All 96 S. pneumoniae species were tested for antibacterial susceptibility and serotyped with Pneumotest latex test and Quellung reaction. Also Multiplex PCR (Polymerase Chain Reaction) was used.

Results. In period of 2011-2014 years 96 S. pneumoniae species from blood and CSF were identified. 68 (70.8%) S. pneumoniae cultures were found in blood, but in CSF – 28 (29.2%). In 2011 21 strain from 96 were identified. In 8 cases it was the 14th serotype, other were found more rare. Penicillin resistant was one 14th serotype, which also was resistant to Trimethoprim/sulphamethoxazole (SXT). In 2012 it was 18 cases. The 14th serotype was identified in 3 cases, the other were identified more rare. All serotypes were susceptible to Penicillin. In 2013 it was 30 cases. From them 7 cases of the 3rd serotype, 5 - 19F, 4 – the 14th, other were identified more rare. 2 14th serotypes were resistant to Penicillin and STX. In 2014 ( till may) S. pneumoniae strains were identified in 27 cases. It was 6 cases of the 4th serotype, 5 cases of 7F serotype and 4 cases of the 3rd serotype. One 35B serotype was identified. All the serotypes in 2014 were susceptible to penicillin.

Conclusions. The results show that all detected serotypes are included in PPSV23, except one 35B serotype. In our study the 3rd serotype which is not included in PCV7 was detected in 11 (11.5%) cases. Also were detected serotypes which are not included in PCV13 (2, 9N, 10A, 11A, 12F, 15B, 20 and 22F), but are included in PPSV23. The use of molecular methods simplify typing procedure, also increase the number of isolates being serotyped in shorter period of time. In our study 3 (3.1%) S. pneumoniae cultures were resistant to Penicillin. But the resistance to SXT was identified more often – in 12 cases (12.5%). So a beta-lactam antibiotics can be used for the treatment against S. pneumoniae.
A case report of marked prolongation of APTT without bleeding disorders

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**Background.** Prolonged APTT without bleeding disorders found during a routine coagulation studies could be a reason of delayed elective surgery. It is very important for both - patient and doctor to find out the actual reason. Prekallikrein (Fletcher factor) is a coagulation protein involved in the early stages of intrinsic pathway activation. Prekallikrein deficiency can cause a marked prolongation of the APTT, but it does not cause a bleeding. The same statement is also valid for factor XII (Hageman) deficiency. Two clinical cases: 1) 35 years old pregnant woman, during routine coagulation study showed prolonged APTT (>160sec, Ref. interval 26-40 sec) and Normal PT (135%, Ref. interval 70-130%); 2) 75 years old male during routine preoperative coagulation study also showed prolonged APTT (170sec, Ref. interval 26-40 sec) and Normal PT (93%, Ref. interval 70-130%). No clinical signs and no history of bleeding disorders in both cases. Additional testing plan was established.

**Materials and methods.** We used PT reagent (Dade Innovin), APTT Reagent (Pathromtin SL), Fibrinogen reagent (Multifibren U), Coagulation Factor Deficient Plasmas (FVIII, FIX, FX, FXI, FXII), Lupus Anticoagulant reagents (LA 1, Screening reagent and LA 2, Confirmation Reagent) on BCS XP analyzer (all from Siemens Healthcare Diagnostics). Prekallikrein screening was made by performing classical APTT assay (Pathromtin SL) and following APTT assay (Pathromtin SL) with extended incubation time (10 minutes) in order to increase the time for surface activation; Prekallikrein deficient plasma shows a shortening of APTT using extended incubation time. Additionally, mixing studies were performed (patient plasma with normal pooled plasma, 1:1).

**Results.** Case 1: PT 132% (Ref. interval 70-130%), APTT >160sec (Ref. interval 26-40 sec), Mixing study (1:1) 33 sec, Fibrinogen 3.8 g/L (Ref. interval 2-4g/L), Factor VIII 148% (Ref. interval 60-150%), Factor IX 88% (Ref. interval 60-150%), Factor XII <5% (Ref. interval 50-200%), Lupus anticoagulant Ratio 1.1 (NEG). Case 2: PT 93% (Ref. interval 70-130%), APTT 117.5sec (Ref. interval 26-40 sec), Mixing study (1:1) 30.9 sec, Fibrinogen 3.3 g/L (Ref. interval 2-4 g/L), Factor VIII 89% (Ref. interval 60-150%), Factor IX 77% (Ref. interval 60-150%), Factor X 84% (Ref. interval 70-130%), Factor XI 109% (Ref. interval 70-200%), Factor XII 100% (Ref. interval 50-200%), Lupus anticoagulant Ratio 1.2 (NEG), Prekallikrein screening: classical APTT 113.4sec shortened to 56.3sec with 10 min. incubation.

**Conclusions.** Diagnostic algorithm for patients with elevated APTT (patient is not on anticoagulant therapy) and normal PT: 1) Mixing study 1:1 (patient plasma with normal pooled plasma). If APTT corrects 2) Perform Factor VIII, IX, XI assays. If factor results fall within the normal ranges 3) Perform Factor XII assay. If FXII results are in the normal range, 4) Perform Prekallikrein screening test. Prekallikrein may also be performed when during routine analyses prolonged APTT evaluation finds no explanation for the prolongation; the APTT is normal in the mixing study; factors VIII, IX, XI, XII as well as PT and Fibrinogen are in the normal ranges; Lupus anticoagulant results are negative.
Biomaterials with biodegradable polymer and antibiotics – efficiency tests in laboratories, practical use, advantages and disadvantages

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Infections continue to spread in all fields of medicine related to the implantation of biomaterials. The most frequent bacteria are *S.aureus*, coagulase-negative staphylococci and other agents of nosocomial infections. Biomaterial-associated infection is a significant clinical problem caused by the adhesion of bacteria and development of biofilm.

One of the ways to protect patients from biomaterial-associated infections would be the use of local antibiotics immediately after the implantation of biomaterial. Antibiotics are significant components for the treatment and prevention of biomaterial-associated infections. To serve this purpose, various biologically degradable polymeric biomaterials have been investigated with an aim to ensure local release of antibiotics. Controlled-release polymeric implants have a potential to reach high concentration of local antibiotics.

The local application of drug delivery systems in the field of bone infections is therefore beneficial due to such poor accessibility for systemically administered drugs. Antibiotics can be delivered locally, conventionally with use of impregnated cement beads, spacers, or pre-moulded implants or via appropriate drug delivery systems.

The antibacterial activity of functionalized biomaterials with antibiotics and biodegradable polymer can be tested by disk-agar diffusion test (also called Kirby–Bauer method), which is classic laboratory method for testing antimicrobial susceptibility of antibiotics. This method is well documented, and standard zones of inhibition have been determined for susceptible and resistant values.

The antibacterial activity functionalized biomaterials with antibiotics and biodegradable polymer also can be estimated in the experiment similar to in vitro drug release tests. When biomaterial samples are placed in 0.5 MacFarland bacterial suspension. The experiment is performed until the day when bacterial growth appeared in the medium.
Antibacterial efficiency of hydroxyapatite biomaterials with biodegradable polycaprolactone polymer, saturated with gentamicin.

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Introduction. One of the major obstacles against a wider use of biomaterial implants is the capacity of bacteria to attach to biomaterial surfaces, which can cause infections associated with biomaterials. These infections can cause many serious problems, as well as poorly treatable biofilm infections.

Materials and Methods. Group A consisted of hydroxyapatite biomaterials with biodegradable polycaprolactone polymer, saturated with gentamicin. Group B consisted of hydroxyapatite biomaterials saturated with gentamicin. Group C consisted of hydroxyapatite biomaterials saturated with biodegradable polycaprolactone polymer. Antibacterial efficiency of all three group biomaterials were tested using S. epidermidis (ATCC 12228) and Ps. aeruginosa (ATCC 27853) bacteria reference cultures. Studied biomaterials of all groups were incubated at 37°C for 24h 2 ml TSB with investigated bacterial suspension. Suspension consisted of 1 ml TSB and 1ml bacteria suspension with an optic density of 0.5 according to McFarland standard. 2ml TSA bacteria suspension with an optic density of 0.5 according to McFarland standard without biomaterial, which was used as study’s control group.

Results. The average antibacterial length of group A biomaterials against S.epidermidis was 336h±12, whereas the average antibacterial length against Ps. aeruginosa was 288h±12. The average antibacterial length of group B biomaterials was 45h ±15.09 against Ps. aeruginosa bacteria culture, whereas the average antibacterial length of group B biomaterials against S. epidermidis culture was 55h ±15.09. Antibacterial characteristics were not observed on group C biomaterials against any of the bacterial cultures used in the study.

Conclusions. By using this type of biomaterials with antibiotics and polymer, the polymer is degraded slowly and it also ensures the slow secretion of antibiotic substances; however in situations when biomaterials are saturated with antibiotic substances and they are not covered by biodegradable polymer, antibiotics secrete rapidly, thus ensuring protection from infection for a shorter period of time.
Implication of cytogenetic methods in to clinical cytogenetic diagnostics: a case report.

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Introduction. Development of aCGH method and implication of this method into clinical cytogenetics had made huge impact in to detection of chromosome deletions and duplications in patients with various congenital malformations and mental retardation. Although aCGH is great tool in diagnostics, FISH and other methods still has diagnostic value.

Case. Proband, 26 years old woman, has referred to Center for Medical Genetics due to aggravated obstetrical anamnesis: she had a newborn with multiple congenital malformations who has died at age of 1 month and spontaneous abortion at 31th week of gestation. Her mother had aggravated obstetrical anamnesis too.

Materials and methods. Chromosomes slides for G-banding and FISH analysis have been prepared from cultivated lymphocytes, DNA extraction for aCGH analysis has been done with standard phenol-chloroform procedure.

Results. Initial karyotype analysis indicated possible derivative chromosome 5. Subsequent subtelomeric FISH analysis revealed reciprocal translocation between chromosome 3 long arm and chromosome 5 short arm. To identify size of translocated fragments aCGH analysis of proband’s second newborn has been performed which showed 31 Mb terminal duplication of chromosome 3 long arm and 33.7 Mb terminal deletion of chromosome 5 short arm. Final cytogenetic analysis result has been 46,XX,t(3;5)(q26.1;p13.3).

Conclusions. Appropriate genetic diagnosis and counseling to the proband have been presented only according to all cytogenetic findings. Presented case shows need of different molecular cytogenetic methods application in cytogenetic diagnostics.
Correlations of cartilage biomarker C2C with clinical parameters in middle-aged patients with knee osteoarthritis

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Research in the last decade has been focused on protein biomarkers which can reflect already pre-radiographic lesions in osteoarthritic joints. We have investigated cleavage neoepitope of collagen type II neoepitope C2C in urine. Its assay was developed for this purpose.

Aims: to test (i) ability of the the biomarker to differentiate between patients with and without knee cartilage lesion, (ii) correlation between urinary C2C output and clinical status of patients with early knee osteoarthritis

Material and Methods. We investigated 180 knee OA patients (68 male, 112 female) aged 36-62 (mean 50) yrs. Standardised radiographs of the tibiofemoral and patellofemoral joints were assessed. The clinical status of OA patients was established by KOOS questionnaire (self-assessment) and by four performance tests (Up & Go test, raising from low chair, stairs-stepping, 30 m walk). In a subset of 51 patients (25 male, 26 female) the degree of cartilage lesion was assessed by an orthopedic surgeon on the Outerbridge (0-4) scales in the femoral, tibial and patellofemoral compartments (Dept. of Traumatology and Orthopaedics, Tartu University Hospital).

The immunoassay used was C2C-HUSA™ (IBEX, Canada) that measures the C2C neoepitope fragments present in human urine samples, not normally detectable in blood.

Results. The uC2C values were significantly higher for patients with tibial or femoral lesion degree 2 or higher both at baseline and 3 yrs after surgery. U-C2C correlated positively with knee symptoms (pain) as well as with limitations of everyday (ADL) and demanding recreative (Sp/Rec) activities only in female patients. Higher output of uC2C correlates with decline of the functional capabilities of lower limb (described by all 4 tests).

Conclusions
1. Significantly higher excretion of uC2C is associated with grade 2 and above cartilage lesion in knee joint.
2. Highly significant correlation exists between increased uC2C output and knee symptoms, as well as between decline in the clinical functional parameters of the lower limb.
3. Some gender-related differences appeared for the above associations.

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Copy-number variations at 16p11.2 in Estonian patients

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There are many copy-number variations (CNVs) associated with genomic disorders with extreme phenotypic heterogeneity. One of such CNVs is duplication-deletion at 16p11.2. This syndrome seems to be as frequent as microdeletion/duplication 22q11.2. Both deletions and duplications at 16p11.2 have been associated with intellectual disability and language impairment. In case of duplication, underweight and intellectual disability is observed contrary to deletion, where obesity and autistic features are main symptoms. It is shown previously that clinical symptoms in carriers of the deletion/duplication represent opposite manifestation mediated by gene dosage.

From January 2012 until January 2013 we performed 667 CNV analyses (HumanCytoSNP-12 v2-1 BeadChips; Illumina Inc.) and found microdeletion/duplication at 16p11.2 in 10 (1.4%). At the same time we diagnosed in 5 (0.75%) patient with microdeletion/duplication 22q11.2. Here we describe 11 Estonian patients with CNV at 16p11.2. Three cases were with duplication and 8 with deletion in that region. The length of changes varies between 0.2Mb-0.8Mb. All patients show developmental delay and language impairment in different severity. As expected, dysmorphic features are presented in very minor scale. In one family two brothers had inherited the deletion from father. In one case the duplication was inherited from mother. All parents are not clinically investigated yet.

16p11.2 microdeletion/duplication syndrome is surprisingly frequent in Estonian population. It seems to be even more frequent CNVs than 22q11.2 microdeletion/duplication. Such finding can be explained by the fact that since 2012 chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities and/or congenital anomalies.
HLA class I and class II haplotypes in diabetic families from Eastern Croatia

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Type I diabetes (T1D) is closely associated with the human leukocyte antigen (HLA) gene products. In order to examine the extent of haplotypic risk and the patterns of HLA associations with T1D in the population of East Croatia, A, B, DRB1 and DQB1 loci were typed at low resolution using sequence-specific primer PCR methodology. Single and multipoint genetic associations were assessed through transmission disequilibrium testing of multilocus haplotypes in 13 families (55 subjects), estimated according to the expectation-maximization algorithm. Empirical P-values and pairwise linkage disequilibrium measures were obtained. No Mendelian errors were detected. Generally, DQB1*02, DQ8 and DQ7 were commonly inherited in cis with DRB1*03, DRB1*04 and DRB1*11, respectively. The most frequent (10.6%) ancestral haplotype (A1-B8-DR3-DQ2) was not associated with risk of T1D. DQ8 allele and two-locus (permuted P=0.047) DRB1*04-DQB1*DQ8 haplotype (85.66% transmission, p=0.0049, RR=6.0) were preferentially transmitted to affected probands, indicating the robust diabetogenic effect of these alleles. Conversely, DQ7 allele (14.3% transmission, p=0.047, RR=0.17) and B*18-DRB1*11-DQ7 haplotype (no transmissions, p=0.0018) were nominally highly protective, being significantly less transmitted to diabetic children. These findings confirm DRB1*04-DQ8 and DRB1*11-DQ7 as major HLA determinants of T1D susceptibility in Eastern Croatia. In conclusion, sample size enlargement, DQA1 loci typing and high resolution testing are required for in-depth information on hierarchy of risk effects at the DR-DQ haplotype levels.
Review of activities of the Estonian society for laboratory medicine

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The Estonian Society for Laboratory Medicine (ESLM) was founded in 1965 and next year it will celebrate its 50th anniversary. The society has 227 individual members, including laboratory doctors, non-medical specialists, trainees, and 5 corporate members.

The main goal of ESLM is to develop laboratory medicine as one of the medical disciplines, to raise the level of knowledge and education among its members, and to facilitate scientific activity.

ESLM organises 2 to 3 general meetings annually. The meetings are free for the participants and usually attended by 100 to 140 colleagues. We also organise joint meetings with other societies (e.g. in 2013 with anaesthesiologists and nephrologists, in 2014 with doctors and nurses in emergency medicine) and laboratory sessions in clinical conferences (a session about tumour markers in 2013).

The tradition of Summer Schools started in 1996. This is a 3-day event with a mixture of science, education and social activities. Up to now 16 Summer Schools have been organised. ESLM has set up 10 working groups devoted to the following fields: antimicrobial susceptibility testing (EUCAST), Logical Observation Identifiers Names and Codes (LOINC), standardisation of terminology, POCT in GP practices, professional standard for non-medical laboratory specialists, quality in laboratory medicine, myocardial markers, renal markers, and laboratory haematology. In 2014 a new working group was formed in the field of diagnostics of urogenital infections.

Every year ESLM gives two grants to young scientists for presenting a poster at international conference. International cooperation includes participation in BALM, IFCC, EFLM, EC4 Register Commission and ESCMID. ESLM also consults the Ministry of Social Affairs of Estonia in development strategies and issues concerning the specialty of laboratory medicine.
Changes in preanalytical quality indicators after implementation of electronic request

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Objectives. Preanalytical errors make up about 70% of all the mistakes in laboratory testing which can negatively impact on patient care. Electronic requesting in the ward can reduce the frequency of preanalytical errors. The aim of this study was to analyse the impact of electronic requesting on the preanalytical quality indicators seen by the laboratory in the tertiary care hospital.

Methods. In sample reception all test requests and samples for clinical chemistry, complete blood count, coagulation, immunology and body fluid analysis were assessed according to the defined acceptance criteria. In 2012 we implemented electronic requesting from the wards and abandoned paper request forms. Preanalytical quality indicators based on IFCC WG Laboratory Errors and Patient Safety model were registered and analysed during two one-year periods (in 2011 and 2013), before and after implementation of electronic requesting. We compared the frequency and types of preanalytical errors between the two periods, using chi-square test.

Results. In 2011, we found 5,070 mistakes from a total of 516,041 requests / sample materials, i.e. a relative frequency of 0.98%. After implementation of electronic requesting there were 3,002 errors out of 575,245 requests in 2013 (0.52%, p<0.0001). The pre-analytical quality indicators that improved the most were missing sample (relative frequency 0.13% before and 0.01% after implementation of electronic request), missing sampling time on the request form (0.016% and 0.003%), no investigations stated or incorrect request (0.06% and 0.01%), missing request form (0.05% and 0.01%), missing data about nurse who had taken sample (0.03% and 0.01%), and mismatch of patient ID (0.013% and 0.004%, all p<0.0001).

Conclusion. Electronic test requesting from the wards improves pre-analytical quality indicators of test requests.
X-linked adrenoleukodystrophy – a clinically heterogeneous syndrome requiring an early diagnosis. Two familial cases

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Introduction: X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disorder with an estimated birth incidence of 1 in 17,000. It is characterised with a disturbed beta-oxidation of very long chain fatty acids (VLCFA, C22) in peroxysomes. The causal ABCD1 gene is located in the X-chromosome and encodes ALDP protein that is involved in the VLCFA transmembrane transport. X-ALD leads to accumulation of VLCFA in tissues, including white matter of brain, spinal cord and adrenal cortex. The clinical spectrum in males is heterogeneous, ranging from isolated adrenal insufficiency and slowly progressive myelopathy to a severe cerebral demyelination. In females the clinical findings are milder and demyelination is very rare. The individual disease course remains currently unpredictable. Follow-up of affected males is important for the early detection of adrenocortical insufficiency and cerebral X-ALD. With cerebral X-ALD the only effective therapy is early allogenic hematopoietic stem cell transplantation.

Clinical cases. We present two adult brothers with molecularly confirmed X-ALD. Both brothers developed adrenocortical insufficiency in early school age and were treated since with corticosteroids. The younger brother (currently 36-ya) has developed progressive gait disturbances from the age of 30-31-ya, difficulties in bladder control, depression and sleep disturbances from the age of 35-ya. In MRI investigation, pronounced symmetrical white matter lesions were found. The older brother (38-ya) has developed mild spastic paraparesis, but can still work and lives an active life. By MRI analysis, he has no white matter lesions in the brain or spinal chord. Both brothers were found to have elevated VLCFA levels in the blood serum and c.1534G>A (p.Gly512Ser) hemizygous mutation in their ABCD1 gene. The clinical findings of the younger brother are too severe to be considered for hematopoietic stem cell transplantation. A regular follow-up is planned for the older, less affected brother. He is a candidate for hematopoietic stem cell transplantation.

Conclusion. Based on the available literature and our clinical cases we suggest that an early VLCFA analysis should be performed in all male cases with adrenal insufficiency of unknown origin. VLCFA testing sensitivity in X-ALD males is estimated to be > 99%. A positive finding requires molecular confirmation by ABCD1 gene mutation analysis. Due to the frequently severe disease course and a potentially treatable nature, X-ALD is a candidate to be included in the newborn screening programmes.
Case Report: Clinical Expression of an Inherited Unbalanced Translocation in Chromosome 2

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The carriers of balanced reciprocal translocations mostly do not have recognizable phenotypic expression. However, they have increased risks of producing gametes with unbalanced chromosomal rearrangements, leading to miscarriages or children with significant clinical expression.

We report a family comprising of healthy parents and their newborn with facial dysmorphism and feeding difficulties. Conventional GTG-banding (G-bands by trypsin using Giemsa) analysis of somatic chromosomes identified a balanced translocation, 46,XX,t(2;6)(q37.1;q25.1), in mother and an unbalanced rearrangement, 46,XX,der(2)t(2;6)(q37.1;q25.1)mat, in the child. The child has inherited a derivative chromosome 2 with loss of the segment 2q37.1qter and gain of 6q25.1qter. The finding was confirmed by FISH (fluorescent in situ hybridization) and array CGH (comparative genomic hybridization) analysis.

2q37 deletion syndrome is a condition that can affect many parts of the body. This condition is characterized by weak muscle tone (hypotonia) in infancy, mild to severe intellectual disability and developmental delay, behavioral problems, characteristic facial features, and other physical abnormalities. 6q25.1qter duplication is not yet described in the literature and it is still unclear how this aberration contributes to the clinical picture.
Prenatal screening of aneuploidies and possible new schemes

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Today’s medical advances have given us the capacity to identify many diseases before they occur and at times to apply preventative measures, so that morbidity and mortality may be avoided. Large scale of tests in pregnancy exists: gestational risk in diabetes, infection, Rhesus factor and also thyroid dysfunctions. Thyroid dysfunction in pregnancy can cause in the outcome of pregnancy and also the development of the foetus with using TSH, TPOAb and FT4 as screening markers. Prenatal screening of the Down’s syndrome (DS) is the most common testing for aneuploidy.

The management of screening is very similar all over the world. The current DS screening strategies involve the traditional second trimester serum biochemistry (AFP, HCG, uE3 and inhibin A), the combine test in the first trimester (ultrasonography markers - nuchal translucency and serum biochemistry - PAPP-A and free beta HCG), or combinations of results from the both trimesters - integrated test, which has the best efficacy (> 90%); as well as safety and cost efficiency. The first-trimester combined screening is better than second trimester screening, both stepwise sequential screening and fully integrated screening have high rates of detection of Down’s syndrome, with low false positive rates. For a true diagnosis, the chromosomes of the foetus must be examined.

In our group of 7,530, 9-11 week pregnant women, were determined TSH, TPOAb and FT4. In a region with sufficient iodine supplementation, a raised concentration of TSH was found in 5.14% of pregnant women; a suppression of TSH in 2.90%. Family or personal history of thyroid diseases was present in 58.3% women with any thyroid test positivity. Minimally 30-40% of women with TPOAb positivity had thyroid disorders after delivery.

Prenatal screening should be universally offered to all women who desire to know the health status of the child they bear. It is on the basis of a mother’s autonomy that Down’s syndrome screening is offered. Our results support the implementation of general screening for thyroid disorders in pregnant women, but also the close follow-up for a prolonged time period after delivery. New developments include the possibility of testing for DS by extraction of cell-free fetal nucleic acids from a maternal serum. The initial study seems to indicate, this could eliminate the need for more than 90% of invasive diagnostic testing.

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