Symposium lectures

S1 - Can we predict the outcome in critically ill patients?

S1 - 1

Ionized calcium: what to report?

Baird Geoffrey

University of Washington, Laboratory Medicine, Seattle, United States

Corresponding author: gbaird@u.washington.edu

Measuring the calcium concentration in blood is often part of the care of critically ill patients. Many disease states disrupt the complex, multi-organ endocrine system that normally regulates calcium concentration, and assessment of calcium concentration permits one to assess and correct any disturbance and thereby prevent untoward sequelae. While calcium can be measured in several ways - total calcium, albumin-corrected calcium, ionized calcium, and pH-corrected ionized calcium - no method is perfect in all respects. Methods that accurately assess the concentration of the physiologically relevant free cation in solution, so-called "ionized calcium", are more costly and manual, whereas methods that measure the total calcium concentration with or without a protein-binding correction factor are less expensive and highly automated. In choosing which method to use, therefore, one needs to consider the factors that introduce errors in the methods that employ corrections or estimations. The confounders of estimated or corrected values of calcium include blood pH and the concentrations of binding proteins, small molecule chelators, and free fatty acids, and the concentrations of these substances are often abnormal in the blood of critically ill patients. Therefore, the most accurate assessment of the (patho)physiologic calcium concentration in a critically ill patient is one that integrates the effect of the variables present in the patient at the time of measurement. Exceptions to this approach include situations in which preanalytical errors, such as exposure to air, have knowingly altered one or more properties of the sample.

S1 - 2

Glucose regulation in ICU patients; Do's en Dont's

Ligtenberg Jack

University Medical Center Groningen (UMCG), The Netherlands, Emergency Medicine, Groningen, Netherlands

Corresponding author: j.j.m.ligtenberg@umcg.nl

The outcomes of intervention studies implementing intensive insulin therapy aimed at tight glucose control (TGC) are yet not conclusive. Currently, four truly randomized clinical trials evaluating the clinical benefit of TGC have been performed. Out of these four trials, three were negative from a mortality point of view. Obtaining TGC in a real-life ICU setting may result in varying, sometimes disappointing, results. There is concern about an increasing incidence of hypoglycemic episodes. Computerized protocols give the best results and fewer hypoglycemic episodes. Point-of-care blood gas/glucose analyzers present the best trade-off between accuracy and speed of measurement. Closed-loop systems are not yet available for clinical use. Clinicians should take care in selecting both the patient group and target blood glucose level. Although consensus is that frank hyperglycemia (i.e. levels over 8.5–10 mmol/L [150–180 mg/dL]) should not be tolerated, the optimal target is far from clear. A target glucose level of 7–8 mmol/L has been is advised, below which the association between glucose level and adverse outcome subsides. As long as doubts remain about the potential benefits, it is important to perform TGC in a safe way. This can be done with a nurse-driven (computerized) protocol, using arterial blood samples measured on a point-of-care blood gas analyzer. Insulin administration should be continuous. Periodical monitoring of performance and modification of the protocol leads to best results.

S1 - 3

Can lactate-guided therapy improve the outcome in critically ill patients?

Funk Georg-Christian

Otto Wagner Hospital, Department of Respiratory and Critical Care Medicine, Vienna, Austria

Corresponding author: georg-christian.funk@meduniwien.ac.at

Sepsis and septic shock are severe conditions with high mortality requiring intensive care treatment. Therefore sensitive and specific markers of therapeutic success or failure are required. Lactate clearance is the percent change of the plasma lactate concentration during a specified time. Multiple observational studies in patients with various forms of shock showed that lactate clearance < 20% over 2 hours is associated with increased mortality. Moreover two randomized trials in critically ill patients have shown the usefulness of lactate clearance for the management of sepsis and shock. Lactate clearance should be used as an adjunct to the standard measures of goal directed sepsis therapy such as venous oxygen saturation, central venous pressure, urine output and hemoglobin. In summary a lactate clearance > 20% over 2 hours should be targeted in critically ill patients with severe sepsis or septic shock and elevated plasma lactate.

S2 - Kidney biomarkers and formulas

S2 - 1

Estimating glomerular filtration rate in 2012: the creatinine-based equations

Delanaye Pierre

University of Liège, Nephrology-Dialysis-Transplantation, Liège, Belgium

Corresponding author: pierre_delanaye@yahoo.fr

The best overall index of renal function is the glomerular filtration rate (GFR). Since measuring GFR can be cumbersome and costly, estimation of GFR is essential for diagnosis and evaluation of chronic kidney disease, defined as kidney damage or GFR < 60 mL/min/1.73m^2 for ≥ 3 months. Serum creatinine is the classical biomarker used to estimate GFR. Because serum creatinine is also dependent on muscular mass, several authors proposed different creatinine-based equations to estimate GFR. Among these equations, the MDRD and CKD-EPI equations have been shown to be the most accurate. We will describe the advantages but also the limitations, including analytical limitations, of such equations. We will comment the differences of performance between the MDRD versus the CKD-EPI equations. We will eventually underline clinical situations where measuring GFR by a reference method is still required.

S2 - 2

New kidney biomarkers

Kes Petar

University Hospital Centre Zagreb, Department of Internal Medicine, Division of Nephrology, Arterial Hypertension, Dialysis and Transplantation, Zagreb, Croatia

Corresponding author: keszpeter@net.hr

An ideal biomarker of acute kidney injury (AKI) or chronic kidney disease (CKD) should identify the primary location of injury, address the duration of kidney failure, identify the aetiology, stratify risk and estimate prognosis, define the course of the disease and allow the monitoring of response to interventions.

Serum creatinine is a suboptimal marker following injury. In the setting of AKI, the delay between changes in serum creatinine and changes in GFR inhibits the ability to accurately estimate timing and severity of injury. Human neutrophil gelatinase-associated lipocalin (NGAL) seems to be one of the earliest markers in the kidney after ischaemic or nephrotoxic injury may be detected in the blood and urine of humans soon after AKI and de-

layed graft function in kidney transplantation. In AKI, urinary excretion of cystatin C has been shown to predict the requirement for renal replacement therapy earlier than creatinine. Kidney injury molecule-1 (KIM-1) seems to be highly specific for ischaemic AKI and not for pre-renal azotaemia, CKD or contrast-induced nephropathy N-acetyl-β-D-glucosaminidase (NAG) has been shown to function as a marker of kidney injury, reflecting particularly the degree of tubular damage. Interleukin-18 (IL-18) is a pro-inflammatory cytokine detected in the urine after acute ischaemic proximal tubular damage. A single marker may not satisfy the requirement of predicting AKI or CKD progression and mortality. It is more likely that a focused panel of biomarkers will be most rewarding.

S2 - 3

Microalbuminuria: immunoassay and/or HPLC

Wittmann Istvan

University of Pécs, Faculty of Medicine, 2nd Department of Medicine and Nephrological Center, Pécs, Hungary

Corresponding author: istvan.wittmann@aok.pte.hu

Urinary albumin excretion is a good marker of development and progression of kidney, cardiovascular, and probably of some gastrointestinal diseases. The uniform use of this marker in these heterogeneous pathologies is questioned in the last a few years, because excreted albumin and albumin fragments, oxidation, carbonylation and other modifications of the albumin are different in each disease. Many methodologies are used for the determination of albumin in the urine, as e.g. immunoassay, HPLC, LC-MS. Current recommendation prefer immunoassay using polyclonal sera and albumin/creatinine ratio, but identified some clinical needs for standardization. Recently a size-exclusion liquid chromatography method has been developed for the identification of intact, monomer albumin in the urine. This new approach using HPLC have shown that there are albumins in the urine not reacting with the antibodies used in the immunoassay. Thus, in diabetic patients, in the normo- or in the microalbuminuric range a 2-3 times higher albumin excretion was detected by HPLC compared to the immunoassay. This raised a novel hypothesis about the existence of the immuno-unreactive, nonimmunoreactive or immunochemically nonreactive albumin in the urine. The exact nature of this immuno-unreactive albumin is not known and the clinical significance is also matter of debate. This HPLC method is not validated in non-diabetic kidney diseases and seems to be not measuring albumin in inflammatory bowel diseases, since albumin peak consisted mainly of alfa1-acid-glicoprotein and Zn-alfa2-glicoprotein.

S3 - The bleeding patient

S3 - 1

Disseminated intravascular coagulation-clinical approach and lab orientation

Paraskevopoulou Penelope

Konstantopoulio General of Nea Ionia, Hematology Lab, Athens, Greece

Corresponding author: pparaskevopoulou@hotmail.com

Disseminated intravascular coagulation (DIC) is an acquired disorder of hemostasis and is estimated to be present in 1% of hospitalized patients. DIC is a thrombohemorragic disease characterized by the systemic (not local) intravascular activation of coagulation, activation or impairment of fibrinolysis, activation of inflammatory cytokines, complement activation and endothelium damage. These conditions lead to excess thrombin generation, intravascular deposition of fibrin, microvascular thrombosis, consumption of clotting factors, inhibitors and platelets, inadequate removal of fibrin deposition in small vessels, tissue ischemia, necrosis and organ failure with clinical evidence of an excessive bleeding.

The clinical diseases related with DIC are sepsis, severe infections, severe trauma, malignancies, transfusion complications, obstetrical complications (HELLP syndrome, placenta detachment, amniotic fluid embolism) etc.

The diagnosis of DIC is based on clinical and laboratory findings. The main clinical finding is sudden bleeding from multiple sites. Lab findings for DIC are: elevated D-dimers (fibrin degradation products), elevated levels of soluble fibrin monomers (sFM), prolongation of prothrombin time (PT), prolongation of activated partial thromboplastin time (aPTT), low fibrinogen levels, thrombocytopenia and presence of schistocytes on blood smear.

The indicated therapy is the treatment of the underlying disease, otherwise blood products are used to manage the bleeding.

S3 - 2

The use of new thrombelastography (TEG) technologies in critically bleeding patients

Johansson Per

Section for Transfusion Medicine, Capital Region Blood Bank, Rigshospitalet, Copenhagen, Denmark

Corresponding author: rh08061@rh.dk

Introduction: Death due to trauma and massive transfusion is the leading cause of lost life years worldwide, with haemorrhage being responsible for 30-40% of trauma mortality and accounting for almost 50% of the deaths the initial 24h. Furthermore, development of coagulopath is associated with a several-fold increase in morbidity and mortality.

Recent findings: Plasma-based routine coagulation tests (RCoT), like prothrombin time (PT) and activated partial thromboplastin time (APTT), are inappropriate for monitoring coagulopathy and guide therapy in critically ill bleeding patients. Instead viscoelastic haemostatic assays such as Thrombelastography (TEG®) should be used. Clinical studies including more than 5.000 surgical patients have consistently reported on the benefit of using the TEG to identify coagulopathy, predict need for transfusions including massive transfusion and enable goal-directed therapy. New TEG assays such as TEG functional fibrinogen and TEG platelet mapping enables identification of coagulopathies secondary to fibrinogen deficiency and the effect of platelet inhibitors respectively and thereby an even more detailed picture of the individual patients hemostatic competence. Also, RapidTEG enables identification of impaired clot development/ strength within 5 min, which is of particular value in patients with life-threatening bleedings.

Conclusion: Routine use of viscoelastical hemostatic assays is recommended by international guidelines concerning patients with critical bleedings.

S3 - 3

Laboratory assessment of bleeding risk

Alberio Lorenzo

Inselspital, Universitätsspital Bern, Universitätsklinik für Hämatologie und Hämatologisches Zentrallabor, Bern, Switzerland

Corresponding author: lorenzo.alberio@insel.ch

During this lecture I will address: 1) The prevalence and type of bleeding disorders among an unselected patient population scheduled for elective surgery, 2) The predictive values of standard laboratory tests for identifying specific haemostatic defects, 3) The utility of a structured bleeding history for identifying patients requiring a detailed pre-surgical laboratory work-up, and 4) The clinical relevance of laboratory-based and point-of-care-based assays for identifying patients at increased bleeding risk.

S4 - Myeloproliferative neoplasms: diagnosing and molecular monitoring of targeted therapies

S4 - 1

Chronic myeloid leukemia – diagnostics and treatment according to ELN recommendations

Mueller Martin M.

Medizinische Fakultät Mannheim der Universität Heidelberg, Scientific Laboratory, III. Medizinische Klinik, Mannheim, Germany

Corresponding author: Martin.Mueller@medma.uni-heidelberg.de

Chronic myeloid leukemia (CML) has become a model disease since the introduction of the first clinically effective tyrosine kinase inhibitor imatinib. The potent reduction of leukemia load gave rise to the need for more sensitive methods of monitoring. These were realized by the establishment and further development of quantitative polymerase chain reaction techniques. The value of different methods of monitoring (conventional cytogenetics from bone marrow metaphases versus RQ-PCR from peripheral blood samples) will be described according to published data. This presentation will review the current ELN recommendations for monitoring and treatment and outline the most recent publications about new drugs and tighter milestones to be fulfilled in the future of modern CML management. Further the prerequisites of treatment discontinuations will be discussed within the scope of pilot study data.

S4 - 2

Measuring response to tyrosine-kinase inhibitors in chronic myeloid leukemia – molecular monitoring

Zadro Renata

University Hospital Center Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: rzadro@mef.hr

Therapy with tyrosine-kinase inhibitors (TKI) in newly diagnosed chronic myeloid leukemia patient progressively reduces the number of Philadelphia positive cells. As the level of bcr-abl1 fusion transcript correlates well with the number of leukemic cells, molecular monitoring of TKI therapy by quantitative polymerase chain reaction (RQ-PCR) is essential part of patient management. According to European LeukemiaNet (ELN) guidelines, optimal molecular response is defined as major molecular response (MMR = 0.1% bcr-abl1/abl1 according to International Scale - IS)) and complete molecular response (CMR). Molecular monitoring is carried out every 3 months until MMR is achieved and then at least every 6 months to confirm MMR and to reach possible CMR. Molecular monitoring is important to predict therapy response as MMR reduces the risk for disease progression. Earlier achievement of MMR means also higher probability to achieve CMR and stability of response. The importance of CMR detection is that CMR is accomplished in higher percentage with second generation TKI. Major tasks of the EUTOS for CML (European Treatment and Outcome Study for CML, supported by Novartis) within the ELN is to promote the standardization of bcr-abl1 detection via RQ-PCR, as well as definition and standardization of CMR. Active participant in this project is Department of Laboratory Diagnostics, University Hospital Center Zagreb which serves as a reference laboratory for Croatia, Bosnia and Herzegovina, Macedonia, Serbia and Albania for the assignment of a conversion factor in the process of harmonization of quantitative results according to IS.

S4 - 3

Classification and diagnosis of myeloproliferative neoplasms (MPN)

Kušec Rajko

University of Zagreb School of Medicine and Dubrava University Hospital, Department of Hematology and Division of Molecular Diagnostics and Genetics, Zagreb, Croatia

Corresponding author: rkusec@irb.hr

World Health Organization classification for hematologic malignancies of 2008 under the term myeloproliferative neoplasms recognizes chronic myelogenous leukemia Philadelphia-positive, chronic neutrophilic leukemia, polycythemia vera (PV), primary myelofibrosis (PMF), essential thrombocythemia (ET), chronic eosinophilic leukemia NOS, mastocytosis and myeloproliferative neoplasms, unclassifiable. In research of biology of these diseases the discovery of frequent point mutation *V617F* in *JAK2* gene has triggered huge effort in explaining its importance in pathogenesis, but also in diagnosing and beyond in MPNs. This led to detection of other JAK-STAT pathway activating mutations: exon 12 of JAK2 in roughly one third of V617F-negative PV, mutations of the MPL gene in V617F negative ET and PMF. Further, mutations of the CBL (Casitas B-cell lymphoma gene) in PMF, but not in ET or PV were recently found. Mutations of TET2 (TET oncogene family member 2) were described in PV, ET, PMF and in post-PV/post-ET myelofibrosis. The EZH2 was detected in smaller proportion of PMF patients, while CMML was observed with a high frequency of TET2, CBL, and EZH2 mutations. Very soon after new genetic discoveries their clinical evaluation in MPN diagnostic workup has been evaluated and the latest algorithms for application of genetic molecular markers will be presented. The basic view for the rationale use of molecular test in MPN is to start with JAK2V617F mutation and investigate JAK2V617F-negative patients for JAK2 exon 12 or MPL mutations, respectively. In case a MPN cannot be established, analysis for TET2, CBL, and EZH2 mutations may be indicated.

S5 - The inflamed gut

S5 - 1

Microbial sensing and epithelial cell function in IBD

Haller Dirk

Technical University München, ZIEL – Research Center for Nutrition and Food Science, CDD – Center for Diet and Disease, Freising, Germany

Corresponding author: haller@wzw.tum.de

Inflammatory bowel diseases (IBD), comprising the idiopathic pathologies ulcerative colitis and Crohn´s disease, are chronic disorders of the gastrointestinal tract. Genetic susceptibility and environmental factors are crucial parameters in the pathogenesis of IBD. Clinical data and experimental studies using gnotobiotic IBD models revealed that the intestinal microbiota plays a major role in the progression of the chronic intestinal inflammation. In this context, commensal bacteria of the intestinal microbiota can have deleterious or protective effects in the disease-susceptible host. First, we identified the 31.5 kDa metalloprotease gelatinase E (GelE) from *E. faecalis* OG1RF as a putative colitogenic structure. In order to investigate the role of GelE in the development of chronic intestinal inflammation, we monoassociated germfree wild type (Wt) and IL-10-/- mice with *E. faecalis* OG1RF and two isogenic mutant strains TX5264 and TX5266, both lacking GelE expression. Ussing chamber experiments with distal colon segments from non-inflamed IL-10-/- and TNFΔARE/Wt mice revealed the effect of GelE on epithelial barrier function before histological changes have occurred in the tissue. Second, we identified a cell-surface protein of the probiotic VSL#3-derived single strain Lactobacillus paracasei (L.p) mediating anti-inflammatory effects in chronic intestinal inflammation. VSL#3-derived L.p expresses a prtP-encoded lactocepin that selectively degrades IP-10 and other pro-inflammatory chemokines in the diseased tissue environment. In conclusion, we identified two bacterial proteases that displayed in

the context of chronic intestinal inflammation pro- or anti-inflammatory activity.

S5 - 2

Is the disease course predictable through biomarkers in inflammatory bowel disease?

Lakatos Peter Laszlo

Semmelweis University, 1st Department of Medicine, Budapest, Hungary

Corresponding author: kislakpet99@gmail.com

Clinical presentation and disease course of inflammatory bowel diseases (IBD) are heterogeneous and variable over time. During follow patients with Crohn's disease (CD) may eventually one day develop a structuring or a perforating complication and ulcerative colitis (UC) patients, and a significant number will undergo surgery/colectomy. Much emphasis was laid in recent years on the determination of important predictive factors. Laboratory markers have been investigated in IBD for diagnosis, assessment of disease activity, prediction of relapse, risk of complications and monitoring the efficacy of therapy. Markers can be classified as short term (e.g. CRP, ESR) or long term (e.g. serology and genetic) markers. CRP is the most studied and has the best overall accuracy. It correlates well with disease activity and risk for relapses in CD and to lesser extent in UC. More recently, it was shown to be associated with the risk of surgery. However, its use should be individualized, since a proportion of IBD patients fail to mount a CRP response. Other laboratory markers, including ESR, leucocyte and platelet count, have been studied either less extensively. Faecal markers (e.g. calprotectin) seem promising and are more specific in detecting active inflammation in the gut. Recent data suggest that the accuracy of calprotectin is superior in UC compared to CD. Long term markers including serology (e.g. ASCA, pANCA, glycans) or genetic markers(e.g. NOD2/CARD15) may assist the evaluation of disease phenotype in selected cases given that they all were shown to be associated with complicated disease phenotype and risk for surgery, especially in CD. In conclusion, laboratory markers are useful and-together with clinical and endoscopic markers-should be part of the global management of the IBD patients.

S5 - 3

Biological therapy for ulcerative colitis beyond anti-TNF

Lowenberg Mark

Academic Medical Center, Dept. of Gastroenterology and Hepatology, Amsterdam, Netherlands

Corresponding author: m.lowenberg@amc.uva.nl

Ulcerative colitis (UC) is one of the two major types of inflammatory bowel disease, along with Crohn's disease. The medical treatment of UC has received more and more attention from clinicians, researchers and the pharmaceutical industry over the last years. Although the management of UC has improved significantly since the introduction of anti-tumor necrosis factor antibodies, a considerable proportion of patients have a poor response to these agents. Fortunately, elucidating molecular pathways in UC pathogenesis is opening avenues for development of novel therapeutic strategies. Various biologicals and small molecules that selectively target inflammatory mediators have been designed and evaluated in clinical trials demonstrating promising outcomes. Vedolizumab is a monoclonal antibody directed against alpha4beta7 integrin and proved to be effective as induction and maintenance therapy for patients with moderate to severely active UC. Anrukinzumab, an antibody that specifically binds to interleukin-13, is currently undergoing phase II trials. Another promising therapeutic agent is the oral JAK3 inhibitor tofacitinib that is being evaluated in large phase II clinical trials for UC. Finally, the potential therapeutic implications will be discussed of the modification of luminal contents by faecal transplantation.

S6 - Early detection of COPD: biochemical mechanism revisited

S6 - 1

COPD heterogeneicity - clinical phenotypes

Popović-Grle Sanja

Zagreb University Hospital Center, Jordanovac Department for Lung Diseases, Zagreb, Croatia

Corresponding author: sgrle@post.htnet.hr

Chronic obstructive pulmonary disease (COPD) is disease with high morbiditiy and mortality. It is estimated that there are 300 billion COPD patients, double than diabetics, prevalence still increasing. About 65% of patients do not have established diagnosis. Reason for underdiagnosing is probably slowly progression of symptoms, as well as development in eldery. Important reason also is COPD hetegeneicity. COPD is a heterogeneous condition in terms of clinical presentation, underlying pathological mechanism, lung function measurements, systemic manifestations, comorbidities or treatment response.

The oldest hetegeneicity in COPD was recognized as different clinical phenotypes before 50 years. A typical patient with chronic bronchitis is obese person with livide lips, always sleepy, called "blue blotter", while typical person with emphysema is cachaexic, rose lips, visibly searching for more air, called "pink puffer". Today we think that the most important COPD characteristics that define different clinical phenotypes are presence or not of clinical symptoms such as cough and expectoration, with dyspnea, presence or not of frequent exacerbations (more than 2 per year), presence or not of cardiovascular comorbidities, osteoporosis, or depreesion, presence or not of cachaexia or obesitas, presence or not of systemic inflammation, and presence or nor of bronhodilator response in obstructive airways. Hetegeneicity in COPD is big challenge for each clinician, as well as for researchers, trying to find what is common to all COPD patients.

S6 – 2

Biomarkers based on pathophysiology

Abstract not provided.

S6 – 3

Airway epithelial cells alarm the immune system in chronic obstructive pulmonary disease

van Oosterhout Antoon JM.

University Medical Center Groningen, Pathology & Medical Biology, Groningen, Netherlands

Corresponding author: a.j.m.van.oosterhout@umcg.nl

COPD is characterized by chronic neutrophilic airway inflammation triggered by cigarette smoke (CS), leading to bronchitis or emphysema. Little is known about the initial phase of COPD pathogenesis. We showed that CS exposure of airway epithelial cells induces oxidative- and ER-stress responses ultimately leading to cell death. Interestingly, CS inhibits caspase-3 and 7 activities, abrogating apoptotic cell death and promoting necrotic cell death. In contrast to apoptotic cell-death, necrotic cell death leads to release damage-associated molecular patterns (DAMPs) that activate innate immune responses. Indeed, increased DAMPs levels were demonstrated after CS exposure of airway epithelial cells. These DAMPs were shown to induce Myd88-dependent IL-8 production in naïve epithelial cells indicating a role for innate immune receptors.

Furthermore, CS-induced neutrophilic airway inflammation in mice is preceded by epithelial sloughing and of the presence of DAMPs in BAL fluid. The profile of DAMPs and neutrophilic inflammation is genetically determined as shown by comparing different inbred mouse strains.

These early inflammatory responses to CS may be an important initial step to COPD development and may thus offer opportunities for early diagnosis and disease intervention.

S7 - Osteoporosis: investigations, fracture risk assessment and monitoring of treatment. A symposium sponsored by the Asian Pacific Federation of Clinical Biochemistry.

S7 - 1

Diagnostics of osteoporosis and fracture risk assessment

Palicka Vladimir

School of Medicine Hradec Kralove, Charles´ University Prague, Hradec Kralove, Czech Republic

Corresponding author: palicka@lfhk.cuni.cz

Osteoporosis is a systemic, progressive disease of the skeleton, characterized by the decrease of bone mineral content, damaged bone microarchitecture, and increased bone fragility. The ideal diagnostic method should be the measurement of bone fragility, which is the main therapeutic target. Bone fragility cannot be measured in clinical practice and we use surrogate markers.

Clinical examination is of limited use as clinical signs are less expressed and are mostly non-specific.

Common and broadly accepted method for osteoporosis diagnosis is the measurement of Bone Mineral Density (BMD) by Dual-Energy X-Ray Absorptiometry (DXA). BMD is well standardized and results (usually expressed as T-score) correspond to the increased fracture risk. On the other hand, BMD could be very different in different sites of skeleton and more fractures are observed in patients with osteopenia than with osteoporosis.

Bone markers reflect bone remodelling. Increased rate of remodelling is connected to increased fracture risk. Low level of standardization and many influences from out-of-the skeleton decrease the usefulness of bone markers in diagnostics; they are mostly used for monitoring of treatment and patient´s compliance.

Direct assessment of bone microarchitecture by histomorphometry cannot be broadly used in the diagnostics as the procedure is invasive and painful.

New methods like trabecular pattern score (TBS) or HRpQCT provided new insights into the bone structure; the relation with fracture risk is under deep investigation. Individual fracture risk assessment could be estimated by calculation tools - FRAX or FRC.

S7 - 2

Bone turnover markers in the management of osteoporosis

Thomas Devika

SA Pathology and University of Adelaide, Chemical Pathology, Adelaide, Australia

Corresponding author: devika.thomas@health.sa.gov.au

Diagnosis of osteoporosis is based on bone mineral density and in many instances the decision to treat depends on fracture risk. Bone turnover markers have been shown to reflect the rate of bone loss particularly in postmenopausal osteoporosis, as measured by decreasing bone density. However, once diagnosed and treatment is commenced, bone turnover markers may be more useful for monitoring treatment response than bone mineral density due to the dynamic nature of bone markers. Both resorption and formation markers may be useful in monitoring response to therapy and in identifying non compliance.

Markers of bone turnover are proteins released by osteoclasts and osteoblasts during the bone remodelling cycle. With the advent of quantitative laboratory measurements of bone turnover markers, they have become useful in predicting and assessing the rate of bone turnover, although they are affected by a variety of physiological and pathological factors. The pattern of increase or decrease in bone turnover makers is not disease specific, therefore they are often not useful in diagnoses. However, the change in the pattern

may be useful in fracture prediction, monitoring treatment response and in identification of non compliance or non response. Various clinical guidelines now incorporate bone turnover markers in monitoring therapy, and the role of bone mineral density in early monitoring of therapy has been questioned. With refined laboratory techniques and methods, more accurate and precise bone turnover marker measurements, they may be incorporated into various algorithms in diagnosis, decision to treat and monitoring disease progression.

S7 - 3

Vitamin D status and osteoporosis: evidence for a role in hip and vertebral fractures

Morris Howard

University of South Australia and SA Pathology, Adelaide, South Australia, School of Pharmacy and Medical Sciences and Chemical Pathology, Adelaide, Australia

Corresponding author: howard.morris@health.sa.gov.au

Current data demonstrate that vitamin D deficiency contributes to the aetiology of at least two metabolic bone diseases, osteomalacia and osteoporosis. Osteomalacia, or rickets in children, is the index disease of vitamin D deficiency and arises from a delay in mineralization. It can be resolved by normalising plasma calcium and phosphate homeostasis even if the vitamin D status remains depleted. The well characterised endocrine pathway of vitamin D metabolism and its activities are solely responsible for vitamin D regulating plasma calcium and phosphate homeostasis and therefore for protecting against osteomalacia.

In contrast, a large body of clinical data supports the concept that an adequate vitamin D status protects bone health by improving bone mineral density and reducing the risk of fracture. The risk of hip fracture is markedly increased at levels of serum 25-hydroxyvitamin D below 50 nmol/L. Meta-regression analyses of data from RCTs of vitamin D supplementation suggest that serum 25-hydroxyvitamin D levels above 75 nmol/L are required to achieve significant fracture reduction. A plausible mechanism for various activities arising from serum 25-hydroxyvitamin D levels greater than 50 nmol/L is provided by studies demonstrating metabolism to 1,25-dihydroxyvitamin D by each of the major bone cells to activate VDR and modulate gene expression to reduce osteoblast proliferation and stimulate osteoblast and osteoclast maturation and mineralisation.

S8 - Laboratory genetics - the critical importance of genotype and phenotype, and their correct interpretation

S8 - 1

MODY - which genes to test?

Ellard Sian

University of Exeter Medical School, Institute of Biomedical and Clinical Science, Exeter, United Kingdom

Corresponding author: sian.ellard@nhs.net

Maturity-onset diabetes of the young (MODY) is a monogenic subtype of diabetes that can be caused by mutations in multiple genes. The most common forms are caused by mutations affecting the glycolytic enzyme glucokinase (GCK gene) or the transcription factor HNF-1 alpha (HNF1A gene) and cause either mild fasting hyperglycaemia throughout life (GCK) or progressive diabetes diagnosed in adolescence/adulthood with risk of diabetic complications (HNF1A). Treatment is determined by the genetic subtype since patients with GCK mutations rarely need pharmacological therapy whereas those with HNF1A mutations are sensitive to sulphonylurea tablets. However, most patients (~90%) are misdiagnosed as having type 1 or type 2 diabetes and access to genetic testing varies 10 fold across the UK.

Selection of patients for MODY genetic testing can be facilitated by non-genetic tests. These include

pancreatic autoantibody and urinary C-peptide tests to identify those more likely to have type 1 diabetes and a web-based MODY predictor tool that gives a likelihood estimate that a patient has MODY.

S8 - 2

The challenges of molecular genetic testing in inherited cardiomyopathy

Thomson Kate

Oxford University Hospitals NHS Trust, The Churchill Hospital, Oxford Medical Genetics Laboratories, Oxford, United Kingdom

Corresponding author: kate.thomson@ouh.nhs.uk

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disorder with an estimated frequency of 1/500. It is a clinically important condition being the most frequent cause of sudden cardiac death (SCD) in young people and trained competitive athletes. HCM is clinically heterogeneous exhibiting variable age of onset, penetrance and expressivity. It is also genetically heterogeneous with over 13 genes reported to be associated. Although generally considered to be an autosomal dominant trait, other more complex patterns of inheritance are becoming apparent. The potential benefits of genetic testing in this condition are widely recognised, however, pathogenic mutations remain elusive in approximately 50% of patients. The challenges of genetic testing in this clinically and genetically heterogeneous condition will be discussed and future analysis strategies explored.

S8 - 3

Familial hypercholesterolaemia: genetic testing helps, but should be used with care

Abstract not provided.

S9 - The poisoned patient

S9 - 1

Modern methods in clinical and analytical toxicology

Grobosch Thomas

Labor Berlin - Charité Vivantes GmbH, Laboratoriumsmedizin & Toxikologie, Berlin, Germany

Corresponding author: thomas.grobosch@laborberlin.com

Background: Acute or chronic poisoning is an emergency situation require immediate, unequivocal and reliable detection and quantification of xenobiotics in blood or plasma/serum in order to establish the correct treatment for the patient. For the detection of unknown substances in such complex biological matrices, only methods providing high specificity and sensitivity are appropriate. Another important aspect is the continuous availability of the complete analytical instrumentation. Basically, toxicological analysis always has to be a compromise between demand of time needed for the procedure and the accuracy of the analytical results.

Methods: Concepts and procedures using e.g. immunoassays/photometric tests, headspace gas chromatography coupled to e.g. flame ionization detector (HS-GC-FID), liquid chromatography coupled with diode-array-detector (HPLC-DAD) or time-of-fight (TOF) analyzer are presented and their perspectives of their future are discussed.

Results: When only a single drug or category has to be monitored, immunoassays/photometry can be used for preliminary screening in order to save time and costs (e. g. THC or lithium). For the systematic toxicological analysis of unknown substances, only the combination of chromatographic systems coupled to a diode-array-detector (identified or classified by their UV-spectra) or a TOF mass analyzer (identified by their accurate mass) will be able to cover the majority of toxicological relevant substances in a reasonable short time. For the quantification of volatile compounds, a HS-GC is absolutely indispensable.

Conclusions: Certain immunoassays/photometric tests, HS-GC-FID, HPLC-DAD, and time-of-fight (TOF) techniques are indispensable modern tools to cover a broad range of xenobiotics and the rising challenges in clinical and analytical toxicology.

S9 - 2

The poisoned patient: quantification in emergency toxicology?

Uges Donald R. A.

University Medical Center Groningen, Hospital and Clinical Pharmacy / Laboratory for Clinical and Forensic Toxicology and Drug Analyses, Groningen, Netherlands

Corresponding author: j.a.tuinstra@umcg.nl

Toxicology is the knowledge of "too much". In other words, toxicology is quantitative science. However, in some clinical toxicology cases, just qualitative analysis in combination with patient's clinical manifestation (toxicodromes) and the available information could be sufficient. To interpret the analytical results, at least one specialist involved should have experiences in pharmacokinetics and toxicokinetics, i.e. an emergency physician, a toxicologist, a hospital pharmacist or a biochemist. Pharmacokinetics is dealing with the time interval of the blood concentration with respect to time of drug intake. Toxicokinetics involves drug exposure and toxicity along with challenges of saturated kinetic parameters and organ failure. Intrinsic low toxic substances can become highly toxic with saturated protein binding. Toxicity can be dependent on at least the dose, concentration, co-medication and patient's clinical condition. The choice and duration of treatment and the prognosis is often concentration dependent. It has to be taken into account that reliable information of the time and route of administration is essential for a good toxicological treatment advice. Nowadays, commonly used analytical methods in clinical toxicology are immunoassays, LC-MS/MS and GC. They all provide both qualitative and quantitative results. In daily practice, therapeutic drug monitoring and clinical toxicology are merging, especially if the cause of poisoning is iatrogenic. Both a too low and a too high dose of an antiepileptic drug can cause seizures.

In conclusion: quantitative analysis can be very useful in clinical toxicology if the right information is provided along with a proper interpretation. (www.bioanalysis.umcg.nl).

S9 - 3

The health risk of recreational drugs and novel psychoactive substances

Wood David Michael

Guy's and St Thomas' NHS Foundation Trust, Clinical Toxicology, London, United Kingdom

Corresponding author: david.wood@gstt.nhs.uk

The use of recreational drugs is common, particularly amongst young people and those who frequent the night-time economy (e.g. bars, raves, discotheques). Recently that has been increasing use and availability of novel psychoactive substances (also known as "legal highs"). These substances include the cathinones (e.g. mephedrone), the piperazines (e.g. 1-benzylpiperazine), the synthetic cannabinoid receptor agonists, the pipradrol-related drugs (e.g. D2PM) and ketamine analogues (e.g. methoxetamine). There is little or no information available on the acute health effects (toxicity) of novel psychoactive substances.

Using a process known as "data triangulation", it is possible to bring together information from a range of different sources to develop an understanding of the overall pattern of acute toxicity for a novel psychoactive substance(s). This reduces the limitations of any one data source, the main one is that typically they is no analytical confirmation of the substance(s) used. These data sources include: Internet discussion fora, where individuals post information on their own personal experiences; case reports/series; poisons centre data series; animal models and human studies.

Classical recreational drugs can clinically be divided into three broad categories based on clinical effects seen with acute toxicity: hallucinogenics (e.g.

LSD, ketamine), depressants (e.g. opioids, gamma-hydroxybutyrate (GHB)), and stimulants (e.g. cocaine, amphetamine, MDMA). Using data triangulation it is possible to conclude that the pattern of acute toxicity of many novel psychoactive substances can similarly be divided: the synthetic cannabinoid receptor agonists and methoxetamine have hallucinogenic-like acute toxicity and the cathinones, piperazines and pipradrol-related drugs have a stimulant-like acute toxicity.

S10 - Education of specialists in laboratory medicine in Europe

S10 - 1

EU perspective in medical specialization

Maillet Bernard

UEMS, Former Secretary-General, Brussels, Belgium

Corresponding author: bernie.mail@skynet.be

In Laboratory Medicine, the big challenge is to harmonise the specialty and the practice in Europe as there are so many different ways in which laboratory medicine is rained and practiced all over Europe.

By proposing a harmonized training program together with the European Federation of Laboratory Medicine in a polyvalent manner, and start to implement this program in an increasing number of EU Member States, the process of harmonization will go hopefully softly and surely forward.

This polyvalency in the training does not exclude that individual practitioners could not have their own field of interest and build by doing so a "Particular Qualficiation" in certain domain(s).

Bearing in mind the revision of the European Directive on the Recognition of Qualifications which is expected to be finalized at the end of 2012, a possibility appears to work on the change of the denomination of the specialty in the Addendum of the Directive.

This will also enable the National Medical Associations and the National Professional Organizations to implement this change at national level and introduce the denomination as well as the common training program as proposed by the UEMS Section of Laboratory Medicine and the EFLM as the national requirement to become specialist in Laboratory Medicine in the different EU Member States.

To be successful, the good collaboration between the different stakeholders in the field is of the utmost importance and this concerns both the European as well as the National level.

S10 - 2

Education and training activities in EFLM

Zima Tomas

Institute of Medical Biochemistry and Laboratory Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic, Prague , Czech Republic

Corresponding author: zimatom@cesnet.cz

The education and training is one of the important activities of EFLM in different areas. Main goals are to assist in knowledge transfer in all areas of profession, to help implement new diagnostic strategies, to produce and assure the quality of EFLM educational materials, support and organized meeting and conferences, to establish the distance learning systems (e-seminars), to coordinate a prepare postgraduate continuous education credits system for our profession. There are many scientific events with strong educational activity as traditional EFLM Postgraduate Course, held in Dubrovnik, Croatia, EFLM Symposium for Balkan Region, Belgrade, Serbia, EFLM -Becton Dickinson preanalytical conference, cooperation with BioRad on conference with topic - Quality in Laboratory Medicine and Accreditation. The main congress in Educational activity is European Joint Congress of EFLM and UEMS in Lisbon and Dubrovnik, now. New attractive instrument is EFLM e-Seminar Series which started in 2010 and for 2012 is prepare 6 seminars. The key topic is harmonization and compensation the differences in specialization education of our profession Specialist in laboratory medicine. The main activity was the education con-

ference held in Prague in March 2012 which should find the consensus in pregraduate, postgraduate and specialization education in Europe in our profession.

S10 - 3

Evaluation/revision of the European Directive on professional qualifications

Zerah Simone

Centre AMP, clinique de la DHUYS, Laboratoire ZTP, Bagnolet, France

Corresponding author: simone.zerah@gmail.com

Background: The first Directive (2005/36/EC) on Recognition of Professional Qualifications had to be transposed by all Member States by 2007. This document is under revision.

Methods: We will give a quick overview of the history to enable understanding of where we are now, after active participation in the numerous meetings and questionnaires, and stress the importance for the future of our profession. We will present the two methods for recognizing qualifications, the automatic system for 7 "sectoral professions" and the "general system". We will report the propositions of the "green paper" and the subsequent draft of the revised directive, published in December 2011, proposed to the European Parliament by the European Commission.

We will explain our strong involvement, and our propositions on the main points: Professional cards, "Common training principles" replacing the common-platforms, back to harmonization instead of identifying differences for compensation measures, CPD, Partial access, rules on language skills.

Results and conclusion: The Deadline for amendments is 15 October. The European professional associations should be well placed to take the lead in devising harmonization frameworks: our aim is the excellence of our profession in Europe. The new Directive is due to be published by the end of 2012.The European Parliament has officially made it a priority. The Member of the Parliament in charge is Bernadette Vergniaud.

S10 - 4

Can we harmonize laboratory medicine in Europe?

Oosterhuis Wytze

Atrium Medical Centre, Clinical Chemistry, Heerlen, Netherlands

Corresponding author: w.oosterhuis@atriummc.nl

The EFLM and UEMS Section of Laboratory Medicine/Medical Biopathology in 2009 have decided to join forces on several projects. One is a survey, with the aim to make a description of the current state of organizations, practices and responsibilities of laboratory professionals within the European Union. Both the EFLM (EC4 curriculum) and UEMS ("Blue Book") work at harmonizing the training of professionals with medical or scientific background. This is closely related to the free movement of people, a major goal of European integration.

In March 2010 a questionnaire has been sent to representatives of both organisations. Delegates of EU countries were asked to answer questions related to the following subjects: the number of professionals (MD, PhD and other academically trained), content of the laboratory specialty, professional organizations responsible for training, official acknowledgment of training and specialties, length of training, relation of scientific organizations with UEMS and EFLM and accreditation of laboratories. The results will be presented, and show a very diverse situation across Europe in many aspects: the relative number of specialists in Clinical Chemistry, the ratio of specialists with a medical and scientific background, official acknowledgement of the Clinical Chemistry specialties, content of the specialty and responsibilities. In some countries the field of Clinical Chemistry is divided in monospecialties, on other countries there is a broader general or "polyvalent" laboratory specialty. A better understanding of the organization and practice of laboratory

medicine will be of help in the harmonization of Clinical Chemistry across Europe.

S11 - Stroke biomarkers

S11 - 1

The role of blood biomarkers in acute ischemic stoke

Katan Mira

Columbia University, Department of Neurology , Divison of Stroke, New York, United States

Corresponding author: mk3270@columbia.edu

Blood biomarkers have begun to play an increasingly important role in the triage and management decisions in several acute medical diseases.

In cardiovascular diseases, some biomarkers are widely accepted and have been successfully implemented into clinical routine, including troponin for the diagnosis of myocardial infarction and BNP to guide diuretic therapy in heart failure.

In acute ischemic stroke several biomarkers have been identified as potential candidates and some have been investigated in clinical settings. However, the utility of a biomarker is defined by its ability to improve clinical decision-making and add timely information beyond that readily available from clinical examination and routine imaging. This aim has not been completely achieved yet for any biomarkers in acute ischemic stroke.

Unique challenges exist in the identification of clinically useful blood biomarkers in ischemic stroke. Stroke is a heterogeneous disease not only etiologically but also in terms of affected cell types. Depending on the clinical question blood biomarkers should reflect distinct pathological processes within the brain and systemically. The blood brain barrier, which separates the brain from the systemic circulation, is an additional concern in this context.

Despite the inherent difficulties to identify useful blood biomarkers for diagnosis or prognosis in ischemic stroke promising data is available and further studies are ongoing. If well-designed studies confirm the incremental value of diagnostic and prognostic blood biomarkers over existing diagnostic and prognostic tools the advantageous opportunity to use these biomarkers to individualize and optimize patient care will arise.

S11 - 2

The significance of uric acid levels in stroke patients treated with thrombolysis

Amaro Sergio

Hospital Clínic, IDIBAPS, University of Barcelona, Functional Unit of Cerebrovascular Diseases, Barcelona, Spain

Corresponding author: samaro@clinic.ub.es

Stroke is a devastating disease with an imperative need for more effective therapies. The thrombolytic molecule rTPA - recombinant tissue plasminogen activator - is the only licensed drug for acute stroke, but its efficacy is limited. The coadministration of neuroprotective drugs could augment the value of thrombolytic therapy, but the evidence in support of this approach is scarce. Uric acid (UA) is the major endogenous antioxidant in blood, and its concentration is almost 10-fold higher than other antioxidants. In acute stroke patients, there is a rapid consumption of UA after stroke and higher UA levels at stroke admission have been associated with less infarction growth at follow-up. Moreover, increased UA serum levels have been associated with better outcome in patients treated or not with reperfusion therapies. In patients receiving thrombolytic therapy, lower UA levels at stroke onset have been associated with a greater incidence of malignant middle cerebral artery infarctions and/or hemorrhagic transformation of the infarction. In experimental ischemia, the exogenous administration of UA has shown neuroprotective properties. Indeed, the coadministration of UA and rTPA has shown to provide synergistic neuroprotection in experimental thromboembolic models and to lessen several biomarkers of oxidative stress in patients with acute stroke. Nowadays, the clinical efficacy of uric acid in acute ischemic stroke

is under investigation in an ongoing phase IIb/3, randomized, placebo-controlled trial.

S11 - 3

Are admission serum lipids predictive of the stroke outcome?

Pikija Slaven

University Clinical Center Maribor, Neurology Department, Maribor, Slovenia

Corresponding author: spikija@gmail.com

Various functions in living organisms are mediated and encompassed with lipid molecules. They are fundamental components of cell barriers thus enabling essential prerequisite for life – compartmentalization. Beside space limiting function, they also serve as messengers in multicellular organisms. Intrinsic properties of high-energy reduced chemical bonds between carbon atoms are recognized by living cell as perfect way to store energy for later use. In equilibrium lipids serve organism well. However, certain pathological states tend to move lipid physiology in unwanted direction. It is well established fact that serum lipid perturbations in long term, along with permissive inflammation state, carries risk for atherosclerosis and all subsequent adverse effects of later. Those facts are best established for coronary artery disease and heightened level of serum lipid – cholesterol. Some favorable effects on stroke volume in patients with heightened levels of triglycerides as well as linkage between low level of cholesterol with greater risk for intracerebral hemorrhage are reported. One hypothesis argues that intrinsic properties of lipid perturbations leads to favorable state of accessible alternative brain food – blood ketones that is known to have multiple neuroprotective effects. Other hypothesis tries to link effects of cholesterol on platelet aggregation and other to effect of TG to ameliorate fatty-acid induced lipotoxicity. Still some have found that high cholesterol can increase gamma-glutamytranferase which can be neuroprotective. It may be as well that intracranial vascular endotel reacts differently to lipid values, specifically when stroke happens, despite gross similarity between atherosclerotic plaques between extracranial and intracranial lesions.

S12 - Diagnosing and monitoring of DM with POCT instruments

S12 - 1

Diagnosing and monitoring diabetes mellitus with POC instruments

Sandberg Svere

Norwegian Quality Improvement of Primary Care Laboratory, University of Bergen, Bergen, Norway

Corresponding author: sverre.sandberg@isf.uib.no

POC instruments for glucose and HbA1c have been used for a long time to monitor diabetes mellitus. In a monitoring situation it is important that the instruments give reproducible results, and therefore that the patients visit the same facility each time. Because of the varying trueness of glucometers, these instruments have – in most countries – not been recommended for diagnosing diabetes mellitus although some GPs and Pharmacists use POC instruments for case finding and, in case of a "positive" result, forward samples to a larger laboratory for further examination. The same has been the situation with u-albumin where case finding for microalbuminuria has been performed, but samples have been sent to larger laboratories for confirmation. Diabetes mellitus can now be diagnosed using HBA1c and an important question is: Can this be done with POC instruments in primary health care? We suggest a model for quality assurance, similar in hospital laboratories and in laboratories using POC instruments. In this model both the trueness and the precision will be registered by an External Quality control Scheme (EQAS), with an allowable deviation in the EQAS of 7 % and where the intralaboratory (within one lot) should be < 2% for the participant to use A1c as a diagnostic criterion. We have established a system to advice and

educate the participants using POC instruments on how they can improve and thereby use the POC instrument for HbA1c to diagnose diabetes mellitus.

S12 - 2

The role of POC instruments for diagnosing and monitoring u-albumin in persons with diabetes mellitus

Gillery Philippe

University Hospital of Reims, Laboratory of Pediatric Biology and Research, Reims, France

Corresponding author: pgillery@chu-reims.fr

Urine albumin (u-albumin, "microalbuminuria" is discouraged) is a marker of kidney damage and is considered a risk factor for renal disease progression and cardiovascular events in diabetic patients. Its accurate measurement is a necessary prerequisite for the correct use of results in global patient care.

Whereas u-albumin may be measured by various immunoassays in clinical laboratories, POCT instruments have been introduced, that measure urine albumin and creatinine, and calculate albumin/creatinine ratio (ACR). ACR is generally accepted as a valid approach to overcome limitations related to urine collection.

Two main questions must be addressed: (i) Are POCT methods for u-albumin as reliable as central laboratory methods? (ii) What is the added value of u-albumin POCT in patient care?

Recent studies have shown that POCT analyzers exhibited analytical performances allowing their use in clinical situations. However, reference values, especially for ACR, may vary between the different devices, and must be taken into account for clinical interpretation. Thus, POCT evaluation of u-albumin may be useful for diabetologists or general practitioners to identify diabetic patients at risk for cardiovascular complications and progression of kidney disease. Although there are still few studies about the relative benefits of POCT *vs*. laboratory assays for u-albumin evaluation, successful approaches have been reported, especially in Australia for community risk assessment, but also for patient management, in large-scale programs dedicated to remote population.

Like HbA1c, u-albumin may be assessed by POCT methods and may represent an interesting alternative in addition to conventional laboratory methods in particular clinical situations.

S12 - 3

Glucose self monitoring by POCT systems with emphasis on the Italian recommendations

Mosca Andrea

Università degli Studi di Milano, Dip. di Fisiopatologia medico-chirurgica e dei Trapianti, Milano, Italy

Corresponding author: andrea.mosca@unimi.it

The term of self-monitoring (SMBG) refers not only to the measurement of blood glucose by patients, usually at home, but also to the interpretation of the results and to some actions to be undertaken in order to improve the glycemic control. Therefore, SMBG is part of an educational trial to be undertaken in collaboration with health care providers. By looking to the evidences, SMBG is essential for type 1 diabetic patients and for type 2 diabetic patients, insulin treated (levels of evidence II, strength A and B, respectively). On the other hand, non continuous SMBG in type 2 diabetic patients under oral hypoglycemic drugs or simply under controlled diet is useful only if an appropriate education is provided, together with the indication of actions to be undertaken to change the therapy on the basis of the SMBG results (strength II, force B). With regards to the choice of the glucometer, a consensus document recently developed by an Italian task force will be illustrated in its major points. Essentially, with regard to the analytical characteristics, only plasma-calibrated POCT have to be used, with compliance to the ISO 15197/2003. Monitoring of the imprecision (minimum desirable CV ≤ 5 %) and bias can be performed by adequate internal quality control and EQAS procedures, in collaboration with laboratory professionals.

S13 - Pre-pre and post-post analytical aspects: communication between laboratory and clinicians

S13 - 1

Improving pre-analytical, analytical and post-analytical phase by EQAS – examples

Aarsand Aasne Karine

Haukeland University Hospital, Laboratory of Clinical Biochemistry, Bergen, Norway

Corresponding author: aasne.aarsand@helse.bergen.no

Even though most laboratory errors are thought to occur in the pre-analytical and post-analytical phases, EQA schemes typically focus mostly on the analytical phase and testing of the non-analytical phases are only rarely performed. NOKLUS, in cooperation with EQALM, runs a web-based post-analytical EQA scheme for automated haematology instruments including more than 400 laboratories in 14 European countries. Participants, mostly medical laboratory technologists responsible for the instruments, interpret cell counts and plots from five-part differential automated haematology instruments in light of an accompanying case history. Results from several distributions show that there are differences between participants and also between different countries regarding how they work, the standard operating procedures used for interpretation of results and plots and the corrective actions taken. To assess all aspects of the laboratory diagnostic process is even more important for rare metabolic disorders, considering the central role played by the laboratory in establishing these diagnoses. The European Porphyria Network (EPNET) runs a clinical case-based EQA scheme covering pre-analytical, analytical and post-analytical aspects for all biochemical analytes relevant for diagnosing the porphyrias. The participants, at present 33 porphyria specialist laboratories throughout Europe, apply diverse diagnostic strategies and large variations in analytical performance are also typically evident. However, most laboratories provide appropriate interpretations and correct diagnoses. Improvements in applied diagnostic strategies and analytical performance have been observed during the time the EPNET EQA scheme has been running, underlining the importance of offering EQA schemes that test the complete laboratory process for diseases requiring complex diagnostic testing.

S13 - 2

Important quality indicators for the pre-analytical phase

Simundic Ana-Maria

Clinical Hospital Center Sestre Milosrdnice, Clinical Institute of Chemistry , Zagreb, Croatia

Corresponding author: am.simundic@gmail.com

Quality of a service or a product is a measure of the satisfaction of its consumers or customers. Quality of a healthcare service may thus be defined as a measure of its impact or effect on the patient care. Quality indicators are tools aimed to measure the quality of a health care service. They need to be evidence based, available, accessible and measurable, objective and relevant to the particular system. They also must be able to indicate the potential for improvement, within the continuous improvement cycle. Numerous organizations have attempted to identify a set of quality indicators and quality specifications to be used universally by any laboratory worldwide, regardless of its size, setting, type and geographical location. However, up to today there is still no universal agreement on the core set of quality indicators for preanalytical phase. Until such agreement has been reached, each laboratory needs to define its own indicators and way to implement it into its quality system. When implementing quality indicators, there are many issues to be addressed, such as: valid and reliable definition of the indicator, its relevance to the service, accessibility, availability, reporting frequency, available corrective measures, acceptance limits and some other. Quality indicators in the preanalytical phase of the laboratory testing relate to the test ordering, patient identification, specimen collection, transport and handling. Currently, data from different laboratories are difficult to compare and standardization and harmonization in that respect is of utmost importance.

S13 - 3

Standardization and harmonization of quality indicators in the pre- and postanalytical phase

Plebani Mario

University-Hospital, Department of Laboratory Medicine, Padova, Italy

Corresponding author: mario.plebani@unipd.it

Quality in laboratory medicine should be defined as the guarantee that every step in the total testing process (TTP) is correctly performed. Whilst current quality indicators in laboratory medicine tend to focus on the performance and efficiency of analytical processes, recent evidence suggests that most errors in the TTP fall outside the analytical phase. The current lack of attention to extra laboratory factors is thus in stark contrast with the body of evidence pointing to the multitude of errors that continue to occur in the pre-analytical phase.

As a result, in 2008, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) launched a working group named "Laboratory errors and patient safety" with a goal to identify and evaluate valuable QIs and related quality specifications in order to address all the stages of the TTP. The prerequisites for selected QIs were: a) relevance and applicability to a wide range of clinical laboratories; b) scientific soundness, with a focus on areas of great importance for quality in laboratory medicine; c) feasibility, both regarding the data availability and the definition of thresholds for acceptable performance; d) timeliness and possible utilisation as a measure of laboratory improvement. 56 QIs have been identified, of which 34 in the pre-analytical, 7 in the intra- and 15 in the post-analytical phase. The aims of, and steps taken in, the IFCC-WG program will be described and preliminary results discussed for better addressing future steps of the project particularly as regards pre- and post-analytical indicators.

S14 - Obesity: from genetic loci to early indicators

S14 – 1

Genetic loci and body mass index

Abstract not provided.

S14 - 2

The obese adolescent: conclusions of the HELENA study

Molnár Denes

University of Pécs, Pediatrics, Pécs, Hungary

Corresponding author: denes.molnar@aok.pte.hu

The HELENA Project was a comprehensive three-year research programme, spanning 10 countries, using a common methodology designed to assess the nutritional status and behavior, as well as the fitness and physical activity patterns, of more than 3000 adolescents aged 12.5 to 17.5 years.

The basic objective of the HELENA cross-sectional multicentre study was to obtain reliable and comparable data on dietary intake, food preferences, anthropometry, serum indicators of lipid and glucose metabolism, vitamin and mineral status, physical activity, fitness and genetic markers.

The aim of the lecture is to present a bunch of the most important findings of the HELENA Study in the light of other, recent international results.

The prevalence of BMI (kg/m^2) categories in European adolescents (N = 3528, male = 1683) was the following: male: BMI < 18.5 = 5.1%; 18.5 ≤ BMI < 23 = 51.7%; 23 ≤ BMI < 25 = 16.3%; 25 ≤ BMI < 27 = 10.9%; 27 ≤ BMI < 30 = 8.6%; 30 ≤ BMI < 35 = 6.0%; 35 ≤ BMI = 1.4%; females: BMI < 18.5 = 6.6%; 18.5 ≤ BMI < 23 = 54.5%; 23 ≤ BMI < 25 = 18.5%; 25 ≤ BMI < 27 = 10.0%; 27 ≤ BMI < 30 = 6.3%; 30 ≤ BMI < 35 = 3.5%; 35 ≤ BMI = 0.6%.

The prevalence of overweight and obesity showed a north to south gradient.

42% of normal weight and only 31.5% of obese adolescents spent 60 minutes/day in moderate-to-vigorous physical activity as measured by accelerometer. Adolescents spent most of the registered time in sedentary behaviors (9 hours/day, or 71% of the registered time).

In the HELENA sample 1.1% of children had metabolic syndrome according to the criteria of the International Diabetes Federation.

The results of the HELENA Study beside their scientific merit help authorities to launch preventive measures on a European level.

S14 - 3

The value of obesity research

Oppert Jean Michel

University Pierre et Marie Curie, University Pierre et Marie Curie, Paris, France

Corresponding author: jean-michel.oppert@psl.aphp.fr

Obesity is the most prevalent metabolic disease worldwide. Despite significant recent research investment, obesity prevalence rates continue to rise throughout most countries of the world, not least in Europe. In recent decades, research has helped improve our understanding of the complex effects of increased fat tissue on the human body and has enabled us to develop better evidence based guidelines to prevent, manage and treat obesity, including better bariatric surgery procedures to treat extreme obesity. A major challenge is to increase the translation of this research into better prevention and treatment strategies. In response to this situation, the European Association for the Study of Obesity (EASO) has now completed a two year consultation process, culminating in a meeting of Europe's leading researchers and main stakeholders in the field with the aim of developing a clear research strategy, with consensus on what the key areas for European obesity research should be, the expected impact of this research and how we can achieve success in these areas in terms of novel approaches. EASO recommends that a first priority is to 'Improve obesity diagnosis (beyond BMI) to categorize individuals for appropriate prevention and management, including critical periods of life'. For all recommended research topics, a major focus is on transdisciplinary approach (integrating social sciences and humanities), societal impact and innovation/economic growth. The key to advance obesity prevention and treatment in Europe now require a political commitment on both member state and EU level.

S15 - New trends in laboratory medicine

S15 – 1

Next generation sequencing for clinical diagnostics

Abstract not provided.

S15 – 2

Population screening for genetic disorders in the 21st century

Abstract not provided.

S15 - 3

Towards fast microbiology

Vila Jordi

Hospital Clinic, Clinical Microbiology, Barcelona, Spain

Corresponding author: jvila@ub.edu

In the last decade a plethora of molecular biology tools have been applied for the diagnosis of infectious diseases. These techniques can provide information from clinical samples within only a few hours. Several multiplex real time PCRs have been applied to detect microorganisms causing sepsis, respiratory

tract infections, sexually transmitted infections, including the detection of virus and bacteria in the two former cases. Finally, multiplex real time PCR panels to detect bacteria, virus and protozoa causing gastroenteritis has also been developed. In this sense specific PCR for individual microorganisms have also contributed to the more rapid diagnosis of infectious disease. When a culture is negative and an infectious diseases is suspected, broad PCR based on amplification and sequencing of the 16S rRNA gene (for bacteria) or the ITS region (for fungi) can be carried out. An important breakthrough in the rapid diagnosis of infectious diseases has been the incorporation of MALDI-ToF mass spectrometry not only to rapidly identify bacteria and fungi but also to detect mechanisms of resistance associated with the degradation of the antibiotic, for instance, detection of beta-lactamases. In addition, ESI-ToF mass spectrometry is also used to identify whatever microorganism is found in the clinical sample by using PCR amplification of specific gene(s) and ESI-ToF to detect the exact size of the amplicon and the number of A, T, C and G. Based on a specific algorithm the microorganism is thereafter determined. Therefore, we are witnessing profound changes in the way that microbiological diagnoses are performed.

PC1 - Pharmacogenetics in predicting anti-cancer therapy response

PC1 - 1

Pharmacogenetics in predicting anticancer therapy response: pro

van Schaik Ron

Erasmus MC, Dept. Clinical Chemistry, Rotterdam, Netherlands

Corresponding author: r.vanschaik@erasmusmc.nl

Prediction of adverse drug effects, or on effectivity of therapy seems most promising in Oncology. In this field, toxicity is often dose limiting. Whereas undertreatment will have fatal consequences. Most anticancer drugs, however, are metabolized by CYP3A4, for which no clinical relevant genetic polymorphisms have been described until recently. In contrast, the anti-estrogen tamoxifen, which is used as adjuvant therapy for breast cancer, needs activation to endoxifen, a process which is heavily depending on the genetically polymorphic enzyme CYP2D6. Approximately 5-10% of the Caucasian population is a poor metabolizer for CYP2D6, with almost no CYP2D6 activity due to inheritance of two inactive alleles. An additional 30% is intermediate metabolizer, with reduced CYP2D6 activity. Based on the theoretical involvement of CYP2D6 in the activation of tamoxifen to endoxifen, one would expect lower endoxifen plasma-concentrations in the circulation of CYP2D6 poor metabolizer breast cancer patients and a lower survival. The correlation of CYP2D6 deficient alleles with lower endoxifen concentrations was indeed confirmed. In addition, several studies showed indeed a lower survival for carriers of one or two defective CYP2D6 alleles. From these facts, it is clear that CYP2D6 genotyping for tamoxifen treatment is THE logical thing to do. In fact, performing NO genotyping would, in view of current knowledge, be completely unethical. Since the alternative for tamoxifen are the much more expensive aromatase inhibitors, cost effectiveness of this approach is clear-cut. We should therefore not withhold breast cancer patients this valuable analysis.

PC1 - 2

Pharmacogenetics in predicting anti-cancer therapy response – contras

Quirke Philip

University of Leeds, Pathology and Tumour Biology, Leeds, United Kingdom

Corresponding author: p.quirke@leeds.ac.uk

Background: Prediction of therapeutic response is critical to progress in personalized medicine. Pharmacogenetics is generally regarded as the study or clinical testing of genetic variation that gives rise to differing responses to drugs whereas pharmacogenomics refers to somatic mutations in tumoral

DNA leading to alteration in drug response. There are issues that must be considered in both areas and these will be addressed including the importance of study design e.g. use of clinical trials, prognostic *vs*. predictive markers, adequate hypothesis generating and validating studies, small numbers etc. The lack of rigour of approval agencies and their changing standards does not help the field.

Pharmacogenomics has come of age and is identifying genetic markers in pathways that identify responders, non responders, the development of induced tumour resistance and is shedding light on mechanisms of drug failure. There is rapid translation from lab to clinic and many examples exist e.g. Her-2 amplification and Herceptin response, Ki-ras and anti EGFr non response, selective mutation of the external domain of EGFr and resistance to anti-EGFr antibodies.

The study of genetic variation in pharmacogenetics has yielded less value, controversial results and few tests are in routine clinical use in cancer.

Results: Data on SNPs cardiovascular disease and colorectal cancer SNP's will be shown and UGT1A1 discussed.

Conclusion: Pharmacogenomics is already here and providing important clinical information. Pharmacogenetics has yet to earn its place in the clinic.

PC2 - Laboratory screening for thrombophilia - some important dilemmas

PC2 - 1

Pro's and con's of thrombophilia testing: pro's

Palareti Gualtiero

University Hospital of Bologna, Angiology and Blood Coagulation, Bologna, Italy

Corresponding author: gualtiero.palareti@unibo.it

Heritable thrombophilia is associated with a tendency to venous thromboembolism (VTE), with a risk that is greater in case of antithrombin, protein C or S deficiency, and lower in presence of factor V Leiden or Prothrombin mutation. The latter are however are extremely frequent, while the former are relatively rare. Altogether their prevalence is about 8-10% in the general population, a situation that nudges up the probability of carrying multiple alterations, with a further increase in thrombotic risk up to 20-fold. Testing in unselected patients with VTE is not indicated. However, testing selected patients may give important information on the risk of recurrences after completion of the standard first course of anticoagulant treatment and help clinicians to decide on the optimal duration of anticoagulation in the individual patient. Patients with a first unprovoked VTE (especially if young), with recurrent VTE, with VTE during pregnancy/puerperium or during contraceptive or replacement hormone therapy, with thrombosis in unusual sites, are candidate for testing. Other subjects can be examined: asymptomatic subjects, with a family history of VTE and a first-degree relative with thrombophilia diagnosis, before important conditions at risk such as pregnancy or hormone therapy. Thrombophilia has also been claimed as possible risk factor for pregnancy complications; women with a history of pregnancy loss, recurrent or late in pregnancy should be tested. Testing should be patient-specific, because results are affected by many preanalytic variables, such as pregnancy or hormone therapy, the acute phase of VTE and drugs used for anticoagulation.

PC2 – 2

Pro's and con's of thrombophilia testing: con's

Abstract not provided.

PC3 - Screening for bowel cancer - FOBT, FIT and/or FLEXI-SIG?

PC3 - 1

Laboratory screening for colorectal cancer

Halloran Stephen

National Health Service, Bowel Cancer Screening Programme, Guildford, United Kingdom

Corresponding author: s.halloran@nhs.net

Colorectal cancer is the second most common cause of cancer deaths in Europe and probably the most common cause in men who do not smoke! Whilst tumour markers largely play second fiddle in front-line cancer diagnosis, the clinical niche for faecal occult blood testing (FOBT) has finally been recognised after decades of making an uncertain contribution to the process of diagnosing colorectal cancer.

Two years ago the EU published guidelines on population screening for colorectal cancer and described how using a simple test can save the lives of many destined to die from colorectal cancer.

The diagnostic attributes of guaiac were recognised in 1864 after it was shown impotent in the treatment of syphilis. Reincarnation of FOBT equipped it for small volume analysis but not for population screening. The practical and analytical deficiencies of guaiac FOBT has thrust faecal immunochemical testing for haemoglobin (FIT) into pole position as the EU-recommended test for colorectal cancer screening.

Whilst Poland and Germany maintain a commitment to colonoscopy, the world is now embracing the evidence behind biomarker population screening.

Can the international biochemistry community guarantee consistent, high quality, professionally-led services for this major public health initiative? As this Cinderella analyte moves centre stage, we are challenged to develop safe systems of analysis, adequate sample preservatives, traceable calibration and internationally agreed units of reporting.

The presentation reviews the strengths and weaknesses of FOBT, reveals its impact in the UK screening programmes and highlights the opportunities and challenges for the future.

PC3 - 2

Endoscopic screening for bowel cancer

Atkin Wendy

Imperial College London, Department of Surgery & Cancer, London, United Kingdom

Corresponding author: w.atkin@imperial.ac.uk

Endoscopic screening of the colon is offered opportunistically in most high-risk countries, but few countries have implemented organised screening programmes. This is probably because there has been an absence of high-quality evidence in the form of randomised controlled trials (RCT). During the past three years, however, three trials have demonstrated the long-term efficacy, safety, and cost-effectiveness of flexible sigmoidoscopy (FS) screening. Two showed that a single FS, offered between the ages of 55 and 64, causes a reduction in colorectal cancer incidence that lasts at least 10 years. The other trial showed a lasting benefit from two FS screenings. England is the first country to adopt a national programme based on a single FS offered at age 55.

FS has been criticised as being 'necessarily short-sighted' since it can reliably examine only the rectum and sigmoid colon. Colonoscopy can examine the whole colon but is a more onerous procedure, requiring full bowel preparation and often sedation, leading to a commitment of at least 48 hours in preparation and recovery time. FS, by contrast, requires only a single, self-administered enema and no sedation. Moreover, there are no RCTs examining the incremental benefit in terms of reduction in incidence and mortality of right-sided colon cancer to justify the use of colonoscopy; two RCTs have recently started, but they are not expected to report for at least 10 years. Other methods of screening the right colon include faecal occult blood testing and new DNA-based stool tests, which are currently under research.

PC4 - Does accreditation improve patient outcome?

PC4 - 1

Laboratory accreditation - where is the real benefit for the patients?

Vukasovic Ines

Medical School University Hospital Sestre Milosrdnice Zagreb, Croatia, Clinical Institute of Chemistry, Zagreb, Croatia

Corresponding author: ines.vukasovic@gmail.com

Accreditation is widely used in developed countries to encourage or enforce improvements in the quality and reliability of laboratories. The prime aim of accreditation is to prove, by objective evidence, its competence to provide a medical diagnostic service to its customers, including healthcare staff and patients. Patient management decisions are based on laboratory data. Medical diagnostic services is far broader than just performing analysis of a certain measurand and include a whole range of activities: advising physicians on selection the most appropriate tests for the specific diagnostic problem, instructions on sampling and pre-analytical variables, testing by analytical methods, reporting and interpreting test results in the clinical context. The ISO15189 standard is recognized as a reliable indicator of laboratory technical competence and helps laboratories to provide the better services for the patients who expect that all tests are carried out according to the highest quality principles. If these tests are not available or are inaccurate, treatment outcomes for patients are likely to be poorer, with higher mortality and more frequent illness. Diagnostic testing involves multistep processes which could potentially be a subject to multiple sources of error leading to significant variance in the accuracy of the reported result, incorrect diagnosis, inappropriate treatment, or withholding of lifesaving therapy. Laboratory should consider all potential errors that could cause the worst outcomes and prevent them. Laboratories that achieve accreditation are recognized for superior test reliability, operational performance, quality management, reduced rates of laboratory errors and ultimately contribute to better patient care.

PC4 - 2

Contra laboratory accreditation: Where is the real benefit for patients ?

Müller Mathias M.

Austrian Society for Quality Assurance and Standardisation, Head Quarter, Vienna, Austria

Corresponding author: mathias.mueller1@chello.a

Accreditation of medical laboratories is based on the ISO 15189 standards describing their competence and quality in the whole diagnostic process. Advantages and or disadvantages for a diagnostic laboratory are important questions, since accreditation and keeping the accreditation status of a laboratory are costly.

The ISO standard describes the important technical laboratory processes, following strictly self-defined quality criteria described in subjective quality handbooks. Administrative processes are implemented, new developments of analytical techniques and their use in clinical diagnostics is prohibited. The discipline of laboratory diagnostics loses its innovation within medical sciences.

During the last 5 to 10 years the structure and the competence of a diagnostic laboratory has changed fundamentally by industry driven mega laboratories thus loosing competence in analytics, pathophysiology, medical interpretation of laboratory results by focusing on economics, and accreditation rules concomitant with an administrative overkill. Accredited laboratories are mainly run by economics. Professional competence is measured on basis of keeping with the annual budget.

Modern quality management has been developed as an important field for quality managers not for patients, physicians and laboratory professionals. Searching the literature, the benefit of accreditation is not documented; the number of correct diagnosis has not increased. The diagnostic laboratory must be an integrated institution in clinical medicine being fundamental for the benefit of patients. These are the real goals of a medical laboratory not being measured by industry standards.