INVITED SPEAKERS ABSTRACTS

Quality and patient safety in laboratory medicine
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The path leading to quality and patient safety in laboratory medicine is infinite, since it must be ensured that each and every step in the total testing process (TTP) is correctly performed, thus guaranteeing a valuable medical decision making process and effective patient care. Laboratory-associated error has a completely different meaning today than it did a century ago. At that time the term referred to defects in the analytical performance of the test itself, the so-called analytic phase. The new millennium has hailed a formidable improvement in the analytical phase with a 100-fold reduction in error rates, thanks to an improved standardization of analytic techniques and reagents, advances in instrumentation and information technologies, as well as to the availability of more qualified and better trained staff. In addition, this achievement is due to the development and introduction of reliable quality indicators (QIs) and quality specifications for the effective management of analytical procedures by adopting internal quality control programs and attending external quality assurance (EQA/PT) schemes. According to recent evidence, most errors fall outside the analytical phase, in fact, the extra-analytical steps (both pre- and post-analytical) have been found to be more vulnerable to the risk of error. It needs, therefore, to evaluate all the steps in TTP, whether or not they fall under the direct control of laboratory personnel, with the ultimate goal being to improve, first and foremost, quality and safety for patients. Quality indicators (QIs) are fundamental tools for enabling users to quantify the quality of all operational processes by comparing it against a defined criterion. According to the International Standard for medical laboratories accreditation, the laboratory shall establish and periodically review QIs to monitor and evaluate performance throughout critical aspects of pre-, intra-, and post-analytical processes. A consensual agreement on a possible list of QIs has been recently achieved after revising the model of quality indicators (MQI) developed by the Working Group on “Laboratory Errors and Patient Safety” of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in two Consensus Conferences organized in 2013 and 2016. The consensually accepted list of QIs, which takes into consideration both their importance and applicability, should be tested by all potentially interested clinical laboratories to identify further steps in the harmonization project. The data collected in the last few years, have already allowed us to establish tentative performance specification for extra-analytical phases and to demonstrate that error rates may decrease after QIs monitoring and performing appropriate corrective actions.

References

Electronic apps and medical diagnostics data management
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Laboratory medicine is a domain which offers a unique opportunity to analyze objective patient laboratory data and enable ready communication to both healthcare workers as well as patients. In recent years, an increasing number of web-based and mobile applications has been developed to improve access to laboratory test information and test result interpretation. They range from simple apps that provide reference lab value information to complex medical diagnostics data management. As examples, the “eLab” developed by Tru-Solutions Inc. is a comprehensive medical diagnostic center and lab management software that provides a user friendly interface and access control. It is linked with MedX.com to allow flexible patient search and selection and includes an eLab Dashboard on mobile/tablet, allowing patients and labs/hospitals access to lab reports online. The Davis’s Laboratory & Diagnostic Tests medical app provides another useful app with a wide-breadth of tests, as well as guidance on how to counsel and collect tests. The app is available on multiple platforms including the iPhone/ iPad, Android and Blackberry. The “LabGear” is a medical lab reference app providing a pocket tool for medical laboratory test and is integrated with MedCalc with normal lab value reference information for over 200+ lab tests. There are several other medical apps that provide reference lab values including CALIPER, MedRef, Normal Lab Values, and Lab Tests. The CALIPER App has been developed in our laboratory for paediatricians, family physicians, and other healthcare workers worldwide. It is a user friendly and easy tool to assess a child’s laboratory test results using the latest reference value database developed based on a study of thousands of healthy children and adolescents. The CALIPER apps allow pediatricians & family physicians to interpret laboratory test results for over 170 medical laboratory tests in children and adolescents using a comprehensive database of pediatric data.

The statistical principles of laboratory data analysis
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The majority of scientists (app 70%) are often afraid of statistics. Because most scientists do not fully understand statistics, they tend to either overestimate or underestimate. Unless concepts of statistics are thoroughly understood and comprehended, critical evaluation of scientific research will hardly be adequate and efficient.

Statistics, as defined by the American Statistical Association, is “the science of learning from data, and of measuring, controlling and communicating uncertainty”. Briefly, statistics is the science concerned with developing and studying methods for collecting, analyzing, interpreting, and presenting empirical data. In this lecture, we will briefly discuss the statistics from the laboratory specialist’s point of view. Descriptive (table, figure, etc.) and inferential statistics (group comparison, correlation, regression, etc.) are two broad categories in the field of statistics. A third group, especially useful for Laboratory Specialists is “specific statistics”, which consists of methods for validation, verification, reference intervals, biological variation, quality control statistics, etc.

One of the difficulties in understanding statistics is the “p-value”. A lower p-value is generally interpreted as a stronger relationship or differences between two and all variables. Nevertheless, statistical significance means that, it is unlikely that the null hypothesis is correct. To understand the strength of the difference between two groups (control vs. experimental) a researcher needs to calculate the effect size. The concept of “effect size” enables the readers to understand the magnitude of differences found; however, statistical significance examines the probability of an outcome to occur by chance alone.

The words “data”, “information” and “knowledge” are sometimes used interchangeably. It is essential to understand how “knowledge” differs from “data” and “information”, and to understand what “knowledge management” can add to clinical practice.

Data Science refers to the umbrella of techniques by which, one is trying to extract information and insights from data. Data mining (DM) is the process of analyzing unknown patterns of data according

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to different points of view for categorization into valuable information, which is collected in common areas, for example, data warehouses, for efficient analysis, data mining algorithms, facilitation decision making, and other information requirements to cut costs and increase revenue ultimately. DM is also known as data discovery and knowledge discovery.

DM and Big Data (BD) are two different concepts. BD is a term, which refers to a large amount of data whereas DM refers to deep drive into the data to extract the critical knowledge from a small or large amount of data.

Data Mining and Big Data are essential for clinical laboratories. A medium-sized laboratory can generate 3 to 4 million patient test results a year and each one of those results has related data that never make it to the chart. Our goal in analyzing these laboratory and clinical data is to see whether we can uncover ways to improve not only laboratory practice, but clinical practice all together. That is, in addition to ensuring the accuracy, precision, and turnaround times of laboratory results.

There are lots of softwares for laboratory statistics; some of which we will be investigating during this course. These are Ep Evaluator, Analyse-It, MedCalc, XLSTAT, QI Macros, Minitab, SPSS, Stata, S4S, R. All of them, except for “R”, are usually expensive commercial softwares. When analyzed in terms of laboratory statistics, it is observed that Analyse It, Ep Evaluator and sometimes MedCalc are more convenient than others. While it may not be the ideal software for advanced statistics and Data Mining studies, Analyse It appears to be the most user-friendly software for basic laboratory statistics. Being an open-source and free software, that enables considerable flexibility; “R” definitely stands out as an advantageous software among the others. However, it is not as user-friendly, and certainly requires significant experience.

As a result;
· The problem of fear of statistics should be overcome.
· It is important not to confuse statistical significance with clinical significance.
· A clear understanding of various information technology tools (computer, software, LIMS, HIMS) will enable appropriate and efficient analysis.
· Each specialist must be able to make and evaluate basic statistics and laboratory statistics.
· Laboratory scientists should learn to apply statistical tools correctly, interpret the findings correctly and get an idea about the possibilities of analyzing research questions using statistics.
· A single software cannot solve all our problems. Sufficient comprehension of and substantial experience in Microsoft Excel and SPSS (or other general software) is a must. Where possible, data mining should also be carried out.
· Big data and data mining are critically important to us. However, it is important to note that analysis of the Big Data alone will not guarantee better outcomes. Overwhelming the clinicians with unrestrained volumes of data bears the risk of complicating the separation of signal from the noise.

Evaluation the Performance of Autoverification Processes Using Six Sigma Approach

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One of the main objectives of the quality is to minimize the error rates to a negligible level. The literature of laboratory errors goes back to 1950s (1,2). In 1999 the report of Institute of Medicine (US) ‘To Err is Human: Building a Safer Health System’ broke the silence on medical errors, and created awareness in the public and healthcare sector. Later in 1915 we learned that the big picture was worse and medical error is the third leading cause of death in US (3). In total testing process (TTP), the error rates are higher in the phases where human interventions are higher such as pre-pre and post-post analytical phases. To decrease error rates of each phases of TTP, we should decrease human interventions and implement laboratory automation and artificial intelligence.

Autoverification (AV) of test results decrease error rate and increase the efficiency of laboratory and patients’ safety. In addition to verification test results, a well-designed AV system use patient-related clinical information, instrumental messages and flags to help physicians to interpret test results correctly and consequently decrease the error rate in post-post analytical phase.

Six Sigma methodology has been evolved from total quality management and created a revolution in quality management in new millennium. It is not only a statistical tool but also provide problem solving methods by using the approach of define, measure, analyze, improve and control (DMAIC) cycle. In each phase of this approach statistical procedures are used to evaluate the performance of the phase and help us to take the corrective actions. In addition to DMAIC, the performance of a process can be measured objectively using sigma metric (SM). SM denotes the number of standard deviations (SD) of the process fit between the target and upper/lower tolerance limits. 6 sigma represents the world class quality and in this process only 3.4 errors or defects occur per million opportunities (DPMO). SM can be converted to DPMO and inversely DPMO can be converted to SM. This flexibility enabled the application of the Six Sigma Methodology to a broad area such as industry, business, healthcare sector etc.

It has been shown that applying the principles of Six Sigma methodology (DMAIC) to AV systems improved turn-around time and reduced time for manual verification (4). For the ideal AV system, DMAIC principles should be taken into consideration while developing a suitable system compatible with the realities of the laboratory, and an objective criteria such as SM should be used to measure the performance of the system. A detailed data analysis using fishbone diagram and Pareto chart can be used to evaluate the performance of AV systems.

In conclusion, AV systems designed on the principles of Six Sigma methodology will increase the performance of laboratory, decrease error rate and contribute patients’ safety significantly. Additionally, the performance of AV systems should be measured using an objective criteria such as SM.

References

Uncertainty in laboratory medicine

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Medical laboratories should guarantee that their measurement procedures (MPs) results are fit for clinical purposes, and this requirement calls for the long-term monitoring of the quality and reliability of results. Since its inception, the International Standard ISO 15189 for medical laboratory accreditation has called for the calculation of measurement uncertainty (MU) to be included in each MP. Interestingly, because MPs are used to describe the whole measurement process, including the specific analytical procedure, all processes which contribute to uncertainty in the test results should be considered when calculating MU. The international vocabulary of metrology (VIM) has defined MU as a “nonnegative quadratically characteristic of the dispersion of the values that could reasonably be attributed to the measured”. For a given test result, MU thus represents the interval associated with a defined probability in which the true result should lie. In addition, this interval should fall within limits which guarantee fitness for the clinical purpose of the tests in question. Measurement uncertainty goals for defining fitness-for-purpose limits may be based on clinical outcome studies, biological variation, state of the art, recommendations from an expert group or professional opinions. The components which require consideration in calculating MU are systematic error (bias) and random errors. Bias is inversely related to the degree of trueness of a measurement, while random error represents imprecision and is defined as the standard deviation of a series of measurements. I would like to provide some usable practical procedures regarding the MU estimation for a series of MPs, routinely used in medical laboratories. In particular, for imprecision component its estimation appears to be a reliable estimation of MU if the correct interpretation of the lab test result is guaranteed on the basis of its clinical purpose. For the bias component, the development of a practical solution for including bias in MU estimation allowed us to derive a standardized approach that considers the source of the bias reference and whether and how bias can be calculated.

In addition, MU is an important information to improve the appropriate interpretation of laboratory results and reduce the risk of errors. In fact, diagnostic uncertainty may derive from incomplete information in laboratory reports, leading to an increased risk of inappropriate interpretation of laboratory data. Therefore, MU has two intended uses: for laboratory professionals, it gives information about the quality of measurements, providing evidence of the compliance
with analytical performance characteristics; for physicians (and patients) it may help in interpretation of measurement results, especially when values are compared with reference intervals or clinical decision limits, providing objective information.

References

Harmonisation in clinical laboratories and the harmonisation activities of EFLM

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Harmonisation is likely to be an important contributor to ensure high quality laboratory testing, thus potentially improving patient outcome. Efforts for harmonisation must be made in the total testing process, from test requesting to communication of the laboratory test results and its consequences to the patient. In this article, suggestions are given about what level of harmonisation is possible at the various steps of the testing process, who could be responsible for facilitating and monitoring the effects of harmonisation, and what are likely barriers to achieving harmonisation. Harmonisation can be achieved at local, national and international levels, and will be most challenging when it involves more than one profession as in the extra-analytical phases. Key facilitators will be laboratory associations, regulatory bodies and accreditation systems, whereas barriers are likely to be reimbursement systems or economic factors, opinion leaders and manufacturers. A challenge is to try to turn barriers into facilitators. Harmonisation effects can in most settings be monitored by external quality assurance organisations provided that schemes are expanded to cover all relevant steps and phases. We must combine our efforts, both within our profession as well as in cooperation with others, to achieve harmonisation of the total testing process, in the best interests of the patient. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has initiated many harmonisation activities in all phases of the examination process. The EFLM is dealing with both the scientific and the educational aspects of harmonization, with the intention of disseminating best practice in laboratory medicine throughout Europe. Priorities have been given (1) to establish a standard for conducting and assessing biological variation studies and to construct an evidence based EFLM webpage on biological variation data, (2) to harmonize preanalytical procedures in molecular diagnostic and (3) to improve test ordering and interpretation, (4) to produce other common European guidelines for laboratory medicine and play an active part in development of clinical guidelines, (5) to establish a common basis for communicating laboratory results to patients, (6) to harmonize units of measurement throughout Europe, (7) to harmonize preanalytical procedures in molecular diagnostics and (8) to harmonize and optimize test evaluation procedures. The EFLM has launched a new database for biological variation study (www.eflm.eu) and also the 5th version of the European Syllabus to help the education of European Specialists in Laboratory Medicine (EuSPLM).

Adding value in thyroid cancer diagnostic: thyroglobulin and calcitonin measurement in fine needle aspirate washout

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The diagnostic approach to thyroid cancer (TC) is one of the most challenging issues in the oncology of the endocrine system because of its growing incidence, the difficulty in distinguishing benign from malignant non-functional thyroid nodules and in accurately establishing cervical lymph node involvement during preoperative staging, as well as identifying later recurrences. Neoplastic transformation can occur in either the follicular cells of the thyroid, generating differentiated tumors (papillary thyroid carcinomas (PTCs), follicular thyroid carcinomas (FTCs), Hurthle cell carcinomas), or rarely, poorly differentiated and anaplastic thyroid carcinomas, or in the parafollicular cells of the gland, producing medullary thyroid carcinomas (MTCs). Accurate selection for surgery of thyroid nodules at risk for malignancy, as well as avoiding the adverse effects of overdagnosis and overtreatment it is of critical clinical importance. Optimal diagnosis and stratification of TCs needs less-invasive, specific, reliable and clinically relevant biomarkers. Fine-needle aspiration biopsy (FNAB) of thyroid nodules or lymph nodes is a useful and safe tool and it is considered the gold-standard method in TC diagnosis and monitoring. Despite its high accuracy, in 10-30% of the cases the cytology is inconclusive. Measurement of biochemical tumor markers (thyroglobulin (Tg), calcitonin (Ct), recently CYFRA 21-1) in washout fluids from FNAB of lymph nodes or thyroid nodules is recommended as an ancillary tool for the management of TC patients. In differentiated thyroid cancer (DTC) Tg measurement in FNAB washout (FNAB-Tg) of a suspect lymph node may increase the diagnostic sensitivity and specificity particularly in those cases in which the lymph nodes are cystic, cytological evaluation of the lymph node is indeterminate, or the cytological and sonographic evaluations are divergent (i.e., normal cytological biopsy of a large lymph node with microlcifications). The diagnostic performance of Tg-FNAB compares favorably with cytology, having superior results in athyreotic patients. For the diagnosis of MTC the recommendations of the American Thyroid Association (2016) is that FNAB inconclusive results should be followed-up by Calcitonin (Ct) measurement in the FNAB washout fluid (FNAB-Ct), in addition to IHC staining of the FNAB sample for several tumor markers (Ct, chromogranin, CEA). However, the lack of standardization of FNAB-biomarkers measurements (patient selection, technique of sampling, standardization of the analytical methods - e.g. washout matrix, samples processing and storage, assays, antibodies and/or biotin interference, cut-off values) rises potential difficulties in interpreting data and have an important impact on clinical decision. We evaluated the analytical performance of FNAB-Tg and FNAB-Ct immunoassays in our laboratory. For both determinations the washout was performed by rinsing the needle with 1 ml saline solution 0.9% immediately after the biopsy’s cellular component was expelled for the cytological examination. No matrix interference was demonstrated with saline solution either for Tg, or for Ct (LOD < 0.04 ng/ml <0.5 pg/ml, LOD = 0.046 ng/ml/0.55 pg/ml, respectively), when measured with an immunonelectrochemiluminiscenc method. Validation parameters (accuracy, precision, reproducibility, recovery, dilution linearity) fulfilled the acceptance criteria. Besides analytical validation, studies for the clinical validation are ongoing, in the attempt to identify the best cut-offs for FNAB-Tg and FNAB-Ct. Our experience so far confirms that a reflex strategy would be most cost-effective: negative or non-diagnostic or indeterminate cytology cases should be reflexed to FNAB-Tg/Ct, while positive cytology cases do not need measurement of tumor markers in FNAB. The management of TC patient may be improved by a genomic approach, various diagnostic and prognostic molecular markers (BRAF, PAX8/PPRG, RAS, TP53, TERT promoter, mutations and RET/PTC rearrangements) being available; the benefit of the extent of their analysis in FNAB samples should be further evaluated. A generally accepted standardization of tumor markers measurement in FNAB is required and the results should be integrated in the context of the full clinical, imagistic and histological picture.

Key words: Thyroid cancer, FNAB, thyroglobulin, calcitonin
Mass spectrometry achieving prominence in clinical medicine

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There is an extraordinary flow of new technologies in medicine nowadays - sophisticated diagnostics based on mass spectrometry, genome assays and cell sorting platforms are driving the technological transfer and promote the entrance of individualized patient management in clinical practice. Mass spectrometry (MS) could be viewed as one of the major tools that achieve prominence in clinical medicine. GC-MS was the starting of MS for biochemical research and clinical analysis, and still remains a working horse for clinical toxicology. LC-MS/MS systems are also employed to resolve challenging analytical demands. MALDI-TOF platforms are routine instruments in medical microbiology laboratories from over 10 years now, which revolutionize diagnostics of infectious diseases, achieving ultimate speed and accuracy. Orbitrap and tandem TOF MS systems transfer proteomic and peptidomic research into clinical diagnostics with unprecedented incite and data to understand deepest pathobiocarbon mechanisms of many illnesses. The great technological advance of LC-MS/MS resulted in the introduction of methods with extreme sensitivity, specificity and extended linearity range, which are simpler to use in the medical laboratories, and are based on the current reference analytical principles. Further, the ability to perform panel profiling with simultaneous measurement of bioactive compounds, their precursors and metabolites in a single sample, enormously amplifies the informative value of results, with significant improvement of patient care. Typical examples include now born screening, TDM, toxicology, endocrinology and others. There is an ultimate demand for clear differentiation of the discovery stages, selection and validation of newer biomarkers, as well as analytical method development and validation of MS techniques that are standardized to meet criteria for clinical use with post validation routine proficiency testing assessment: CLSI has issued guidance for validation and performance characteristics of LC-MS/MS methods for clinical use, which is much more stringent, compared to industrial requirements. Currently, MS is the preferred technique in central laboratories, where the expertise and the larger sample workload provide cost-effectiveness and reliability in applications. Clinical MS will flourish in the near future, with the introduction of certified commercial LC-MS assay kits, and automated analytical platforms closely resembling routine clinical chemistry analyzers. In addition, clinical MS will meet and get together chemical and anatomical pathology. MS imaging and MS-based guidance in surgery, although still in research phase, open new horizons for personalized treatment and individualized patient care, with ultimate impact on precision medicine. Precision medicine (also referred to as personalized medicine), employs patient's genotype and phenotype information to establish individually tailored drug treatment. While genetic testing allows the physician to choose appropriate medicine, the pathologist of MS assays provides the patient's actual phenotype, with all of the environmental, pharmacological and pathological variables. Therefore, MS is essentially important technology for personalized patient management.

Significance of systemic inflammatory markers in patients with systemic diseases

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Systemic diseases are generally an interdisciplinary challenge in clinical practice. Systemic diseases are able to induce tissue damage in different organs with ongoing duration of the illness. The heart and the circulation are important targets in systemic diseases. A wide variety of systemic diseases may affect the heart by a number of different mechanisms, including increasing demands on the heart, causing arrhythmias, affecting the structure of the heart or promoting cardiovascular disease and therefore coronary heart disease.

Coronary heart disease (CAD) also known as atherosclerotic heart disease, coronary heart disease or ischemic heart disease (IHD), has been defined as a progressive disease process that causes focal thickening of large- to medium-sized muscular and large elastic arteries. Atherosclerotic vascular diseases are the number one cause of death globally, accounting for 30% of all deaths worldwide.

New scientific evidence from the last two decades including epidemiological, in vivo and in vitro assays support the notion that the immune system significantly contributes in the development and progression of atherosclerosis.

This new theory proposes that any potential noxious challenge to the host immune response could be related to the pathogenesis of atherosclerosis.

Traditional risk factors for atherosclerosis and consequent CAD, such as hypertension, hypercholesterolemia, diabetes mellitus, marked obesity, smoking and physical inactivity, do not account for fully half of all cases of atherosclerosis.

Inflammation and the systemic immune response are believed to play a central role in the initiation and progression of atherosclerosis.

Inflammatory response and cytokine elaboration are integral components of the host response to the tissue injury and an active role after myocardial infarction.

Elevated values of circulating inflammatory markers such as CRP, serum amyloid A, IL-6, and IL-1 receptor antagonist commonly accompany CAD. Such elevations correlate with in-hospital and short-term adverse prognosis and may reflect not only a high prevalence of myocardial necrosis, ischemia-reperfusion damage, or severe coronary atherosclerosis but also a primary inflammatory instigator of coronary instability.

The acute-phase response is a non-specific process that may occur in the initial host response to injuries, infections, ischemic necrosis or malignancy. It is initiated by the activation of local macrophages and other cells leading the release of mediators such as TNF-alpha, interleukin-6 and interleukin-1 beta. These in turn cause systemic changes including hepatic release of a range of plasma proteins, including CRP, activation of complement proteins and various of metabolic changes. IL-6 also promotes induction of fibrinogen, haptoglobin, a1-antitrypsin and a 2 macroglobulin among others.

The purpose of our study was to assess the serum levels of high-sensitivity C reactive protein (hs-CRP), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) between patients with and without coronary heart disease.

These results demonstrated that inflammatory markers are significantly higher in patients with coronary heart disease compared with healthy group, especially for hs-CRP.

CRP is the best studied of the inflammatory biomarkers in CAD. CRP is not only a powerful inflammatory marker, but increasing evidence suggests that CRP may also directly participate in the inflammatory process of atherogenesis.

Keywords: inflammatory markers, systemic diseases, CAD

Low grade inflammatory response to obese and non-obese subjects, facts and promises

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While researched the inflammatory response induced by diet, we have come across two terminologies that describe it. Generally they are the two most commonly used as, chronic inflammation and Low-grade inflammation response, but the latter is more appropriate for conception and preferred. It is generally known that there is a greater presence of inflammatory elements in obese individuals, but how could be readouts in term of a ‘low grade of inflammation’ compared to non-obese subjects, we tried to find out in this study review.

Many inflammatory mediators are released by adipose tissue, more expressed at obese subjects. Many of inflammatory markers are present at obese people in higher concentrations of lean people do. Infiltrations of macrophages in fatty tissue of the obese individuals seem to be a clear relation between obesity and pro-inflammatory tendency. Therefore, it is believed that many of these mediators of inflammation are the trigger of many metabolic diseases, which begin as reactions at the cellular level and until the onset of metabolic syndrome where its insulin-resistance and the appearance of diabetes are at its center.

Likewise, hours following the consumption of a meal, there is an elevation in the concentrations of inflammatory mediators in the bloodstream which is exaggerated in obese subjects and in type 2 diabetes although this is quite
Assessment of Vitamin D status deficiency in Albanian pregnant women

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There are many evidences suggesting that vitamin D deficiency is related with mother problems during pregnancy such as pre-eclampsia, gestational diabetes mellitus, metabolic disorders, increased risk for caesarean section and also with fetal complications such as impaired fetal growth, lower bone mineral density, respiratory infections, small size for gestational age, etc. Serum levels of 25-hydroxyvitamin D (25-OH-D) were evaluated in 185 Albanian healthy pregnant women aged 18–47 years old, which are presented between July to December 2018. A general information form was completed for each pregnant woman included in the study. In this form, for every pregnant woman, collected general demographic data (self-reported) regarding age (in years), weeks of pregnancy, place of residence, number of pregnancy, education, use of multivitamins and/or vitamin D, smoking, alcohol etc. All participants with a history of chronic diseases were excluded from the study. The gestational age of the participants was a 3-41 week. 25-OH-D levels were evaluated on a blood sample obtained by venepuncture in a plain tube. Serum level of 25-OH-D was measured using the CMIA method in Abbott Architect i2000 platform. We used the Endocrine Society recommendation cut-off of 25-OH-D to define vitamin D status: <20 ng/mL deficiency; 20-30 ng/mL insufficiency; 31-50 ng/mL adequate Vitamin D status. Of 185 Albanian pregnant women participating in our study we found that: 9 (4.9%) participants result with vitamin D severe deficiency <10 ng/mL as cut off). High percentage (74%) of pregnant women had vitamin D levels ≤30 ng / mL (75nmol / L) and only 26% had normal levels >30ng/mL (75nmol / L). It is important to note that the factors affecting vitamin D levels in our study are: Season: the prevalence of vitamin D deficiency is higher in winter (100%) and decreases towards summer (62%); Age: with age increases, the prevalence of vitamin D deficiency decreases; Gestational age: the prevalence of vitamin D deficiency is lower in the third trimester of pregnancy; Vitamin D levels increase with increasing intake of multivitamin and/or vitamin D supplements. While the least important factors resulted, engagement at work, education level, number of pregnancies.

Vitamin D deficiency in Albanian pregnant women is in significant percentage, up to 40.5% are vitamin D deficient and 74% had vitamin D levels ≤30 ng/mL. It is necessary to elaborate a national screening and treatment strategy to detect vitamin D status, especially in high-risk groups such as pregnant women.

Anti Müllerian Hormone: New roles for an established biomarker of ovarian reserve

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Anti Müllerian Hormone (AMH) is a homodimeric glycoprotein that belongs to transforming growth factor b (TGF-b) superfamily. In females, AMH is secreted by primary, secondary, pro-annul and small antral follicles (< 7 mm). Since its serum concentration is strongly correlated with the ultrasound marker antral follicle count (AFC), AMH represents a reliable biomarker of ovarian function, having also the advantage of low variation within and between cycles. In our days, AMH plays an increasing role in the forecasting of reproductive lifespan, the prediction of menopause onset, ovarian response to stimulation in ART techniques, iatrogenic amenorrhea due to ovarian surgery or gonadotoxic cancer treatment, and has also proposed as a marker of Polycystic Ovary Syndrome (PCOS).

In serum, AMH is found in different forms: an inactive non-cleaved form known as pro-AMH and a cleaved, biologically active form AMH composed by N- and C-terminal fragments. Both Pro-AMH and AMH are detected by immunometric assays. Until recently, enzyme-immunoassays (mainly Beckman Gen II, EIA/AMH Immunotech, and Anshlab assays: Ultrasensitive (AI-105) and Pico-AMH) were used for the determination of AMH concentrations. Since 2014, automated techniques have been developed (Roche Elecsys AMH and Beckman Coulter Access AMH) and have improved the sensitivity and reproducibility of AMH measurements showing 15% to 20% lower values compared to manual assays.

In normo-ovulatory women, a peak of AMH secretion is observed between 20–25 years of age with AMH values decline thereafter until menopause. It is estimated that 34% of total AMH variation is due to age. A recent study suggested median age-specific values of AMH for normo-ovulating women with Elecsys assays: 4/3.13/2.81/2.0882 and 0.071 ng/mL for age ranges respectively: 20–24/25–29/30–34/35–39/40–44 and 45–50 years. Similar median AMH values were also found in our study with Roche Cobas e411: 6.73/9.23/6.80/4.04/1.11 for the same age ranges.

The estimated age of menopause is important for women seeking fertility individualized counselling, or oocyte preservation. So far, no marker enough reliable exists to assess the onset of menopause. AMH may be a more effective marker than FSH, menstrual irregularities, or maternal age alone. AMH levels decrease from 5.6% per year, and become undetectable during the 3–5 years before menopause onset. A meta-analysis showed that AMH associated to age was more effective in the prediction of early menopause than age alone. However, a specific AMH threshold for menopause is still under debate.

Treatments such as chemotherapy (CT), radiotherapy, ovarian surgery are known to have detrimental effects on female fertility. Recent studies have suggested that AMH could be used to predict ovarian follicle loss for CT patients. In a large prospective study, mean basal AMH levels were 4.19 ng/mL and 4 months after CT completion, AMH levels were of 0.78 ng/mL. Moreover, a prognostic score was created to estimate the time to recovery of ovarian function following chemotherapy was developed based on age, AMH and BMI.

It remains unclear whether low AMH levels are predictive of lower spontaneous fertility. A prospective study conducted on patients aged from 30 to 44 years old found lower fertility rates in patients with AMH levels under 0.7 ng/mL.
Conversely, by measuring biomarkers of ovarian reserve (AMH, FSH and Inhibin B), another study showed that women with low AMH levels (<0.7 ng/mL) did not have a significantly different predicted probability of conceiving compared to other women, after 6 or 12 cycles. AMH appears to be a weak independent predictor of qualitative outcomes of assisted reproductive technology (ART) such as implantation, pregnancy, and live birth. Meta-analysis has shown that the predictive accuracy of AMH on live birth in women undergoing IVF was poor. Although different AMH values (from 0.4 to 2 ng/mL) have been proposed, no clear AMH threshold exists to conclude on a low, normal or increased ovarian reserve, nor on the chances of a future pregnancy.

Since AMH is associated with AFC, may be the best predictive marker of hyper or hypo-response to ovarian stimulation. Since 2013, dosing AMH before IVF is recommended by ESHRE (European Society of Human Reproduction and Embryology) and NICE (National Institute of Excellence for Health and Care) to individualize strategies for ovarian stimulation.

Concerning 5 to 10% of women, PCOS is the most common cause of chronic anovulation and hyperandrogenism in young women. Since a solid correlation exists between AMH and AFC, AMH may play a role in the diagnosis of PCOS. Its use is yet not recognized in clinical practice. In vitro, AMH production by granulosa cells was found to be 4-fold higher in normo ovulatory PCOS and 75-fold higher in anovulatory PCOS compared to normal ovaries, suggesting that AMH in PCOS women is not only explained by the increase of pre-antral and small antral follicles. However, no AMH threshold exists to define PCOS. Despite this absence, American Association of Clinical Endocrinologists proposed that AMH might be an interesting alternative, while the new ESHRE guidelines do not recommend the use of serum AMH levels as an alternative for the detection of PCOS, nor as a single test for the diagnosis of PCOS.


Evidence of HbC disease in Albania - Clinical heterogeneity related to combination with other hemoglobin disorders

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Albania is one of the Mediterranean countries where inherited hemoglobin disorders (thalassemia and hemoglobinopathies) are considerably widespread and constitute a major concern for public health even today. Screening studies have noted a high frequency of β-thalassemia carriers in the western lowland areas. Besides the β-thalassemia, all screening studies conducted on the Albanian population have found a high presence of another hemoglobin disorder, hemoglobin S (sickle cell disease), in various areas of the country. The frequency of HbS has been found to be particularly high (up to 12%) in the central areas of the western lowland. Studies have also identified carriers of Hb O-Arab, Hb Lepore, double heterozygotes HbS/β-thalasaemia and some carriers of alpha thalasaemia.

In this presentation we report our data about the presence of haemoglobin C variant in Albanian population and we describe some of the distinctive clinical features of the disease related to the combination with other haemoglobin disorders.

A retrospective study was conducted. Data were collected from the results of the anemia screening and diagnosis unit of the Laboratory Department, University Hospital Center “Mother Teresa” between 2006 and 2018. Clinical data relating to geographical origin, place of birth, age, disease onset, comorbidity, and past and ongoing treatments were collected.

Laboratory tests were performed as part of a routine diagnostic evaluation. CBC (complete blood count) and biochemical parameters were determined by automated routine procedures. Hemoglobin electrophoresis was performed in alkaline and acid agarose gel using Hyrys Hydrasys SEBIA system.

From 2006 to 2018 we have identified 15 cases with presence of HbC. 80% of our patients were women and 20% were men. Only 1 patient was in pediatric age. The median age was 33 years (range 10-52). 14 patients were Albanian from central and south areas of western lowland. 1 patient was from Nigeria.

53% of our patients (8 cases) result with HbC trait. We have found 5 cases with Hb SC disease, 1 case with Hb C homozygote and 1 case with HbC/β+ thalassaemia. Clinical picture for our HbC trait patients was nonspecific anemia. In this group, general hematological findings didn’t reveal any important or evident change. Painful crisis, acute chest syndrome, cholelithiasis with icterus, pain and fever were the main clinical features in our Hb SC disease cases. Our patient with HbC/β+ thalassaemia was followed-up for several years in the Hematology Department of our University Hospital for anemia symptoms with splenomegaly, abdominal pain crises and recurrent weakness.

The presence of HbC is a rare event in Europe and Mediterranean region where thalassemia and HbS are more frequently encountered. The rarely diagnosed cases are linked with the migration of people from West-Central Africa and their movements in the trade routes that connected these areas with Europe in centuries. The subjects found in our population do not refer any descendent indicative for mutation migration. An additional reason for HbC presence in Albania might also be the past presence of malaria. Until the mid-twentieth century, malaria has been the principal medical and social cause influencing the reduction of the number of the Albanian population. This disease was endemic in western lowlands, which was the origin of the above mentioned patients.

The clinical presentation, as also confirmed in our suspected and diagnosed cases at an adult age, is discrete and unclear. HbC carriers might never be diagnosed because they are asymptomatic. The most serious clinical presentation belongs to HbC/HbS forms where sickling phenomenon might lead to pulmonary complications, cholelythiasis, retinal phenomena, osteonecrosis, etc. From morbidity and mortality point of view, HbC presence, particularly when combined with HbS or thalassaemia, is problematic during gestation, especially in the perinatal period.

The correct diagnosis of HbC presence can’t be confirmed with standard methods used for the screening of thalassemia and sickle cell disease in our country. In literature is emphasized that hematological changes might be absent or to a degree that is not an indication for diagnosis. The changes in the peripheral blood smear, although characteristic, do not confirm the diagnosis because they can also be encountered in other forms of hemoglobinopathy. The common electrophoresis in alkaline pH gives information only about the presence of a band that migrates in Aα position which can be HbC, HbO-Arab or HbE. Electrophoresis at acidic pH confirms the diagnosis because not only identifies HbC but gives exact data on the relative percentage of HbC and the other fractions of the patient. In cases where it is suspected the combination of HbC with alpha or beta-thalassaemia, the diagnosis confirmation can be achieved only by molecular biology methods. Chromatography is also a method of choice to correctly diagnose hemoglobinopathies including HbC presence, due to the short time of examination and comparable cost regarding to other methods.

HbC seems to be a hemoglobin variant widespread in areas reported as endemic of hemoglobinopathies in Albania. Due to the morbidity and complications it may manifest in patients, it is necessary the application of neonatal screening programs for HbC and HbS, at least for the subjects whose origin is from the areas with high prevalence of thalassemia and hemoglobinopathies.
Analytical performance goals

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Quality laboratory results are one of the factors involved in patient safety. Discussing performance specifications has a long history of more than 70 years in the laboratory medicine because it is long realized that it is impossible, and rather non-productive, to discuss quality in laboratory medicine unless analytical quality specifications (goal, analytical goals, or analytical performance goals) are set a priori.

Analytical performance specifications are required for many purposes, including: 1) to assist laboratories in choosing and evaluating new assay methods; 2) to assist the organizers of EQA/PT schemes; 3) to help the manufacturers of instruments and reagents, in design, construction and marketing; and 4) to encourage laboratories to decide which particular examinations require improvement.

First universal initiative to harmonize goal setting was reflected in the 1999 Stockholm Consensus statement in which a 5-level hierarchy was proposed. In 2014, in the Milan Congress, the Stockholm hierarchy was reduced to three models based on: 1) clinical outcome; 2) biological variation; and 3) state of the art.

Depending on how quality of performance is defined, analytical performance specifications can be presented as separate goals for bias and imprecision or as combined goals in the form of allowable total error. Total error model has the advantages of: 1) compatibility with Six Sigma concept, and 2) usability in internal and external quality control. Therefore, Even if separate goals are preferred, when it comes to QC planning and Six Sigma, allowable total errors is needed.

Quality management: illuminating the path to ISO 15189 accreditation - A view from the Republic of North Macedonia

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In the Republic of North Macedonia the work of the diagnostic medical laboratories is regulated by the Law of Health Care. There is an urgent need for better development of an evidence-based, scientific, and sustainable national strategy for the improvement of health laboratory service. Clear indicators of improvement must be established. A key indicator should be the number of laboratories that have achieved, and can maintain accreditation.

The Macedonian Society of Medical Biochemistry and Laboratory Medicine (MMSMBLM) recommends that the quality system established meet the requirements of the International Standard for medical laboratories (Medical laboratories: Requirements for quality and competence [EN ISO 15189:2012]), which has been accepted as the fundamental standard for the accreditation of medical laboratories in European countries. EN ISO 15189 was developed as a baseline standard for the Quality Management System (QMS) in medical laboratories and is recognised as the connecting standard for all disciplines in laboratory medicine. With the acceptance of the ISO standard, the need of countries for their own QMS for laboratory medicine no longer existed.

In 2013, the Standardisation Institute of the Republic of North Macedonia accepted the standard as the Macedonian norm for quality assessment of medical laboratories (MKS EN ISO 15189:2013). MMSMBLM, as the professional society of specialists in medical biochemistry, is responsible for the translation of international guidelines into national guidelines. These guidelines have to be in agreement with the standard EN ISO 15189. For that purpose, cooperation between MMSMBLM and the National Accreditation Body (Institute for accreditation of the Republic of North Macedonia), as well as cooperation between international medical laboratory organisations, such as International Federation of Clinical Chemistry (IFCC), European Federation of Laboratory Medicine (EFLM) and international accreditation bodies, such as International Laboratory Accreditation Cooperation (ILAC) is essential.

The accreditation of Macedonian medical laboratories is not mandatory; the decision for accreditation is voluntary. Accreditation is accessible to every client submitting an accreditation application to the Institute of Accreditation, which has been a member of ILAC since 2008. In 2013, the first medical biochemistry laboratory was accredited in the country. So far, nine medical laboratories have been accredited according the MKS EN ISO 15189:2013. Four of them are public sector laboratories. Flexible scope is not yet started for the ISO 15189 accreditation process in North Macedonia. The medicalized steps, including test's selection advice and interpretation of results are not included in accreditation process.

Diagnostic laboratories of the Institute of pathology, Medical Faculty-Skopje and Research Center for Genetic Engineering and Biotechnology “Georgi D. Efremov (Macedonian Academy of Sciences and Arts) are also using ISO 17025 as additional standard.

The low number of accredited laboratories could be the result of the shortage of financial resources, poor government attention to laboratory service, the shortage of qualified personnel and/or the lack of a national laboratory policy. The experiences of laboratory professionals from accredited laboratories, who have a high level of knowledge, skills, and competence, are crucially important to the process of developing a competent laboratory service within the national health system.

The implementation of the Laboratory Quality Management system (LQMS) requires support of laboratories by the MMSMBLM and close collaboration between specialists in laboratory medicine (medical biochemistry), technical assessors, and consultants. Each of them will give a different perspective on what should be prioritised. Implementation of a QMS should be a stepwise process but it is necessary to start with changes that can be easily accomplished and have the biggest impact. All quality essentials must be addressed. Appropriate laboratory facilities, infrastructure, and equipment for each laboratory tier level are essential to enable safely and efficient performance. Strong programs supporting quality assurance, quality control, and quality improvement should exist. They are fundamental for the establishment, maintenance and improvement of laboratory quality systems. SOPs must be well-written, understood, and implemented; laboratory personnel should routinely perform IQCs; and laboratories must be required to participate in EQA or proficiency testing (PT) programmes.

Future directions: The globalisation of markets and migration of health professionals requires improving the laboratory diagnostic process. A quality laboratory system is the foundation of a strong national health system. Laboratory workforce, infrastructure, and quality management system are vital for the delivery of quality laboratory services. Coordination with the Ministries of Education and Health is essential for maintaining standards of education and levels of knowledge. The competency of laboratory professionals has to be maintained through mandatory participation in continuous medical education (CME). For Government, Ministry of Health, professional association(s) and stakeholders, accreditation of medical laboratories according to ISO 15189:2012 should be a high priority. They should act together and undertake coordinated efforts to integrate accreditation programs into national health policy, planning, and health development programmes.

Key words: accreditation, ISO 15189, Quality Management System

Quality Control in Research Laboratories: Perspectives on Standardization

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Basic or applied research is based on scientific assessments aimed at unraveling new facts using new inventions and/or innovations of techniques. The quality control (QC) in the routine analysis in clinical laboratories is well established. For example, staff training and ongoing competency, maintenance of equipment, written document control, and method validation/verification are some of the important requirements in clinical laboratories. However, in research laboratories the culture regarding quality is immature although the resulting data is substantial. There are limited specific standards for research laboratories (1) and implementing the existing standards is difficult due to the peculiar characteristics of research laboratories. In a typical research laboratory, quality management systems are most commonly not a priority, the professionals’ performances are measured on the publications and teaching activities and most of the staff in research laboratories are temporary (graduate students and visiting scientists). In addition, the costs of quality assurance (QA) might cause a significant loss of research time. All the mentioned issues and many other peculiar features of research laboratories impede the execution of potential quality management systems at research laboratories.

Despite the problems stated above, there is extensive interest in setting up a concept
for QC in research laboratories since it has become increasingly substantial that the researchers conduct experiments at the highest standards. In the late 1990s, it became recognized that researchers were in need of practical guidance about the best way to implement existing QA applications to non-routine analytical work. Therefore, a guide was produced by a EUROCHEM working party in order to promote QA applications in research and development and non-routine analysis (2). According to the guide, basic measurements are conducted in accordance with the Valid Analytical Measurement (VAM) (3) and supported by technical and operational quality elements. The EUROCHEM’s guide advises controls at organizational, technical, and analytical levels (2). The research laboratories that implemented QA applications based on EUROCHEM’s guide, had indicated some critical factors for achieving success in research laboratories. For example, it was suggested that the QA documentation system should be simple, QA system should add value to the organization, and be self-sustainable in order to keep the maintenance of the QA system due to the presence of temporary staff (graduate students and visiting scientists) (1).

As expected, every research process has its own characteristics based on the targeted objectives and experimental design. Still, the quality in the research process can be divided into three common features that represent key quality aspects; namely the quality of the objective, quality of the research approach to reach that specific objective, and the quality of the results (4). For example, the quality of the objective can be judged by a funding agency according to the research proposal in view of the aims, scientific interest, and approaches. Similarly, the quality of the results can be evaluated in panels or assessed in refereed high-impact journals. Besides, the assessment of the research approach quality is dependent on scientific and technical competence, and the presence of a quality system. Importantly, the implementation of QA systems requires a certain degree of flexibility, in which the limits determined by standards, in order to reach success in research laboratories. The need for flexibility arises from the inherent nature of research since observations and approaches in research processes cannot be defined and predicted precisely. In order to assure effective QC in research laboratories, it is also substantial to embrace pre-analytical, analytical, and post-analytical phases just like routine measurements in clinical laboratories. In typical basic research practice, the storage conditions, the sampling time of the biological material, and sample preparation methodologies are major pre-analytical aspects. The analytical phase consists of the analytical process itself and other related approaches to obtain an analytical result. In an analytical perspective, the validation of the standard operating procedures (SOPs) and protocols represents an important issue in research laboratories in order to obtain repeatable results. Using a standardized workflow including pre-analytical, analytical, and post-analytical aspects will increase the reliability of the data produced in research laboratories. Research laboratories have intrinsic quality criteria, namely reliability of data, reproducibility of methods, and monitorability of the research process (5), and mainly act upon it. However, what is new and necessary in research laboratories is to construct structured and pre-planned QA systems. Therefore, there is a need for development of specific QA systems for research consisting of national and international standards (2). It should be further noted that the acceptance and commitment to the potential QA systems are the initial steps for success. These systems need to be embedded in an organization’s culture and therefore; it should be taught early at the undergraduate and postgraduate levels in universities.

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Bias in clinical chemistry
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“Error” of a single measurement result consists of random and systematic components. The “error” may be determined by comparison with the result of a reference measurement procedure or by participating in proficiency testing, but neither the systematic nor random error can be elucidated as such from a single measurement result. The average of repeated measurements is needed for estimating both biases.

A qualitative concept measurement trueness is the “closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value”. It is quantitatively expressed as bias. Another qualitative concept measurement accuracy describes the “closeness of agreement between a measured quantity value and a true quantity value of a measurand. It includes all causes of systematic and random error components. A more accurate result has a smaller measurement error. It is on the average more true when the bias is small and more precise when the random error is small. Precision is expressed quantitatively as its opposite – imprecision using the unit standard deviation or relative standard deviation (e.g. %CV).

The reasons for bias in clinical chemistry are numerous and vary between measurement methods e.g.: - Bias when taking samples, e.g. when samples are sometimes taken when the patient has been walking around and sometimes when he/she has been lying down. When the regulatory systems of the body adapt to gravity, the blood plasma volume is reduced to about 10% from a lying to a standing position thus increasing the concentration of macromolecules and cells in the blood of the patient. - Instability of the sample during transport or storage, e.g. during transport in extremes of heat and cold and mechanical effects on cells and blood gases when transporting samples through pneumatic tubes in hospital transport systems. - Uncorrected loss of measurand at extraction e.g. when preparing samples for measurement using high-performance liquid chromatography or mass-spectrometry. - Errors when the calibrator is prepared, including errors in volume measurements or in weighing of calibrators in the laboratory. - Using sample matrix which differs from the matrix in the samples e.g. using defatted and lyophilized stable materials for internal quality control or proficiency testing programs. - Interferences in the samples, e.g. the color of hemoglobin and bilirubin in hemolytic and icteric samples or the presence of high concentrations of proteins or lipids in the sample (myeloma or hyperlipidemia). - The presence of molecules which specifically interfere with the reagents used in the measurement process, e.g. heterophile antibodies (e.g. human antibodies against mouse IgG). - Specificity for different epitopes in macromolecules of antibodies used in immunochemical measurement methods e.g. when measuring macromolecules including prostate- specific antigen, troponins and protein- or peptide hormones. - Metrological traceability is a property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty. Traceability is crucial for standardization of measurement results and for minimizing bias. A crucial and frequently underestimated factor in achieving traceability is commutability. Commutability is a property of a reference material that expresses the closeness of agreement between results for the reference material and results for patient samples when measured by two or more measurement procedures. Lack of commutability is commonly due to matrix effects which are the combined effects on the measurement results of all other molecules than the ones you intend to measure.

Automation has substantially reduced repeatability imprecision when measuring patient samples in clinical chemistry. Reproducibility imprecision has not been reduced to the same extent probably since it is more challenging manufacturers to improve reproducibility. The Joint Committee for Traceability in Laboratory Medicine (JCTLM, http://www.jctlm.org/) was established in 2002 in response to the implementation of the European Community Directive on in vitro medical devices. Its founding organizations are the International Committee of Weights and Measures (CIPM), the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC), and the International Laboratory Accreditation Cooperation (ILAC). The JCTLM publishes list of higher order reference materials, reference methods and reference laboratories. They are joined in this effort by other corresponding organizations including the FDA, National Metrological Institutes (NMI) etc. in other parts of the world. Though far from easy, through perseverance we are likely to see a bountiful harvest of the work done by JCTLM, especially as products of reagents and systems and organizers of proficiency testing programs increasingly adopt the facilities that JCTLM brings together.

The American Association of Clinical Chemistry (AACC) in 2010 initiated the International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR, https://www.harmonization.net) organizing a global effort to
harmonize test results especially in the instances where standardisation is not feasible. Amongst the activities of the consortium is the publication of a toolbox of approaches and procedures to be used when developing a process to achieve harmonization for a measurand. Further developments in reference measurement systems is likely continue to play the major role in minimizing bias in clinical chemistry in the decade ahead. Reference measurement systems are, however, unlikely to solve the most complex bias issues, e.g. in the fields of immunochemistry. Natural patient samples are commutable and in abundant supply in the laboratories of clinical chemistry. They represent an asset that is likely to be increasingly used for minimizing bias using harmonisation methods which promise to minimize bias and measurement uncertainty in clinical chemistry still further.

Value and impact of the clinical laboratory in healthcare
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Laboratory medicine is the branch of medicine that provides objective data to clinicians and other healthcare workers to guide appropriate clinical decision making. Laboratory medicine is integral to many clinical decisions on prevention, diagnosis, treatment, and disease management (CLB 2017). It supplies health care professionals with evidence-based data necessary to provide high-quality, safe, effective and appropriate care to patients. Unfortunately, this critical role of laboratory medicine is not widely recognized within healthcare organizations, leading to poor visibility both within the field of clinical medicine and externally with the public at large. The laboratory is viewed as a black box where patient specimens are sent and test results are magically generated. There is very little understanding of the laboratory testing process not only with patients but also physicians and other healthcare workers. This is in large part due to the low visibility of the important work carried out in clinical laboratories and the poor recognition of the major developments in laboratory testing technology that have contributed to an increasingly vital role in evidence-based clinical decision making.

Systematic evidence for the contribution of the clinical laboratory to the overall assessment, diagnosis, and management of patients is not readily available. Establishing this evidence is vital to all promotional activities by the IFCC and other organizations involved in laboratory medicine. There is a critical need for both a systematic review of the available evidence in the published literature as well as the initiation of new retrospective and prospective studies to more clearly establish this crucial evidence. The IFCC established a new taskforce to evaluate the published evidence on value and impact of laboratory medicine on clinical outcomes and healthcare delivery, and if necessary propose new studies to more clearly establish this evidence. I will review the evidence supporting the key role of laboratory medicine in clinical management and outcomes and identify the gaps requiring new studies. This will be followed by a discussion of data demonstrating the value of laboratory testing from a clinical and economical perspective. I will also review the key activities of the IFCC in promoting the visibility of the field of laboratory medicine among healthcare professionals, hospital administrators, governmental regulators and funders, and the general public.

Lipid guidelines: emerging evidence on importance of non-fasting and postprandial lipids
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With the current eating patterns in Western societies, the fed state predominates over the course of a day, with the typical individual only in the fasted state for a few hours in the early morning. Nevertheless, the fasting lipid profile has been a standard assessment of cardiovascular disease (CVD) risk. There are two primary reasons for traditionally measuring fasting triglycerides (TG): to reduce the variability in TG concentration following meal ingestion and to accurately calculate low-density lipoprotein cholesterol (LDL-C) using the Friedewald equation. However, nonfasting (i.e. random blood sample measurement irrespective of time since last meal) TG levels have been reported to fluctuate only modestly within the same individual. Additionally, calculated LDL-C has been shown to change minimally after food intake and measured and calculated LDL-C are highly correlated between fasting and nonfasting state. As nonfasting TG levels are independently associated with cardiovascular event, a paradigm shift towards assessing lipid parameters in the nonfasting or postprandial (i.e. blood sample measurement at specified time points following a standardized meal) state is occurring. In fact, postprandial TG levels obtained after consuming a standardized high-fat meal, better predict coronary artery disease compared to fasting TG levels. Several clinical guidelines have included nonfasting lipid testing in the primary prevention setting, including Denmark in 2009, UK in 2014, as well as the European Atherosclerosis Society (EAS) and European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and the Canadian Cardiovascular Guidelines in 2016. The nonfasting lipid panel has become the clinical standard in Denmark, offering physicians the option to measure fasting lipids when TG >4.5mmol/L, while in Canada it is recommended to obtain a fasting measurement when TG >4.5mmol/L. Furthermore, the EAS/EFLM guidelines state that nonfasting and fasting measurements should be complementary and not mutually exclusive. The option of nonfasting lipid testing has also been included in The 2011 National Heart, Lung, and Blood Institute (NHLBI) Guidelines specific for the pediatric population. Assessing the postprandial lipid profile can provide a better indication of an individual’s capacity to metabolize lipids following a meal, reflecting their metabolic efficiency.

Apolipoprotein profiling for addressing residual cardiovascular risk: in search of a personalized and metrologically sound answer to the latest dyslipidaemia guidelines
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An elevated low-density lipoprotein cholesterol (LDLc) concentration is a classical risk factor for cardiovascular disease. This has led to pharmacotherapy in patients with atherosclerotic heart disease or high heart disease risk with statins to reduce serum LDLc. Even in patients in whom the target levels of LDLc are reached, there remains a significant residual cardiovascular risk; this is due, in part, to a focus on LDLc alone and neglect of other important aspects of lipid profile metabolism. According to the latest dyslipidaemia guidelines, a more refined lipoprotein analysis is advocated, especially for secondary prevention, which provides additional information on the accumulation of very low-density lipoproteins, intermediate density lipoproteins, chylomicrons, chylomicron remnants and Lp(a). Instead of measuring the overall cholesterol and triglyceride content of lipoproteins, measurement of their apolipoproteins is more informative. Apolipoproteins are either specific for a particular lipoprotein or for a group of lipoproteins. Measurement of apolipoproteins in atheregenic particles is more biologically meaningful than the measurement of the cholesterol concentration contained in these particles. Applying serum apolipoprotein profiling will not only improve characterization of lipoprotein abnormalities, but will also improve definition of therapeutic targets. Apolipoprotein profiling aligns with the concept of precision medicine by which an individual patient is not treated as ‘average’ patient by the average (dose of) therapy. This concept of precision medicine fills the unmet clinical need for stratified cardiovascular medicine. The requirements for clinical application of proteomics, including apolipoprotein profiling, can now be met using robust mass spectrometry technology which offers desirable analytical performance and standardization.

Keywords: Dyslipidaemia, mass spectrometry, clinical proteomics, metrological traceability, serum apolipoprotein profiling

The importance of cholesterol synthesis and absorption markers determination in healthy subjects and patients with ischemic heart disease
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According to the World Health Organization, worldwide incidence of cardiovascular diseases (CVDs) is everincreasing, and CVD are among the leading causes of morbidity, co-morbidity and mortality. Atherosclerosis is a chronic, focal disease of the blood vessel intima. Atherosclerosis is the underlying
cause of the most CVD, including coronary artery disease (CAD). Even though many etiological factors are involved in the pathogenesis and progression of atherosclerosis, dyslipidemia has the key role in atheroma development. Statins represent a hypolipemic of choice in primary and secondary CAD prevention. In addition to the inhibitory effect on cholesterol synthesis, statins also have numerous pleiotropic effects. Basic lipid parameters are used for diagnosing dyslipidemia and monitoring the statin therapy response in clinical practice. Elevated plasma total cholesterol (TC), LDL-cholesterol (LDL-C) and triglyceride (TG) concentrations in different subclasses of HDL-cholesterol (HDL-C) concentrations represent well-documented risk factors for CVD. However, in order to examine the overall cholesterol metabolism and monitor its homeostasis, it is necessary to examine the efficiency of cholesterol synthesis and absorption, its distribution between lipoprotein particles, and the preservation of the reverse cholesterol transport function. Cholesterol homeostasis represents the balance between cholesterol synthesis and absorption. Many studies have shown that cholesterol synthesis and absorption are in equilibrium. Increased cholesterol synthesis leads to reduced absorption and vice versa, in order to maintain balance. Cholesterol synthesis is divided into two different pathways, that may be independently regulated (80% via the lathosterol - Kandutsch-Russel pathway; 20% via the desmosterol - Bloch pathway). Non-cholesterol sterols (NCSs) represent cholesterol synthesis precursors (desmosterol and lathosterol) and cholesterol absorption surrogate markers (phytosterols - campesterol, stigmasterol and β-sitosterol). Knowing that the plant sterols are absorbed in the same way as the intestinal cholesterol, plant sterols are used as surrogate markers of cholesterol absorption efficiency. These markers can indicate early development of dyslipidemia and predict response to statin therapy. NCSs concentrations in plasma are 200–1000 times lower compared to cholesterol levels and relatively low NCSs concentrations represent a specific problem for their quantification. This represents the additional reason to conduct an extensive method validation for NCSs determination, as well as to resolve pre-analytical and analytical factors of influence. In order to contribute to a better understanding of cholesterol metabolism and the statin effects on cholesterol homeostasis, the objectives of this study were: establishing and validating the method for NCSs determination; determination of NCSs concentrations in healthy subjects (CG) and CAD patients; determination of cholesterol homeostasis patterns and their association with basic lipid parameters and distribution of low-density lipoprotein subclasses (LDL) in examined groups. The study included 31 healthy controls (CG), 32 statin-treated patients and 47 statin-naive CAD patients. Method optimization, validation and stability studies were executed in human serum and plasma. Freeze-thaw cycles were done with and without antioxidant. Gas chromatography-mass spectrometer (GC-MS) was used for NCSs determination and plasma oxidative stress markers such as AOPP, TOS, MDA (P<0.001) and PAB (P<0.05) were determined whether increased levels of OS in PCOS are due to the syndrome itself or related to its characteristics (hyperandrogenism, insulin resistance (IR), obesity and abdominal obesity that significantly contribute to OS development). Chronic low-grade inflammation is an important feature of PCOS (participates in its pathogenesis and development). Numerous evidence supports the concept of feedback formation, where inflammation induces reactive oxygen species (ROS) formation, while OS exacerbates inflammation as described in endothelium and adipose tissue. High-density lipoprotein (HDL) particles are present in the circulation in the form of different subclasses that differ in size, density and lipid composition. The antioxidant/anti-inflammatory role of HDL depends on the presence of antioxidant enzymes. Paraoxonase 1 (PON1) is an antioxidant enzyme associated with apolipoprotein A1 on HDL particles whose activity and concentration may be oxidized in OS, further increasing the risk of developing cardiovascular disease (CVD). Although there are different opinions about the PON1 distribution between HDL 2 and HDL 3 subclasses, PON1 is assumed to follow the reverse cholesterol transport. The study included 114 PCOS patients and 50 healthy females (control group, CG), of similar age (18 – 39 years). The CG participants had normal glucose metabolism, were non-smokers and zero alcohol consumers with no signs of hyperandrogenism. Patients were analysed during the early follicular phase of the menstrual cycle, or at any time if they had severe oligomenorrhea or amenorrhea. Systolic and diastolic arterial blood pressure (SBP and DBP), anthropometric, biochemical and oxidative stress parameters were determined in all study participants using standardised assays. Plasma HDL particles were separated using a non-denaturing 3-31% polyacrylamide gradient gel electrophoresis method, previously described by Rainwater et al. which was optimized in the laboratory of the Department of Medical Biochemistry, Faculty of Pharmacy, Belgrade. Following HDL lipoprotein electrophoresis, PON1 activity on HDL 2 and HDL 3 subclasses was determined using the Trinder reaction according to Gugliucci et al. As we wished to examine the mutual effect of the most important risk factors in PCOS patients, we calculated the DOI score as a sum of dyslipidemia, OS and inflammatory scores. High-density lipoprotein cholesterol (HDL-C) was significantly lower in patients with a higher BMI (P=0.01) compared to healthy controls (P=0.01) with no significant differences in triglyceride (TG), non-HDL-C concentration and atherogenic index (TG / HDL-C) values. Total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations were not elevated in women with PCOS. In contrast PCOS patients had significantly higher CRP (P=0.001). More pronounced oxidative stress markers such as AOPP, TO8, MDA (P<0.001) and PAB (P<0.005)
combined with a concomitant reduction in the concentration of total SH-groups and lower PON1 antioxidant activity (P<0.001, P=0.05, respectively) were found in patients compared to healthy controls. All individual scores: oxy-score (P<0.001), dyslipidemia (P=0.05) and inflammation (P<0.001) scores were significantly higher in patients compared to healthy controls. Consequently, the DOI score was significantly higher in comparison to healthy controls (P<0.001), highlighting the significance of this result for assessing cardiometabolic risk in patients.

The analysis of HDL size and HDL subclasses (HDL 2 and HDL 3) distribution showed that HDL particle size did not differ between patients and healthy controls. However, normal weight patients had significantly higher HDL 2 subclass than normal weight and obese controls (P=0.05). HDL particle size analysis within the PCOS group showed that obese patients had significantly smaller HDL diameters (P<0.05) and a greater HDL 3 subclass than normal weight patients (P<0.05). This was consistent with previous findings that showed a decrease in HDL particle size in patients with higher CVD risk. The proportion of HDL subclasses did not differ between patients and healthy controls. Obesity had no influence on PON1 distribution within HDL subclasses in patients. However, in patients with small LDL particle size the relative proportion of PON1 on HDL 2 subclasses was significantly higher (P<0.001) and the relative proportion of PON1 on HDL 3 subclasses was significantly lower (P<0.01) than in patients with large LDL particle size. As patients had a higher antioxidant score, the relative proportion of PON1 on HDL 2 subclasses increased (P<0.01) while the total proportion of PON1 in the HDL 3 subclasses decreased (P<0.05). Increased oxy-score was accompanied by an increase in the total proportion of PON1 on HDL 3 subclasses (P<0.05).

The results demonstrate that PCOS women have elevated levels of OS markers i.e. products of their action, and decreased values of antioxidant protection parameters. Patients have higher OS, dyslipidemia as well as chronic low-grade inflammation compared to healthy women that indicates currently low cardiovascular risk. Obese PCOS patients have significantly smaller HDL diameters and a higher proportion of small HDL 3 subclasses (associated with a high risk for CVD) compared to normal weight PCOS patients. Based on this, we can assume that obesity in PCOS affects the profile of HDL subclasses, while PCOS itself has no effect on the HDL subclasses profile. A comparison of PCOS women, according to the LDL particle size, indicated that PON1 activity on small HDL 3 subclasses was significantly lower in women with small, denser LDL particles, which is a sign that the antioxidant ability of HDL 3 subclasses is decreased in PCOS in conditions of increased risk for CVD.

Sensitive assessment of white blood cell functionality by novel hematological parameters

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Cellular and morphological analysis is an integral part of modern haematology analyzers. A unique combination of techniques permits to separate cell populations based on lipid composition of cell membranes, the fluorochrome RNA labels, cell volume and intracellular structure. In addition, the DNA of the nucleus is labeled by the fluorescence reagent penetration. The intensity of the fluorescence signal is directly proportional to the nucleic acid content and the strongest signals are shown by immature and activated cells. Sensitive assessment of cell functionality or activation status depends on cholesterol- and glycosphingolipid raft in the plasma membranes that play important roles in protein trafficking and cellular signalling. The information about membrane lipid rafts and cytoplasmic RNA is analyzed with proprietary algorithms that deliver sensitive detection of reactive or pathological cells in a blood sample. Modern hematology analyzers are designed with improved gating and optimization of leukocyte clusters including immature granulocytes (IGs). Moreover, results including the presence and concentration of IGs become available within minutes – and are included in the complete CBC+DIFF analysis, making it a valuable sixth subpopulation of the white blood cells. The measurement of the immature cells, which combine promyelocytes, myelocytes and metamyelocytes, is considered clinically useful for the diagnosis of infections, especially neonatal sepsis, inflammation, myeloproliferative diseases, tissue necrosis and acute transplant rejection at a very early stage. The results were excellent considering the low levels of IGs observed and the well-known limitations of manual differentials and rare cell events. Modern analyzers are much more sensitive than the manual differential counting method in the detection of leukemic blasts and they provide more cell population data than the manual differential count, including blast lineages. Distribution and appearance of lymphocyte population quantify the numbers of all reactive, antibody-synthesizing and malignant lymphocytes. For instance, several studies showed that lymphocyte RE-LYMP and AS-LYMP counts (Sysmex XN series) were mainly increased in viral infections. Monocyte population provides information for screening and differential diagnosis of malaria and dengue by a calculated malaria factor. The modern analyzers are characterized with high sensitivity and specificity for detecting atypical cells in the samples and smear reviews can be reduced by approximately 20% in routine laboratory. The possibilities of current haematology analyzers for better screening, diagnosis and monitoring of reactive and malignant diseases are increased out the need for clinically irrelevant follow-up tests.

The future of Cytometry in Europe

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The field of Cytometry is a recent discipline which emerged through developments in the fields of Physics (optics and fluidics) and Computer Science (electronics and informatics). Flow cytometry is today an established field that deals with the quantification of cellular characteristics. To envision the future of the field of cytometry in Europe we navigate through the past and the present in an effort to describe the state of the art on the field, based on the recent advances.

From the dawn of Cytometry during the mid-20th century till today many pioneer European cytometrists have largely contributed to the field. Among them, Wolfgang Göhde is considered as the founder of European cytometry and is the one that developed the first fluorescence-based cytometer. The field has further evolved after Kholer and Milstein (Nobel prize in Physiology or Medicine in 1984) described the development of monoclonal antibodies. During this period Flow Cytometry attracted many pioneer scientists from diverse disciplines and scientific backgrounds, including: Claude Curties, who applied cytometry in oceanographic studies and in discovery of eukaryotic organisms; Andrew Ridell, founder of the European Cytometry Network; Gerda Schmidt, founder of EWGCCA (European Working Group on Clinical Cell analysis); Günter Vallat, Pioneer of multiparametric flow cytometric analysis and one of the earliest promoters and applicants of the current concept of Cytomics and its application to Predictive Medicine; Phillip Sansonnet, organizer of the First International Workshop on Flow and Image Cytometry.

The continuous application of novel methodologies widened the scope and target audience of Flow Cytometry in both clinical and research laboratories. Such developments led to initiatives that resulted in the formation of organized Working Groups and Societies in individual Countries and in Europe as a whole. First, ISAC (International Society for Advancement of Cytometry) was founded in 1983, followed by ISAC Europe in 1995, including the First European Course in Clinical Cytometry in Athens, Greece. ISAC Europe is also an established network in the field of HematoOncology, by J.J.M. Van Dongen (chair) and Alberto Orfao (co-chair), including 19 diagnostic research groups and one SME, with a vision to connect experts in the field of flow cytometry in Europe, with a network of European Cytometry Network has been recently established, as an initiative of European Molecular Biology Laboratory EMBL, Heidelberg, 2008, in order to establish communication, cooperation, education and promotion of Cytometric science and techniques among its members. The European Cytometry Network (ECN) has been created with the aim to support modern infrastructure and to build up connections between professionals in Cytometry. EuroFlow Network is also an established network in the field of HematoOncology, by J.J.M. Van Dongen (chair) and Alberto Orfao (co-chair), including 19 diagnostic research groups and one SME, with a vision to connect experts in the field of flow cytometry and molecular diagnostics. The European Working Group on Clinical Cell Analysis (EWGCCA) established cooperation and training in Cytometry since 1995, including the First European Course in Clinical Cytometry in Athens in 2005. As a continuation of EWGCCA activities, European Society for Clinical Cell Analysis has been established as a scientific society in 2006. ESCCA holds Annual Conferences in collaboration with local societies, annual EuroCourses (Education programs), Schools on cytometry (winter, summer, autumn), flow events (international and guidelines meetings). More recently ESCCA provides Certification exams for Cytometry operators and Cytometry specialists and also ESCCA databases, an organized database of flow cytometry results and analyses.

The development of European and local societies, along with the organization of Conferences and educational courses have been necessary, based on the rapid recent developments of technology and methodologies. During recent years

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Multimarametric Flow Cytometry made it possible for more cell characteristics to be examined from the same cell population leading to the establishment of Cytometry. Recent developments in the field also include: Mass Cytometry, a combination of flow cytometry and mass spectrometry to interrogate up to 100 parameters from a single cell; Imaging Flow Cytometry, Using a microscope that also analyses cell shape and the relative position of different epitopes (allowing for colocalization analysis); Spectral Flow Cytometry, where newly developed multichannel detectors allow for the simultaneous analysis of more parameters minimizing the problems of spectral overlap. The state of the art in the field include Cytometry methodologies that are among the standard techniques practiced in both clinical and research laboratories. The combined efforts of cytometrists throughout Europe guaranteed the advancement of the field up until today. Knowledge and data dissemination are critical parameters in the era of information and informatics. Our suggestion is that the future of the field should be based on cooperation, openness in knowledge sharing and organized cytometry courses for educating the new generation of cytometrists.

Significance of the determination of biomarkers of bone resorption and formation in patients with end stage renal disease

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End stage renal disease (ESRD) is associated with various mineral and bone disorders. Guidelines for improving the quality of life and education of patients with ESRD Kidney Disease Outcomes Quality Initiatives, KDOQI, published by the U.S. National Kidney Foundation (NKF), indicate the importance of biomarkers of bone metabolism that should be analyzed in ESRD patients. Routine parameters that are determined in most laboratories are indirect indicators of bone turnover, such as: calcium ions (Ca), inorganic phosphate ions (Pi), magnesium ions (Mg), the total alkaline phosphatase (ALP), an intact parathyroid hormone (iPTH) and 25-hydroxy vitamin D (25D). On the other hand, direct indicators of bone metabolism are products of bone cells. The activity of osteoclasts, the cells responsible for bone formation, is well expressed by the levels of bone alkaline phosphatase isoenzymes (BALP), which is highly specific for bone tissue. The activity of osteoclasts, the cells responsible for bone resorption, specifically reflect levels of tartrate resistant acid phosphatase (TRAP). A good marker of bone resorption is the beta-carboxy terminal telopeptide of collagen type I, beta-CrossLaps (beta-CTx). The aim of this study was to evaluate the usefulness of biomarkers of bone resorption and bone formation in ESRD patients. The study included 40 predialysis patients (18 women and 22 men) aged 25−79, 141 patients on continuous ambulatory peritoneal dialysis (CAPD) (49 women and 65 men) aged 30−84 and 112 patients on hemodialysis (HD) (53 women and 59 men) aged 25−79. Average duration of the HD and CAPD treatment was 76 and 35 months, respectively. The analyzed biomarkers of bone formation and resorption were determined in the serum of patients on the day of sampling: for predialysis and CAPD patients when they came to the routine check-ups, and for HD patients immediately before dialysis therapy. To determine the reference intervals, analyzed biomarkers were measured in a group of 50 healthy volunteers (25 women and 25 men) aged 20−70 years. ALP, TRAP, Ca, P and Mg were determined using spectrophotometry (Olympus AU2700 ISE). BALP values were determined using zone electrophoresis (SEBIA Hydrasis), beta-CTx and iPTH concentrations were determined with ECLI A (Elecsys Rosche) and 25D concentrations were determined by HPLC with reversed phase detection (HPLC ChromLine®Clinical software Version 4.20). Determination of the analyzed biomarkers is considered reliable based on the coefficients of variation (CV) obtained by precision testing in the series (CV: 0.6%−3.3%) and from day to day (CV: 1.0%−3.6%). We established the normal distribution of the values for each of the analyzed biomarkers in predialysis and dialyzed patients. There was significant impact of gender on iPTH, P and Ca×P values in the all analyzed groups. However, the effect of age was observed only on the values of BALP. Duration of the dialysis had impact only on the values of ALP and BALP in HD patients and on Mg concentrations in CAPD group. BALP values were significantly lower (P<0.001), and beta-CTx and TRAP values were significantly higher (P<0.05 and P<0.01) in ESRD patients, compared to the control group. The effect of the dialysis, regardless of the dialysis mode, was confirmed with lower BALP values in dialysis patients compared to the predialysis patients (P<0.05). However, we obtained much lower beta-CTx concentrations in HD patients as compared to predialysis patients (P<0.05). The most significant change considering the iPTH concentrations (<150 pg/mL, 150−300 pg/mL and >300 pg/mL) was observed in the BALP values in all three groups of patients. There were parallel changes in the values of BALP and iPTH in all three groups of the patients. There was significant difference in the BALP values, regarding the 25D concentrations (<50 mmol/L and >50 mmol/L) in CAPD patients (P<0.05). In order to determine diagnostic accuracy of direct and recommended biomarkers in relation to the recommended value of the iPTH (<100 pg/mL) for detection of adynamic bone disease in ESRD patients, we performed ROC analysis. When we analyzed all three studied groups of patients and HD patients separately, we found calcium had highest diagnostic value. The areas under the curves (AUC) were significantly different in comparison with other biomarkers analyzed (AUC<0.701, P=0.0001 and AUC<0.631, P=0.007, respectively). In the group of predialysis and CAPD patients, the highest diagnostic value had BALP (AUC=0.668 and AUC=0.588), although there was a marginal significant difference with other analyzed biomarkers (P=0.058 and P=0.053). This study support other reported data, that examined biomarkers (BALP, TRAP and beta-CTx) have comparable diagnostic accuracy as well as the recommended biomarkers (Ca, P, Mg, ALP, iPTH and 25D) to determine the level of bone metabolism in ESRD patients. On the basis of our results we can conclude that bone markers, generally, may be an appropriate alternative to invasive method of bone biopsy.

CEA monitoring in colorectal carcinoma - to the limit of the guidelines and beyond

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Carcinoembryonic antigen (CEA) is a well-established serum tumor marker in colorectal cancer. It is used in preoperative prognostication, disease stage prediction, immediate post-resection assessment and, to some extend as an adjunct in treatment response monitoring. The diagnostic and screening value of CEA is definitely poor, but the pre-operative determinations of the marker for risk stratification in patients with diagnosed colorectal cancer is widely discussed in the literature and is considered to be recommended. The discussion for the preoperative testing is much more connected with the clinical value of the marker, i.e. whether the patients with higher preoperative values to receive adjuvant therapy, due to the poorer prognosis, based on these higher values of CEA or not. The disease stage prediction, based on CEA is accepted and recommended by NCCN and ASCO, but it should be mentioned that the tumor marker values must not be considered as an indication for adjuvant therapy, but only for a basis for intensive follow-up of patients in high risk of recurrence. The data for the immediate post-resection assessment of CEA is under discussion and although there is preclinical research on the basis of these higher values of CEA, the value of the marker is not considered proven and is not recommended in the official guidelines. The usage of the marker in the recurrence monitoring has a proven influence on the surveillance of patients with colorectal cancer. The major role of the marker, however, is in the monitoring/follow up of patients with colorectal cancer, treated with curative intent. CEA value in follow up of those patients is addressed in the guidelines and beyond.
CEA levels may occasionally experience a recurrence despite negative results from the extensive work-up. Close CEA levels monitoring is essential in this scenario with the results going in two directions; those with further rising CEA levels almost invariably recur while those with high but stable CEA levels rarely experience recurrence. The absolute CEA value is also important with serum levels of CEA of more than 10ng/ml being predictive of recurrence in very high proportion of patients. However one should bear in mind that CEA levels rise in a variety of benign conditions and could reach excessive absolute values without a proven malignancy. The most often mentioned benign diseases connected with CEA elevation are chronic hepatitis, cirrhosis, chronic kidney failure, colitis, jaundice. So neither the rise alone nor the absolute value but the trend of rising is predictive of recurrence if so-far work up has failed to localize disease. Even though guidelines and official recommendations are mostly clear about the role of CEA in the monitoring of colorectal cancer patients, treated with curative intent and the consecutive conventional and high-end imaging, the management of the patients with no recurrence detected is less clear. In these cases combined assessment with Ca 19-9 may be attempted, but with the clear idea that Ca 19-9 performs suboptimal in colorectal cancer and is a subject of broad spectrum of non-specificity. Other problem is that Ca19-9 and CEA may rise simultaneously in similar benign processes which limits the use of the combination of markers as differential diagnostic tool. In the present era of molecular and genetic testing attempts are made to correlate the rising monitoring CEA levels, such as circulating tumor cells, circulating free tumor DNA (cfDNA), methylated DNA (e.g. septime 9), reporter mRNA etc. Attempts in this direction have been made also in the group of CEA positive FDG negative patients. None of the tests has however reached routine clinical use.

The development of new imaging methods and tests for detecting recurrence in patients with colorectal cancer puts on discussion whether and how underestimated is in fact the positive predictive value of CEA, including the levels below 5 ng/ml and whether in patients with higher levels of the marker the oncologist should make a great effort for clearing the reason, i.e. accepting or rejecting a recurrence, or the patient should be followed up using only conventional methods till appearance and verification of clinical symptoms of recurrence.

Although not perfect in predicting recurrence CEA is still the monitoring tool of choice when it comes to colorectal cancer patients. Patients with rising CEA levels should be chased to prove recurrence by conventional imaging, endoscopy and FDG PET CT. Those with no proof of recurrence should be followed up strictly to define any upward trend of CEA values and in case of such should be reassessed again.

Amino Acid and Organic Acid CRMs for Newborn Screening
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The Certified Reference Material (CRM) is utilized in chemical measurements as a useful tool for proving traceability of measurement result and enhances measurement quality. Organic acid and Amino acid concentrations are frequently measured for treatment and diagnosis purposes of inborn error of metabolism (IEM) which is a permanent and inherited biochemical disorder generally caused by organic acid, amino acid metabolism distortedness. Early diagnosis of metabolic diseases is very critical and they should be evaluated through reliable screening tests. The use of CRMs is required to ensure the quality of the chemical measurements. Particularly, it is important to use CRMs, having the same chemical compositions (matrix matched CRM), for the detection of subject quantity in the mixtures (matrix), such as body fluids, containing more than one metabolite. In this way, through the use of CRM in measurements, metrological traceability chain can be ensured. Production and the certification of the CRMs are carried out according to the technical requirements of ISO Guide 35. Quality management system based on ISO/IEC 17025 and ISO Guide 34. IDMS method was applied as primary method of measurement for the characterisation of the materials. Amino acid concentrations in lypohilized human plasma are certified in UME CRM 1314. Organic acid concentrations in lypohilized urine are certified in UME CRM 1315. Two new certified reference materials were produced and certified to be used in newborn screening tests and routine clinical measurements for 32 amino acids in human plasma and 47 organic acids in urine.
Keywords: Certified Reference Materials (CRMs), quality control, newborn screening, metabolic disorder
ID-MS based reference measurement method for small analytes: vitamin D, creatinine, glucose, cholesterol, amino acids

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Isotope Dilution Mass Spectrometry (IDMS) is a primary method capable of providing accurate and precise results directly traceable to the International System of units. IDMS is an analytical technique based on the modification of the natural isotope composition of compounds after the addition to the sample an isotopically labeled form of the analyte. In this study, the use of LC-IDMS in analysing Vitamin D, Creatinine, Cholesterol, Glucose in serum and amino acids in diluted HCL is described. Certified reference materials were provided from Nist. Liquid Chromatography-Isotope Dilution Mass Spectrometry method was used for quantification. 25-hydroxy vitamin D3 in human serum were ranging from 25.84 to 37.82 ng/g with an expanded uncertainty of 1.84 to 2.71 ng/g for 25-hydroxy vitamin D3.

Creatinine in human serum were ranging from 7.47 to 7.485 µg/g with an expanded uncertainty of 7.45E-02 to 7.74E-02 µg/g. Glucose in human serum were ranging from 1.15 to 1.16 mg/g with an expanded uncertainty of 1.23E-02 to 1.39E-02 mg/g. Cholesterol in human serum were ranging from 2.27 to 2.31 mg/g with an expanded uncertainty of 6.19E-02 to 6.59E-02 mg/g.

Phenylalanine, Leucine, Isoleucine and Proline in diluted HCL were 482.59, 206.64, 218.98 and 47.24 µg/g with an expanded uncertainty of 7.20, 3.24, 7.00 and 1.62 µg/g respectively. Primary method techniques are capable of providing accurate and precise results. The reliability and performance of the method was demonstrated by uncertainty budget and method validation.

Keywords: vitamin D, creatinine, glucose, cholesterol, amino acids

Reference methods for quantification of peptides & proteins: β-amyloid in CSF, hCP, oxytocin, HbA1c, insulin, hGH

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Over the past two decades there have been important developments in the diagnosis and treatment of diseases with the increase in the number of biomarker molecules. There are now many endogenous peptides and proteins used as biomarkers and/or drugs. Amyloid-beta (Aβ) peptide, oxytocin, growth hormone, C-peptide, HbA1C are just a few of them. These molecules are used for therapeutic purposes as well as reference material for the diagnosis of the disease from plasma or serum. The use of certified reference materials (CRM) and validation of the measurement methods is a technical and regulatory issue deserves close attention. In TÜBİTAK UME Laboratories, the peptide impurity analysis is performed by PICAA (Peptide Impurity Corrected Amino Acid Analysis) method. PICAA analysis involves AAA Isootope Dilution Mass Spectrometry (AAA-ID-MS / MS) and the intact peptide analysis using High Resolution Liquid Chromatography MS (LC-HR-MS/MS). Impurities from the peptide content determined by intact peptide analysis are used to correct the results of AAA analysis. An SI traceable method was developed and validated for the impurity determination of several peptides and proteins in our laboratories. The analytical run was assessed determining, linearity, within-run accuracy and carryover. Matching the acceptance criteria the Correlation coefficient (r) of the calibration curve was found more than 0.995. The accuracy of 90% of the analyzed Quality Control was between 85.0% and 115.0%. The PICAA method is an alternative method to the Total Mass Balance method used in peptide impurity analysis and can be performed with much less peptide/protein.

Keywords: CRM, Quantification, Peptide, Protein, Traceable

Latest developments on NMR; reference method for purity determination of small analytes and peptides

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Nuclear magnetic resonance spectroscopy (NMR) is a very significant analytical method which has been routinely used by chemists for the determination of structures of compounds. Besides this, quantitative nuclear magnetic resonance spectroscopy (qNMR) has great importance in various fields, such as drug industry, manufacturing of reference materials, food analyses and metabolite determination in human body fluids. Moreover, applications of quantitative NMR involve determination of purity of a compound and amount and concentration of a sample inside a matrix. The aim of this study is to determine the purity of some small molecules by qNMR method. It is also to obtain very useful information to be used with the mass balance method for the purity determination of larger molecules such as peptides: The purity assessment of estradiol, folic acid, human C-peptide and oxytocin were done by quantitative nuclear magnetic resonance (qNMR). Internal standard purity was determined by UME CRM 1301 chloramphenicol with a certified value of 99.58 ± 0.15% (k=2 (TÜBİTAK UME, Gebze, TR)) within the traceability chain. All NMR experiments were performed at 298.15 k on a Varian VNMRS 600 spectrometer operating at 599.747 MHz for proton (1H) resonance frequency equipped with a 5 mm One NMR probe using 5 mm sample tubes. The softwares VnmrJ 4.2 and MestReNova 11.0.0 were used for data acquisition and data processing, respectively. The purity determination experiments performed within CCQM comparisons have been successfully completed. The results of these comparisons were published in the Metrology journal and in the BIPM key comparison database (kcdb.bipm.org/). The purity of folie acid and human C-peptide were reported as 909.78 ± 2.56 mg/g, and 853.15 ± 8.06 mg/g respectively by qNMR. NMR is the unique method, which can determine, with one analysis, a small molecule, having a single proton or a peptide possessing multiple protons. In this study, the advantages of NMR as a quantitative technique are mentioned.

Keywords: qNMR traceability purity

Development of a Reference Method for Transfering Quantification in Serum

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Iron is one of the metals that are thought to be involved in development of Alzheimer’s Disease (AD). Recent developments revealed that metalloproteins transport the metals to the brain across the blood-brain barrier. Hence, reliable measurement method for determination of transferrin (TRF) in body fluids is needed for investigating the influence of TRF in AD development. This study aims to develope and validate a reference method for TRF quantification in serum.: Triple species-specific HPLC isotope dilution mass spectrometry (SS-HPLC-IDMS) approach was used for determination of TRF in serum and CSF. ERM-DA470k/IFCC (IRM) and pooled CSF sample were used for method development and the method was validated using ERM-DA470k/IFCC. Firstly, 57Fe-TRF spike was synthesized and characterized. In triple SS-HPLC-IDMS approach, two calibration blends were prepared with 56Fe-TRF solution (traceable to NIST SRM 3126a) and the synthesised 57Fe-TRF spike solution. The sample blend was prepared with 56Fe saturated ERM-DA470k/IFCC and 57Fe-TRF spike. The measurement of 56Fe/57Fe ratios in all blends were performed on HPLC-ICP-MS system using bracketing sequence. The instruments used for the measurements were Agilent 1100 Biotiner HPLC and Agilent 8000 ICP-MS Triple Quad (Agilent Technologies). MonoQ 5/50 GL column (5 x 50 mm i.d., GE Healthcare) is used for separation of TRF sialofomer. In the validation study, 3.0% repeatability has been obtained in measurements of 5 replicate measurements of ERM-DA470k/IFCC. The trueness of the method was tested, and varying recoveries in the range of 99.8%–105.9% were obtained.: A traceable quantification method for TRF in serum was developed and validated.

Keywords: Cerebrospinal fluid, Serum, Transferrin, SS-HPLC-IDMS
A Reference method for genetic mutation quantification of KRAS
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The TUBITAK National Metrology Institute (UME) is a member of the International Bureau of Weights and Measures (BIPM). The aim of the Bioanalysis Laboratory is to develop primary measurement methods in the field of biometry and life sciences, to give primary level measurement service, to produce certified reference materials and to carry out proficiency testing needed especially in our country. The aim of this study is to describe newly developed measurement methods with digital PCR (dPCR).

Digital PCR instruments enable the calculation of DNA amount with the help of the amount of specific DNA molecules in a sample. This method has a lot of potential advantages compared with direct methods. The processes are faster, cheaper and do not involve patient inconvenience, discomfort or the risks associated with generating new patient health information. Indirect methods also use the same preanalytical and analytical techniques used for patient management and can provide very large numbers for assessment.

Interest has been renewed in the topic as a result of the following regulatory s. In the present-day era of evidence-based practice, the challenges are even greater. Developing a roadmap for laboratory test utilization management program
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Utilization management has been a traditional approach to control costs in clinical laboratory services for several decades. Following utilization management, best practices results in the highest quality care at the lowest cost, supports Lean and Six Sigma initiatives, and saves significant time and money. In fact, appropriate utilization reduces patient risk and empowers organizations to provide the highest quality of care. While it is good to have an understanding of utilization management, IFCC Committee on Clinical Laboratory Management has recently conducted an international survey to investigate what does this mean for the laboratory leaders and examined the state of medical laboratory test utilization management and relevant practices which are country-specific from a laboratory leader perspective. The findings of this survey revealed that the recognition of test utilization management, current practices, and maturation of those programs are significantly diverse among countries. It is relatively well established in most developed nations. However, the findings have confirmed that the need to develop a roadmap and to construct essential strategies for managing laboratory test utilization is a common interest. With this regard, it is of importance to select the right management tool to implement an optimal laboratory test utilization. This presentation will address the following key points for implementing utilization management initiatives:

- Structure of effective communication
- Infrastructure to assist implementation
- Establishing a laboratory formulary
- Gatekeeping mechanisms
- Clinical decision support
- Benchmarking and management metrics
- Consultative and Interpretive services

Keywords: IFCC, C-RIDL, reference intervals, multicenter studies, EP28-A3c guideline, decision limits, indirect methods
In this presentation, measures that need to be taken against biochemical health issues, establishing specialized response teams and a laboratory response chemical threat, some proposals may include in fields of concern such as: sharing training activities, including medical units, procurement of protective measures and detection assets. Lack of coordination and preparedness at national, regional and international levels of chemical in use.

Modern threats of biochemical terrorism lead to development of methods for more rapid identification of these agents than conventional methods used in this field, from which chromatography techniques can be employed for hostile purposes and planned to cause disease or death in human, animals or plants. Under the pre-incident preparedness measures, A rapid and coordinated medical response should be based on main integrated areas of interest including training and research on preparedness and prevention, detection and surveillance system, diagnosis and characterization of the agents and emergency management involving epidemiologic investigation, medical treatment and prophylaxis for affected people and decontamination measures. Modern threats of biochemical terrorism lead to development of methods for true and fast detection of chemical weapons. Currently, there are many types of methods used in this field, from which chromatography techniques can be employed for more rapid identification of these agents than conventional laboratory analytical methods. However, analysis is often challenging because of the limited size, quality, and purity of the biological target for the verification of chemical in use. Lack of coordination and preparedness at national, regional and international levels can have some dramatic consequences. Defense against chemical threat is such a complex issue that requires highly qualified experts from various organizations including medical units, procurement of protective measures and detection assets and being aware of the current treatment approaches accompanied with extensive training activities. This is why the medical management involves first responders’ organizations (including health experts) in charge of the protection and mitigation of the effects of chemicals.

To enhance coordination and effective medical response against regional chemical threat, some proposals may include in fields of concern such as: sharing medical preparedness plans while also contain emergency medical and public health issues, establishing specialized response teams and a laboratory response network, exchange information and publications which are not confidential, organizing scientific meetings to increase the awareness amongst medical personnel. A Balkan common understanding may play a vital role in coordinating and conducting these mentioned measures.

In this presentation, measures that needs to be taken against biochemical terrorism and concepts are to be reviewed from the Turkish medical perspective and potential items which are supposed to be ruled in this event to be outlined.
Cardiology Foundation and the American Heart Association recognized the value of Gal-3 testing and included it into the Guideline for the Management of Heart Failure, because it has been proven that Gal-3 could provide useful information for optimisation of HF patient care decisions. Namely, Gal-3, as a biomarker of myocardial fibrosis, is predictive of hospitalization and death and may provide incremental prognostic value over natriuretic peptide levels in patients with HF. Gal-3 has also been proven as a useful diagnostic marker for the differentiation of benign and malignant thyroid nodules, whereas its value for the diagnosis/ prognosis of other malignant and chronic diseases, e.g. diabetic nephropathy, is under intensive investigations.

Due to its important roles in different pathologies, Gal-3 has also been recognised as a potential therapeutic target. However, designing selective Gal-3 inhibitors is challenging because of the shared homology of the carbohydrate-recognition domains among not only galectins, but also other lectins. Yet, several Gal-3 agonists, either plan-based (GCs-100, GM-CT-01, GR-MD-02, modified citrus pectin) or synthetic (TD139) are in different phases of clinical trials as a potential drugs for different chronic diseases, e.g. NASH advanced fibrosis, chronic kidney disease, idiopathic pulmonary fibrosis, osteoarthritis, etc. as well as malignant diseases, e.g. chronic lymphocytic leukemia, melanoma, colorectal cancer, metastatic melanoma, etc.

Our long-standing interest in Gal-3 has recently been directed on its involvement in the adaptation response of cardiovascular system (CVS) to recreational SCUBA diving, which represents a special form of physical activity, due to the body exposure to low temperature, hypoxemia and elevated pressure. Our studies of the effects of single dive and repeated dives on CVS, showed significant changes not only in Gal-3 plasma concentration, but also in the levels of other CVS biomarkers, such as hs-TnI, NT-proBNP, VEGF, endothelin-1 and myoglobin. Although transient, these changes suggest extensive activation of adaptation mechanisms, which in some aspects could possibly have a positive effect of SCUBA diving on CVS.

Serum non-coding RNA profiling as a promising diagnostic approach

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Serum non-coding RNAs (ncRNAs) have been identified as paracrine and endocrine messengers of different diseases. It has now been widely acknowledged that ncRNAs a new area in the field of biomarkers has emerged. ncRNAs are RNA molecules of different sizes that are transcribed as independent genes or as part of protein coding genes and are not translated, therefore they do not produce proteins. They have been classified according to their size and function and include micro RNAs (miRNAs), piwi RNAs (piRNAs), piwiRNAs (piRNA), short ncRNAs and long non-coding RNAs (lncRNAs). These non-coding RNAs are present in different cell compartments participating in multiple cell functions, but they have also been identified in biological fluids, also known as cell-free or circulating ncRNAs, where they can be detected in exosomes, bound on lipoproteins as well as free circulating molecules. The role of circulating ncRNAs is still under investigation but are believed to be paracrine or endocrine messengers to systematically deliver signals between cells and tissues. Extensive studies have implicated a family of ncRNAs, this of miRNAs in disease pathogenesis and their potential as diagnostic and prognostic biomarkers of diseases. Recent evidence have identified additional families of ncRNAs such as piRNAs or lncRNAs as potential diagnostic tools both in the serum and in tissues. Detecting ncRNAs in biological fluids has opened a new field in Clinical Chemistry utilizing them as biomarkers of diseases or prognostic markers for different pathological conditions. To date, individual ncRNAs or groups of ncRNAs are being used to facilitate disease diagnosis. Nevertheless, diversity between individuals and pathogenetic mechanisms limits their specificity for most conditions. As high throughput analyses are becoming wider used and more affordable, ncRNA profiling is emerging as a diagnostic and prognostic approach. Profiling utilizes next generation sequencing approaches and allows screening of all ncRNAs in biological fluids or cell extracts, thus providing a comprehensive view of the changes in any particular patient. Serum ncRNA profiling coupled with bioinformatics analyses that identify targets and functions associated with the target genes, provides evidence for a direct impact of the circulating ncRNAs on disease pathogenesis. A recent example published by our group has shown that ncRNA profiling identified miRNAs and piRNAs as biomarkers of male subfertility and associated those with hypogonadism.

Additional examples in cancer patients have indicated that changes in serum ncRNA profiling reflects changes in cancer growth and may predict disease outcome. Thus, profiling of ncRNAs will provide a diagnostic tool that allows global understanding of changes occurring in diseases. Thus, ncRNA profiling coupled with proteomics analyses in patient samples is the foreseeable future in diagnostics.

Ethical issues in (pharmaco) genetics

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Apart from genetic testing for diagnostic purposes, application of genetics in human medicine encompasses genetic interventions and pharmacogenetical testing which are becoming more frequently utilized in clinical practice, as well as genetic studies employed in the process of research and drug development.

It’s been widely known and accepted that application of a drug in equal dosing regimens for treatment of the same disease in different patients, doesn’t produce equal results regarding achievement of a therapeutic effect and/or occurrence of side effects. Investigating the genetic cause for interindividual variations in patients’ drug response and toxicity, pharmacogenetics holds valuable prognostic and predictive value in tailoring the pharmacological treatment of various diseases according to the principles of precision medicine.

But, just as any other medical testing, genetic analyses impose ethical risks which in this case are even more serious due to the following specific features of these tests and the obtained data: the “mutual” ownership of the genetic information by individuals from the same family, the lack of precise phenotype-genotype correlation and the influence of epigenetic and environmental factors on the phenotypic expression of genetic information, the balance between the right of an individual “to know” and the right “to not know” as well as the enormous potential for discrimination. The rapid advancement of high throughput technologies delivering a mass of detailed data on an individual’s genome introduces a lot of advantages in scientific and clinical applications, but also threatens with a tremendous risk for misuse of these data in various settings.

The lecture discusses the fundamental ethical principles applicable to genetic analyses/studies including respect of the individual’s autonomy and privacy and commitment to providing confidentiality, beneficence and justice. The informed consent as well as the levels of anonymization in genetic testing as measures to satisfy the above mentioned principles will be addressed. Special emphasis will be placed on the ethical issues regarding orphan and rescued drugs emerging in the pharmacogenetical testing within clinical studies in drug research and development. Philosophers of science claim that science is morally neutral, it is actually the use and implementation of science that can have positive or negative impact. Hence, it is crucial to understand that achievement of our aim for humane application of (pharmaco)genetics can only be accomplished if technological and clinical advances in this field advance at a similar rate with the corresponding ethical considerations.

The relationship between adiposity parameters and hsC-reactive protein values in overweight and obese women

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Overweight/obesity has become an important health problem in developed countries and as a result of the rising epidemic of obesity, understanding body fat distribution and its clinical implications is critical to timely treatment. Adipose tissue is anatomically distributed in different proportions throughout the human body, but the percentage of adipose tissue is higher in women, the elderly and overweight individuals. Visceral adipose tissue is a hormonally active component of total body fat, which possesses unique biochemical characteristics that influence several normal and pathological processes in the human body. It has been distinctly linked to several pathological conditions including impaired glucose and lipid metabolism, insulin resistance, several malignancies, increased incidence of infections and non-infectious complications, and increased mortality

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Photodynamic activity properties of novel BODIPY compound against colorectal cancer cell line

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Colorectal cancer (CRC) is the third most common cancer type and the second leading cause of cancer-related mortality worldwide in 2018 according to World Health Organization reports. Photodynamic therapy is a well-established clinical modality for treating various types of cancers. BODIPY compounds are promising molecules for diagnostic and therapy usage in cancer. In this study, photodynamic activity potential of water soluble novel BODIPY compound bearing pyridine group using different techniques were investigated. The photochemical and CT-DNA binding properties of water soluble novel BODIPY compound bearing pyridine groups (6a) were investigated absorption titration, competitive ethidium bromide and viscosity experiments. The DNA cleavage activities and topoisomerase I and II inhibition properties of compounds were investigated using pBR322 DNA on agarose gel electrophoresis. The cytotoxic and phototoxic effects of the compound were tested against human colorectal (HCT-116) cell line using MTT assay and flow cytometer. The singlet oxygen quantum yield of 6a was 0.21 in photochemical studies. The DNA binding experiments suggested that 6a interacted with DNA via non-covalent modes. 6a significantly cleaved pBR322 plasmid DNA forming singlet oxygen with light irradiation. The topoisomerase studies suggested that 6a inhibited enzymes in a concentration-dependent manner. In the cell culture studies, 6a had lower cytotoxic and higher phototoxic effects. In addition it induced apoptosis on HCT-116 cells. The results suggested that it was thought that 6a had a promising photosensitizer agent for CRC.

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Metabolomics and biomarkers in inborn errors of metabolism

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Inborn errors of metabolism are inherited disorders resulting from mutations affecting functional proteins such as an enzyme/transport/activator protein in the metabolic pathways or organelle function that cause an interruption of protein, fat, carbohydrate, steroid, nucleic acid, membrane, neurotransmitter etc. metabolisms. To date, over 1000 inborn errors of metabolism have been identified. Although they are individually rare disorders, the cumulative incidence is 1/1000 live births. Age of presentation can vary from infancy to adolescence with a wide clinical spectrum, the more severe forms appearing in early childhood accompanied by significant morbidity and mortality. Nowadays, treatment options including enzyme replacement, substrate reduction, cell and organ transplantation and gene therapies are available and early diagnosis is becoming important for early treatment. In recent years, with the development of high-throughput technologies, metabolomic studies have advanced and new biomarkers have started to emerge for early diagnosis and treatment follow-up. Metabolomics is comprehensive analysis of metabolites (<1500Da) in a biological specimen that can enable precision medicine at a number of levels, including the characterization of metabolic derangements and metabolic phenotypes that underlie disease, discovery of new therapeutic targets, and discovery of biomarkers that may be used to either diagnose disease or monitor activity of therapeutics. Structural and functional information on 247 metabolites associated with 147 inborn errors of metabolism and 202 metabolic pathways involved in various inborn errors of metabolism have been reported in the human metabolome database (HMDB). Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)-based technologies are reference methods for extracting comprehensive and unbiased chemical information from complex mixtures of metabolites. Both targeted and untargeted mass spectrometry-based metabolic approaches have been used to expand the range of disease-associate metabolites. In the targeted approach, specific metabolites are detected, quantified and compared to establish reference ranges. The untargeted approach consists of analysis of all detectable metabolites known and unknown in a single test performed on a biological sample to determine any perturbation of single or multiple metabolites and of related biochemical pathways. Among the first well-known targeted metabolomic for inborn errors of metabolism include acylcarnitine, amino acid and organic acid analyses in biological samples for screening of the disorders. Aminociclopeptides, organic acidurias and fatty acid oxidation disorders were investigated by using the targeted metabolomic analyses. Beside them, analyses of oxysterols (Cholesterol-3β, 5α, 6β triol and 7-ketocholesterol) as biomarkers for Niemann-Pick Type C, and bile acids in the diagnosis of hereditary bile acid metabolism defects have been performed by targeted mass spectrometry-based analyses in Central Laboratory of Hacettepe University Hospitals. Classical bile acids, hydroxylated bile acids, 3β-hydroxy-Δ7-bile acids, 3-oxo-Δ7-bile acids, short-chain bile acids, long-chain

Pharmacology abstracts of novel BODIPY compound against colorectal cancer cell line

in hospital. Visceral obesity itself is an independent component of metabolic syndrome and the magnitude of obesity directly relates to the prognosis of this condition. It may be related to presence of low-grade inflammation in white adipose tissue. Precise mechanisms of chronic inflammation induction in obesity as well as the relation between obesity and inflammatory markers are yet to be explained. So far, the importance of high sensitive C-reactive protein (hs-CRP), as the most versatile inflammatory marker, is still in the spotlight. hs-CRP outstands as independent risk factor, apparent from traditional risk factors such as increased total cholesterol, increased levels of glucose and homocysteine, hyperinsulinemia, high body mass index (BMI), smoking and physical inactivity. As a hormonally active, VAT releases different bioactive molecules and hormones, such as adiponectin, leptin, tumor necrosis factor, resistin and interleukin 6 (IL-6). Among these hormones, adiponectin is of particular significance owing to its protective antiangiogenic activity. In addition, in obese persons, white adipose tissue is infiltrated by macrophages with increased local production of proinflammatory mediators. These factors promote acute phase reaction and chronic inflammation in obese persons. However, some authors proposed the existence of a subgroup of obese persons who are metabolically normal (without increased risks of heart diseases, type 2 diabetes, hypertension, stroke, cancers, etc.). They hypothesize that in this subpopulation obesity seems to be uncomplicated and is characterized by early onset, hyper-plasticity of otherwise normal adipocytes, and peripheral type of fat distribution. The inflammation in these persons should be absent, and they supposed to have normal levels of inflammatory marker. The aim of this study was to investigate the levels of inflammatory marker CRP and adiponectin and their relation to standard anthropometric parameters [body mass index (BMI) , waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHRi)], in population of apparently healthy overweight and obese females. This study enrolled 76 overweight (BMI between 25 and 29.9 kg/m² ) and 45 obese (BMI ≥ 30 kg/ m²) females, non-smokers, aged 18-45 years, without any comorbidities, and with regular menstrual cycles. Standard anthropometric measurements were performed: body weight (BW), body height, WC and hip circumference and followed parameters were calculated: BMI [kg/m² ], waist-to-hip ratio (WHR), waist-to-height ratio (WHRi). Quantitative determination of hs-CRP was determined using particle enhanced turbidimetric assay on the Cobas Integra 400 plus autoanalyser. The measuring range of hs-CRP was 0.1-20 mg/L, with a lower detection limit of 0.1 mg/L. Levels of the total adiponectin were measured by an ELISA competitive enzyme immunoassay for quantitative measurement of the human adiponectin, using commercially available kits (BV51001 Human Adiponectin). Average hs-CRP was 5.36 ± 2.43 mg/L, and significantly positively correlated to all investigated anthropometric parameters. Statistical analysis showed the significant difference between the overweight and obese group for all investigated anthropometric parameters, except for the age as well as CRP values. Average adiponectin was 9.88±4.4 and correlated both negatively and significantly with the waist circumference, BMI (p<0.001). The major characteristic of our results is significant difference observed between overweight and obese subjects in almost all important features. Besides the anthropometric differences which were almost all important features. Besides the anthropometric differences which were expected (BW, BMI), in the overweight group we recorded significantly lower values of parameters that reflect the metabolic risk: WC, WHR, WHRi, as well as significantly lower values of inflammatory marker CRP. Our results confirmed that CRP is a valuable marker of metabolic risk in obese females, and BMI, although not so new, is still a reliable parameter of adiposity.

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bile acids, differential bile acids can be screened in urine samples by LC-MS/MS. An increase in 3-oxo-4-α-bile acids in Δ4-3-oxo-steroid 5β-reductase deficiency, an increase in 3β-monohydroxy-Δ4 bile acids in oxysterol-7α-hydroxylase deficiency, an increase in bile acid alcohol in peroxisomal diseases can be observed. Decreasing in unusual bile acids can be observed during treatment and follow-up. With targeted metabolic approaches using MS technology, biomarkers for different inborn errors of metabolism can be detected. Beside oxysterols, lysylo-pingomyelins-509 for Niemann-Pick Type C, glycosaminoglycans for types of Mucopolysaccharidoses, globotriaosylphosphoglycerine (LysyGib) for Fabry, Lyso-Gb1 for Gaucher are examples for biomarkers of some inborn errors of metabolism that can be detected by targeted metabolomic analyses. The best approach to metabolic study of complex inborn errors of metabolism may be the combination of untargeted approach, that span the breadth of metabolome and perform pathways analysis, with targeted approach, that measures specific metabolites and establishes their reference intervals. The integration of genomic with metabolomic data will improve diagnosis and prognostication of inborn errors of metabolism.

Key words: Metabolomics, mass spectrometry, biomarkers, inborn errors of metabolism

Genetic Technologies in Inborn Errors of Metabolism

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Inborn errors of metabolism (IEM) are inherited single gene disorders caused by a deficiency of an enzyme, its cofactor or a transporter protein in synthesis or catabolic pathway of proteins, carbohydrates and fatty acids. Neurometabolic diseases, which are also considered as a subgroup of metabolic diseases, result in neuronal damage due to inability to synthesize essential biochemical substances, abnormal accumulation or formation of toxic metabolites. Although these disorders are individually rare, collectively they account for a significant portion of childhood genetic disorders. The incidence of metabolic / neurometabolic diseases is generally reported as 1/4 000 - 1/5 000 in the worldwide. The rate of consanguineous marriages in Turkey is 21.4% resulting in a population with relatively high frequency of metabolic/neurometabolic disorders compared to other countries. Metabolic/ neurometabolic disorders are considered rare diseases in other countries however these disorders are much more common in Turkey. IEM are quite heterogeneous and severe genetic diseases with a variety of overlapping or unspecific clinical phenotypes. Even though primary diagnosis of IEM is done by clinical suspicion and biochemical tests, genetic investigations play a significant role for appropriate patient treatment and genetic counselling as indispensable tool.

Since the sequencing of the first human genome in 2001, genomic technologies have made a huge impact across many fields such as medicine, computational and information technology, and healthcare. For many years there have been a variety of technologies and tools used in genome analysis. However, only in the past decade there has been rapid revolutionizing progress and improvement in high-throughput methods. These methods are ranging from traditional conventional laboratory genetic techniques of microscopic cytogenetics, fluorescence in situ hybridization (FISH), southern blotting, denaturing gel electrophoresis, single stranded conformation, restriction fragment length polymorphism (RFLP), mapping and genotyping studies using microsatellite markers and many other nonsequencing genetic laboratory methods to more complex systems, such as microarrays and next-generation sequencing. Developing these new methodologies allow rapid, high specificity and high-throughput and cost-effective analysis of a large number of samples from small amount of biological material. Also, utilization of advanced genetic technologies applications/analysis has led to the rapid and accurate diagnosis of the diseases and as a result several novel diseases have lately been defined.

IEM are generally inherited as autosomal recessive although dominant, mitochondrial and X-linked types of inheritance are also possible. The genetic defects include point mutations, deletions, insertions or chromosomal abnormalities that result in loss- or gain-of-function of mutant enzymes or proteins.

Depending on the variant type and locus, there are numerous different genetic methods and tools for the variant detection. For example, due to its simplicity the most frequent method for the analysis of a large (>5 Mb) chromosomal aberration is karyotype analysis by using the GTG banding technique. Other molecular genetic methods, such as microarray-based comparative genomic hybridization (aCGH) or fluorescent in situ hybridization (FISH), could be applied for a more accurate analysis. Moreover, for detection particular variant another molecular genetic methods might be applicable, which include restriction enzyme assay of specific DNA sequence. Direct DNA sequencing method (Sanger sequencing) is accepted as the “gold standard” for the identification of known as well as unspecified variants such as point mutations, small deletions and duplications in the genomic DNA. Although the majority of the mutations accounting for IEM are point mutations, sometimes, large deletions and insertions and copy number changes can be causative. For this purpose, the most commonly used technologies are “duplex ligation-dependent probe amplification (MLPA)” and “comparative genomic hybridization (CGH).” High-throughput single-nucleotide polymorphism (SNP) microarray is also produces genome-wide results that designed for genotyping a patient’s DNA for genome-wide association studies (GWAS) and co-segregation studies to determine linkage between a disease locus and a chromosomal region These results are very useful in complementing the next-generation sequencing results. Furthermore, SNP array platforms may now also include a large number of probes specifically for the detection of copy number variants. SNP arrays and aCGH arrays also provide accurate tools for detecting small deletions and duplications.

In recent years “next-generation sequencing technologies” have enabled investigation of hundreds and thousand of targeted genes, even the whole exome and whole genome, at single base pair resolution. Large deletions and insertions can also be detected by this technology. The use of whole exome sequencing (WES) has facilitated the identification of the many novel genes responsible for metabolic/neurometabolic diseases. In terms of rare genetic diseases, the identification of the genetic basis of disease is reached in 20 to 40% of patients using WES. The most important and crucial part of exome and genome sequencing, is to find the disease-causing mutation among the thousands of variations. The structural or functional effect of the variation on protein or enzyme determines whether the nucleotide changes are a mutation that causes disease pathology. Base changes that result in amino acid changes (missense mutations) are evaluated by software programs including PolyPhen-2, SIFT, and Mutation Taster that rate the pathogenicity of the amino acid change. These programs estimates of how tightly the base is conserved over evolution, whether the amino acid changes charge, size, or conformation, and, sometimes, where in the protein the amino acid change reside. Functional studies may be required to demonstrate the pathogenic effect of a mutations. Genome databases, disease databases, mutation databases can be used to investigate clinical phenotypes caused by different mutations.

Depending on the location and type of mutations, their effects on protein and clinical findings in patients may vary widely. Mutations that cause clinical phenotype are now being investigated by comprehensive methods including collectively evaluating all changes at the genomic level, searching for modifying genes, epigenetic changes and genome-wide bioinformatic analysis methods. Molecular diagnostic methods are of increasing importance in many medical applications such as elucidating the pathophysiology of the disease, identifying patients and carriers and providing genetic counseling services, identifying asymptomatic individuals, differential diagnosis of atypical patients, and developing effective treatment and follow-up for patients.