Abstracts*)

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Moderne Diagnostik: akute hämatologische Neoplasien

V01 – Talk Haferlach

New WHO entities in hematology: from phenotype to genotype

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The recent ten years demonstrated revolutionary steps in the diagnosis of leukemias and lymphomas. This is triggered by insights into biology of respective diseases and definition of new entities. The techniques in place are such as cytomorphology, immunophenotyping, histomorphology, Cytochemistry and histochemistry as well as standard cytogenetics based on chromosome banding analysis. But especially molecular methods have paved the way. Using techniques such as next generation sequencing (NGS) new molecular markers have been identified in a large cohort of patients helping to delineate several new entities from each other and decipher new biological subgroups of patients. Thus, the WHO classification will move into the field of the molecular defined diagnostics and will describe new or separated entities: Subclassification of AML using molecular markers such as DNMT3A, IDH1, IDH2, ASXL1 or RUNX1. The of TP53 mutation in AML and ALL leading to new subclasses according to chromosome banding and molecular based definitions. In CLL new genes such as NOTCH1 or BIRC3 have been discovered. For hairy cell leukemia BRAF mutations where detected and in immunocytoma MYD88 mutations where found. For large granular lymphocyte leukemia STAT3 mutations now can be investigated. For large granular lymphocyte leukemia STAT3 mutations now can be investigated. In chronic neutrophilic leukemia CSF3R mutations have been found and in atypical CML SETBP1 mutations are present. In conclusion, new entities have been discovered. This is based on morphology, combined with cytogenetics and immunophenotyping but finally was possible due to new findings in the molecular field of leukemias and lymphomas, in many cases by the application of NGS.

V02 – Talk van de Loosrecht

Flow-Zytometrie beim MDS: Why and how?

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The pathological hallmark of MDS is dysplasia. Flow cytometry (FC) can identify aberrancies in antigen expression and differentiation patterns indicative of dysplasia. FC is regarded instrumental and recommended in the diagnostic work-up of (suspected) MDS. The WHO-2008 classification recommends the presence of three or more FC aberrancies as highly suggestive for MDS. Minimal requirements to analyze dysplasia by FC have been proposed by the European LeukemiaNet (ELNet) working group (ELNet iMDS-Flow). Within the ELNet-iMDS-Flow group, a score based on four cardinal parameters was validated in MDS patients with <5% blasts and non-clonal cytopenic controls. It was demonstrated that this score also separates distinct subgroups with respect to prognosis within IPSS-R risk groups. Most flow scores mainly incorporate markers that cover the myelomonocytic lineage, e.g. the flow cytometric scoring system (FCSS). The latter separates patients with no and mild-to-moderate dysplasia from those with severe dysplasia. The FCSS has recently been shown to add significantly in separating patients into low or high risk disease in the revised IPSS subgroups. Flow profiles based on the myelomonocytic lineage may fail to recognize MDS patients that exclusively show erythroid and/or megalakaryocytic dysplasia. Recent advances shows that markers such as CD71, CD36 and CD117/CD105 may add significantly to the diagnostic score of MDS by FC. Aberrant FC profile of myeloid progenitors has been associated with high transfusion requirements and disease progression and with short duration of response or lack of response to growth factors and azacitidine. Studies on the value of FC combined with SNP and NGS are ongoing.

V03 – Talk Bär

Next-Generation-Sequencing: Forschung oder Routine?

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Objectives: Diagnosis and prognostication of haematological malignancies are changing gears. The description of diseases is on the way from a phenotype to a molecular genotype.
Methods: The rapid development of Next Generation Sequencing (NGS) techniques revolutionized haematological diagnostics. Different platforms are used from whole genome sequencing to ultra-deep targeted re-sequencing of single amplicons.

Results: Whole exome or even whole genome sequencing revealed frequently mutated genes in haematological malignancies. Many of these were found to be important for the underlying cellular biology and attracted notice to molecular pathways like epigenetic regulation (DNA methylation, chromatin modification) or splicing. Next, massive parallel amplicons sequencing or gene panel testing allowed analyzing genes of interest in larger cohorts, leading to a reshaped mutational landscape in diseases such as AML and MDS. Finally, ultra-deep massive parallel sequencing of individual loci showed highest sensitivity to identify low level mutations (ten-times higher than Sanger sequencing) and consequently opened the possibility for early detection of small mutated cell clones or minimal residual disease.

Conclusion: It should be our aim to place results from NGS on the background of techniques such as morphology, immunophenotyping, cytogenetics, FISH and standard molecular approaches in order to understand challenges and opportunities of the malignant genome.

Epigenetik und Phänotyp

V04 – Talk Linhart

De novo DNA Methylation in Colorectal Cancer

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Objectives: The aim of our study was to identify DNA regions with tumor associated de novo methylation in colorectal cancer that can be used as diagnostic biomarkers.

Methods: 50 DNA methylation hotspots previously identified in a transgenic mouse model with DNA Methyltransferase 3b (Dnmt3b) overexpression were analyzed in 50 paired human colorectal cancer/control samples. Four candidate regions were further validated using 170 colorectal cancer samples and twenty control samples. DNA methylation was measured using Mass Array analysis and 454-bisulfite sequencing.

Results: 34/50 candidate regions previously identified in the Dnmt3b transgenic mouse model also showed significant de novo methylation in human colorectal cancer. Two candidate genes LMX1B and IKZF1 were combined as diagnostic classifier for colorectal cancer: The area under the receiver operating characteristic curve (ROC-AUC) for the LMX1B/IKZF1 classifier was 0.99 for the training data set and 0.96 for the validation data set.

Conclusion: Our results support the hypothesis that Dnmt3b is involved in tumor associated de novo methylation and that this process is conserved between mammalian species. Using murine data we were able to identify DNA methylation biomarkers that allow diagnosis of human colorectal cancer tissue with high sensitivity and specificity.

V05 – Talk Winnefeld

Skin aging is associated with distinct epigenetic modifications

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In recent years epigenetic changes have been considered to play an important role in the process of aging. Besides histone modifications DNA methylation is currently the best understood epigenetic mechanism. Moreover, epigenetics is one of the key mediating factors between the environment and an organism’s genome. Since human skin is strongly exposed to environmental influences, this organ represents an interesting system for the analysis of epigenetic changes. Human skin samples of differently aged volunteers were analyzed using an array-based chip technology (Illumina) to determine the effects of aging on the methylation pattern. Our results show a significant hypermethylation of various CpG-loci in aged skin samples. The validation of several array-predicted methylation values via bisulfate sequencing confirmed that the array produced reliable results. To further characterize the impact of these age dependent methylation changes on gene expression we correlated methylation and gene expression data sets and identified specific genes that became hypermethylated and silenced with age. Therefore it can be speculated that the observed age-related alterations in the methylome might be of functional relevance for the aging phenotype.
Klinisches Biobanking in der Laboratoriumsmedizin

V06 – Talk Illig

ISO/TC 276: News from the working group Biobanks and Bioresources

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Objective: Goal of this initiative is to develop standardized recommendations for biobanks on a European level, which might be important for future accreditation or certification processes of biobanks.

Methods: DIN (Deutsches Institut für Normung) has initiated an application for the establishment of an ISO (International organisation for standardisation) technical committee (TC) on biotechnology. This TC (TC276) consists of 4 work packages (WPs) and includes one WP on biobanks and bioresources.

Results: ISO/TC 276 Biotechnology will work closely with related committees to identify standardization needs and gaps. It will also collaborate with other organizations to avoid duplications and overlapping standardization activities. The norms shall be finished in 36 months with an international standard.

Conclusion: An international valid standardisation protocol for the accreditation or certification of biobanks shall be developed in the next 3 years.

Biomarker des Knochenstoffwechsels

V07 – Talk Meier

Die Erwartungen des Osteologen von der Labormedizin

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The long-term result of imbalances in bone turnover (i.e. osteoblastic bone formation and osteoclastic bone resorption) is a change in bone mass, bone strength and structure which ultimately leads to osteoporosis and fragility fractures. Considerable progress has been made in the isolation and characterisation of cellular and extracellular components of the skeletal matrix, which in turn have facilitated the development of biochemical markers that specifically reflect either bone formation or bone resorption. These biochemical indices are non-invasive, comparatively inexpensive and, when applied and interpreted correctly, helpful tools in the diagnostic and therapeutic assessment of metabolic bone disease. In contrast, however, bone markers cannot be used for diagnosis of osteoporosis. Bone turnover markers (BTM) predict the risk for fractures, independent from bone mineral density. Furthermore, bone markers may be useful for monitoring anti-osteoporotic treatment as they provide pharmacodynamic information on the response to osteoporosis treatment. Baseline measurements of resorption markers are useful before commencement of anti-resorptive treatment and can be checked 3-6 months later to monitor response and adherence to treatment. Similarly, formation markers can be used to monitor bone forming agents. However, their clinical value for monitoring an individual patient is limited by inadequate appreciation of the sources of variability, by limited data for comparison of treatments using the same BTM and by inadequate quality control.

V08 – Talk Bieglmayer

Biochemical markers of bone metabolism

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Objective: To give a review on selected bone turnover markers (BTM) like type 1 collagen teleopeptide (CTX), tartrat resistant acid phosphatase isoenzyme 5b (TRAP), N-propeptide of type I procollagen (PINP), bone specific alkaline phosphatase (BALP) and osteocalcin (OC).

Methods: Manual and automated assays like ELISA, RIA, (electro)chemiluminescence or immunocapture methods.

Resorption markers
CTX: marked circadian fluctuations, influences by food intake, stable in EDTA plasma (1 day 20°C, 3 months at -20°C). Diseases of kidney and liver interfere. IOF and IFCC recommend CTX for clinical studies.
TRAP: instable at 20°C (storage at -80°C for few months); TRAP can be used as BTM for patients with kidney insufficiency.

Formation markers
P1NP: small circadian variability, minor effects of food intake, stable in EDTA plasma (1 day at 20°C, 6 months at -20°C). P1NP is elevated in hepatic cirrhosis. IOF and IFCC recommend P1NP for clinical studies.
OC: minor circadian rhythm. Mostly the rather stable N-mid fragment is measured from EDTA plasma. Kidney disease interferes.

BALP: faint circadian variability, stable in serum (2 days at 4°C, 2 months at -20° and -80°C for long time storage). Liver disease interferes. KDIGO recommends BALP as BTM for dialysis patients.

Conclusion: The scientific benefit of BTM is well acknowledged by many clinical studies. Due to analytical restrictions only some guidelines claim BTM to be useful for the estimation of fracture risk and/or therapy monitoring.

Pharmakogenetik 1

V09 – Talk Eap

Pharmacogenetics in psychiatry: is there clinical relevance?

C. Eap

Unit of Pharmacogenetics and Clinical Psychopharmacology, Centre for Psychiatric Neurosciences, Department of Psychiatry, Lausanne University Hospital, Prilly-Lausanne, Switzerland

Objective: To discuss the clinical relevance of pharmacogenetics in psychiatry

Methods: Review of published studies

Results: Multiple genetic factors can influence the response to treatment with psychotropic drugs. At the pharmacokinetic level, it has, for example, been advocated to genotype cytochrome P450 isoforms before starting a treatment so to adapt the dose according to the patient’s metabolizing status. At the pharmacodynamic level, genetic testing is currently more promising for predicting rare idiosyncratic adverse drug reactions. (e.g. HLA polymorphisms and clozapine-induced agranulocytosis or carbamazepine-induced epidermal necrolysis / Stevens-Johnson syndrome) than for predicting response to treatment. However, until now, with the notable exception of carbamazepine and HLA-B*1502 in Asian patients, none of the published results reached a level of clinical significance or brought strong enough scientific evidence to support a generalization of pharmacogenetic tests in routine clinical practice in psychiatry, in particular when taking into account a cost-benefit evaluation. On the other hand, over the past few years, there has been a very rapid and strong decrease of costs associated with genetic analysis, which will influence the cost-benefit ratios and modify the paradigm on the usefulness of pharmacogenetic tests.

Conclusion: Pharmacogenetic tests are presently seldom used in psychiatry, and mainly retrospectively. However, due to the affordability of such tests and to the continuous discovery of new pharmacogenetic factors, a strong increase of their use is expected in the future.

V10 – Talk Maitland-van der Zee

Pharmacogenetics in Cardiovascular Disease: is there clinical relevance?

A. Maitland-van der Zee

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Objectives: To give an up-to-date overview of the research in pharmacogenetics of cardiovascular disease, and the clinical implications of this research.

Methods: In this lecture I will focus on these groups cardiovascular drugs where many pharmacogenetics studies have been performed (including statins, coumarins, clopidogrel).

Results: Many studies have been performed in this area of research. These pharmacogenetic studies have been dealing with many methodological issues, such as power issues, issues with exposure and outcome definition etcetera. This is also at least part of the reason that many gene-drug associations that were initially reported were not replicated. For some gene-drug interactions trials have been performed to study the clinical utility of implementing genetic testing.

Conclusion: Even though many studies have been performed for many different drugs currently there are hardly any clinical implications in cardiovascular pharmacogenetics.
Minimale Resterkrankung bei Tumoren

V11 – Talk Joosse

Molecular characterization of circulating tumor cells (CTCs)

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Cancer metastasis is the main cause of cancer related death. Administration of systemic therapy against cancer metastasis treatment is presently based on personal statistical risk and pathological characteristics of biopsies of the metastasis. In the presence of multiple and/or badly accessible metastases, circulating tumor cells (CTCs) extracted from peripheral blood could aid diagnostics and clinical management. For this so called “liquid biopsy”, sensitive methods have been developed to capture the CTCs from the peripheral blood at the single cell level. Individual tumor cells can be investigated using novel technologies such as Next Generation Sequencing to determine copy number alterations and gene mutations. Such data could provide us with information about the genetic heterogeneity of the metastases and possible identification of therapy sensitive and resistant clones.

To establish single cell genetic characterization, we have used the breast cancer cell line SKBR3 spiked in blood of an apparently healthy donor to reflect CTCs in a cancer patient’s blood. Tumor cells were first enriched based on their density and identified by keratin immunocytochemistry followed by capturing of the single cells by micromanipulation. An important aspect of single cell genomic analyses after whole genomic amplification is the quality control of the amplified material. We could show that longer fragments produced from the WGA product will produce better quality NGS data as compared to material that resulted in short PCR fragments only. We were able to reliably and reproducibly generate NGS data from single tumor cells.

Advances and Challenges of Extended Newborn Screening

V12 – Talk Hoffmann / Talk Autti-Rämö

Expansion of Newborn Screening Programs for Rare Metabolic Diseases: New Concepts and Possibilities

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Over the last decade improved knowledge and new technical platforms, especially high-throughput methods for multicomponent analyses, have transformed newborn population screening. From 1995 tandem mass spectrometry expanded newborn screening programs were developed in the USA, Europe and Australia. They became one of the most promising preventive approaches in medicine. Parallel the detection, treatment and possibly prevention of individually rare genetic diseases have become one of the major challenges in developing countries. A child with a severe handicap because of a late diagnosed metabolic disorder in Germany spends 40 - 60,000 Euros every year of life for direct health care costs only. The overall frequency of all disorders detected by the extended neonatal screening program in Germany is now about one affected baby out of every 1,000 newborns. The cost relationship for direct health costs in Germany is one million Euro invested in newborn screening against savings of > 70 million Euro of avoided direct health costs. Comparison of extended screening programs in different parts of the world suggests that the incidences are universally = l:1000 in modern populations with a mixed gene pool and a low rate of consanguinity. This rate rapidly increases in countries with traditional societies, especially influenced by the rate of consanguinity, e.g. to 1: 800 in Turkey and 1: 400 in Qatar. By 2014, 12 European countries, the USA, Australia, Canadian provinces as well as Qatar have expanded newborn screening programs, and more are considering the expansion. As a problematic development the number of disorders screened for by MS/MS range from two disorders (PKU and MCADD) in England to 20 in others (up to 60 in the USA). The number of live births investigated per screening center varies from 18,000 to 200,000. Few programs have reported the number of positively identified cases and technical data, although many participate in quality assurance and proficiency test schemes. This does not reflect major differences in the genetic background of populations or estimated prevalences, but rather highlight different approaches to the estimation of risks and benefits and just lack of evidence. We are lacking detailed knowledge about the natural course of many diseases and their variants, information on middle- and long-term outcome after early treatment initiation. Harmonisation of disease screening panels, spectrum of metabolites analysed, sizes of screening laboratories, analytical procedures, and proficiency and quality testing are all urgently warranted on the European level (and beyond). The huge difference of recall rates illustrates one obvious and important area for improvement. There is a need for universal metrics to allow interlaboratory comparisons, quality assurance schemes, approved training schemes for provider competence in interpretation, as well as inter laboratory cooperation in second-tier strategies (e.g. possibly one specialized second-tier test in one laboratory only). An important
issue is the development and evaluation of uniform follow-up and confirmatory testing of patients leading to uniform guidelines. The long-term benefit of all programs has to be evaluated thoroughly which for most of these rare diseases can only be achieved through close and coordinated international collaboration on long-term follow-up and outcome. By now these challenges appear more complex than technological hurdles. Finally, following high-throughput methods for multicomponent analyses high-throughput methods for molecular analyses are almost ready to be practically implemented into newborn screening programs with identical as well as a whole array of new challenges and possibilities.

**Qualität und Akkreditierung im klinischen Labor**

**V13 – Talk Kaiser**

*Laboratory quality assurance in the context of transplantation medicine*

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**Objectives:** The model for end-stage liver disease (MELD) score is used to prioritize organ allocation for patients who require orthotopic liver transplantation. It is calculated from the results of creatinine, bilirubin and international normalized ratio (INR). Consequently, high validity of the results is crucial for equitable and fair organ allocation. The aim was to study the impact of different laboratory methods and locations on the organ allocation process.

**Methods:** We implemented a quality assurance for MELD diagnostics with individual validation and report. Samples were analysed with two different creatinine assays (enzymatic and Jaffé based). A proficiency test for MELD diagnostics was introduced and performed two times at German transplant centers with the Reference Institute for Bioanalytics. Samples from patients with liver diseases were used and clinical case reports were provided.

**Results:** Parallel creatinine measurements showed significantly higher MELD scores with Jaffé compared to the enzymatic assay. The proficiency test for MELD diagnostics showed maximal differences (median) of 8 points between the 19 centers with deviations due to bilirubin and creatinine with a maximum of 4 and 6 MELD points. Analysis of the INR results showed a possible inhomogeneity of the material.

**Conclusion:** As a consequence of our studies, recommendations had been addressed to the German Medical Association: firstly, laboratories at transplant centers should perform MELD diagnostics with individual validation and reporting of the MELD score; secondly, enzymatic creatinine assays should be used and thirdly, further studies are needed to improve or replace the interference-prone INR diagnostics for allocation of liver grafts.

**Labormanagement in der Stammzellspende**

**V14 – Talk Bornhäuser**

*Labormethoden in Rahmen der Stammzellspende*

*M. Bornhäuser²*

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Hematopoietic stem cell transplants are performed with increasing frequency in order to cure pediatric and adult patients with hematologic malignancies or inborn deficiencies. Throughout the recent decades, the laboratory methods required to facilitate the mobilisation of hematopoietic stem cells as well as the quality controls have been standardized. Still, basic hematologic technologies like white blood cell counting are required but most emphasis is put on the enumeration of vital CD34+ cells using dual or single-platform FACS technologies. The combination of dye exclusion and CD34+ enumeration has outperformed traditional clonogenicity assays like colony-forming unit (CFU) assays traditionally performed in parallel. These CFU assays have been difficult to standardize but still represent a valid technology for research purposes. Additionally, sterility testing has gained more and more relevance since it has become the second most important release criteria. Commercially available microbiological testing reagents may be used but need to be validated for cellular products like bone marrow, mobilized blood and cord blood, separately. Specific functional assays used for immunotherapeutic applications are antigen-specific multimer-based assay and cytotoxicity assays which are currently in the process of being standardized but are still not part of routine procedures.
Validierung von Stammzellprodukten

M. Bornhäuser

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Hematopoietic stem cell transplantation (HSCT) has become an important therapeutic option for pediatric and adult patients suffering from inborn diseases or hematologic malignancies. The main indications for allogeneic HSCT are acute myeloid leukemia and myelodysplastic syndromes. Sources of HSC for allogeneic transplantation are bone marrow, peripheral blood and cord blood. As HSC products are regulated by the drug law, the donation, manipulation and cryopreservation requires validated processes which have to be documented in order to achieve a manufacturing license by the local and federal authorities. Important parameters which are part of the validation process are: nucleated cell counts, percentage and absolute count of vital CD34+ precursors, sterility, clonogenicity and recovery of cells after cryopreservation and thawing. In addition, infectious disease markers have to be validated for donor clearance and sterility testing has to be validated specifically for HSC grafts. Parameters like nucleated cell count and CD34+ have specifically optimised for use with HSC grafts. Although the microbiological control of cellular products has now also been incorporated in the european pharmacopoeia, the material and reagents used for sterility testing have originally not been developed for cellular products and therefore need specific validation with test bacteria. This has to be performed for fresh and cryopreserved products and for various graft sources individually.

The validation of the above mentioned parameters has become more and more standardized accross most institutions but still is far more heterogeneous and less reproducible than other laboratory techniques applied in medicine.

Programmed ways to die or not

Cell death pathways in cancer

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Deregulated cell death pathways are an important feature of many diseases including cancer and translational cell death research is about to enter clinical medicine. Increased expression of apoptosis blockers (e.g. IAP proteins, Bcl-2 family proteins, MDM2/p53 axis) and/or increased activation of anti-apoptotic survival pathways provides molecular targets for selective therapeutic intervention. Also, induction of cell death by e.g. death receptors has been considered as a potential therapeutic approach. A number of clinical studies have been initiated to address the efficacy of apoptosis inducing and/or modulating therapy with heterogeneous responses. Targeting one single pathway may not be sufficient to overcome intrinsic cell death resistance and preclinical models that come close to the disease in patients are mostly lacking. In addition, classical apoptosis may not be the only regulated cell death program. Necrosis considered as accidental cell death and necroptosis have been shown to be governed by molecules of the TNF-receptor signaling complex, and autophagy has been implicated in both, the survival state of cells under starving conditions as well as cell death mechanism. Also, cells may die in the senescence program. Despite this complexity, targeted therapy and identification of relevant biomarkers related to cell death pathways are becoming increasingly important, and modulation of cell death pathways will be a key feature in future therapeutic interventions in many diseases.

Labordiagnostik der Demenzerkrankungen

Labordiagnostik beim M. Alzheimer

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Objectives: Currently, only symptomatic treatment is available for Alzheimer’s dementia (AD) with an unmet need for preventive therapies. Preventive treatment, however, calls for predictive diagnostics of incipient dementia, that is the reliable diagnosis of preclinical AD in
high risk cohorts, e.g. patients with mild cognitive impairment (MCI). Recent research on Cerebrospinal Fluid (CSF) dementia biomarkers has clearly validated that CSF based neurochemical dementia diagnostics (CSF-NDD) can identify MCI patients at risk for AD with negative and positive prediction values exceeding 90%. Due to a prevalence of preclinical AD in MCI of 20-25%, CSF-NDD has become a powerful diagnostic tool to assist the recruitment of preclinical AD patients in MCI cohorts for clinical trials which aim to develop novel preventive therapies.

**Method and Results:** We will screen the recent literature to present an overview regarding CSF-NDD applied for the molecular diagnostics of preclinical and early AD. Moreover, promising novel findings regarding blood based diagnostics of preclinical or early AD will be critically discussed.

**Conclusion:** Recent scientific evidence (validated on S3 level) clearly shows that CSF-NDD does allow predictive and improved early and differential diagnosis of AD. However, there is a strong need for improved quality control and standardization of assay performance. First blood assays for early AD seem feasible but have not been validated yet.

### Entwicklung und Klinische Anwendung des Next-Generation Sequencing

**V18 – Talk Wiemann**

**Herausforderungen für den Einsatz der NGS in der medizinischen Praxis**

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Groundbreaking advances in technology have paved the way towards rapid sequencing the genomes of individuals. The 1000genomes project has established a catalogue of natural variation that now contributes to the classification of mutations identified in cancer sequencing projects like the International Cancer Genome Consortium (ICGC) and the The Cancer Genome Atlas (TCGA). Particularly these projects have unraveled the huge level of variation in genomes and the complexity of formerly assumed uniform tumor entities and subtypes. Every patient is individual and so is his/her tumor. Tumor heterogeneity and evolution, which continues during treatment, complicate matters so that, on the longer run, diagnostic sequencing that is performed for therapy decision needs to be complemented by disease monitoring of therapy response. Numerous studies are ongoing trying to show the benefits clinical sequencing brings to the patient, also to eventually transform NGS from application in clinical studies into routine cancer diagnostics. However, deep knowledge of the genetic markup of individuals as it is obtained in genome analysis is associated with challenges in data management and protection of personal rights. Who should gain access to the data? How should additional findings be treated? What rights should patients have to their most personal information? The solutions that are vividly and controversially discussed in different countries where the application of genome-wide genetic analysis of individuals is right in the process of being transferred into clinical practice substantiate the need for standards that should, ideally, be harmonized.

**V19 – Talk Werner**

**Clinical application reaches beyond mapping and annotation: Principles and approaches of level 2 NGS data analysis**

*T. Werner*

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There are two types of NGS data that are preferentially used in clinical settings: genotyping (resequencing, detecting mutations) of a patients genome and RNA-seq (changes in RNA-levels). Mapping and annotation readily identifies genes affected by changes in both cases but does not identify changes relevant for diagnostics or therapy. Filtering out relevant results is the main objective of level 2 analysis. Biomedical relevance usually arises out of context, thus the focus is on changes of gene-gene relations, which is best achieved in functional cascades exemplified by pathways and networks. Our approach towards this is to merge results from NGS data with the existing knowledge from databases and the literature in general in order to identify disease-correlated mutations and to construct patient specific networks. We were successful in identification of relevant mutations in the CLARITY challenge (Boston Children’s hospital) and we were also successful constructing expression based patient-specific networks in many cases. The underlying principles and selected results will be shown. Translational application of NGS data requires filtering of the vast amount of primary results for biomedical relevant results. This filtering has to be done on the data analysis side in order to close the gap between molecular level data (omics) and the clinical level at which clinicians need to work. While there is no ready-to-apply pipeline there yet, current approaches are very promising.
V20 – Talk Dreher

Personalised Cancer Treatment by Computational Modelling Based on Next-Generation Sequencing (NGS) Data

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Objectives: Every tumor is different on molecular level and thus responds differently to drugs. Typically only $\sim 25\%$ of patients benefit from treatment with mechanistic drugs. NGS allows to analyze a tumor in great detail, hence to handle every tumor as unique disease and to treat it with an individual therapy. Thus systems are needed able to integrate complex NGS data and to derive clinically relevant predictions.

Methods: ModCell is a systems biology modelling system that comprises information on molecular processes in tumor onset and progression. It contains data on cancer-relevant signalling pathways covering over 700 genes. It is able to integrate individual genomic data allowing to build models for the prediction of tumor-specific effects of drugs using a Monte Carlo-based modelling approach.

Results: The computational modelling platform ModCell allows the prediction of individual drug effects using large-scale genomic and transcriptomic data in cancer cell lines. We provide evidence that ModCell is able to reproducibly predict the effects of individual drugs with an accuracy of $80\%$ independent of the tumor tissue type of origin.

Conclusion: We show a strategy for the prediction of drug response in individual cancer patients based on an deep molecular characterization of tumor/patient and the modelling system ModCell. This opens the way for future computer-assisted personalised medicine to improve oncological practice, which could lead to more efficient treatment of cancer patients.

SGKC-Symposium Biomarker Research in Swiss Institutes of Clinical Chemistry

V21 – Talk Mooser

Biobanking as the basis of biomarker research

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New biomarkers will be essential for enabling a more personalized, preventive, predictive and participative (the 4P) medicine. Access to high-quality samples and data from large number of properly informed and consenting individuals is central for the discovery of new biomarkers and, equally importantly, for their proper validation and qualification. Lausanne University and University Hospital have sponsored the creation of a new hospital-based biobank (www.chuv.ch/biobanque), which parallels a local population-based cohort (www.colaus.ch). Each patient admitted to the Hospital is systematically invited to participate in this Biobank. Participants consent for the general use of their data and samples for research (general consent), including whole genome sequencing. In addition, patients are invited to consent for future contacts, in case clinically actionable incidental findings are made on their biological material, and to be invited in future clinical trials. A total of 10 ml of blood is collected and processed immediately for isolation of buffy coat and plasma, which are stored at $-80^\circ$C. Over the first 19 month of recruitment, a total of 11868 patients have consented to participate in this project, out of 15572 patients contacted (participation rate 76%), with 92% of the participants consenting for future contacts. Overall, this data shows that a systematic biobank in a large university hospital is feasible and that patients are generally interested in genomic research.

V22 – Talk Hornemann

Atypical Sphingolipids for the diagnostics of cardiometabolic diseases

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Cardio-metabolic diseases are the major cause of mortality and morbidity, despite the progress in risk prediction and stratification. Besides the known risk factors, sphingolipids are emerging as novel players in the pathogenesis of metabolic diseases. Sphingolipid biosynthesis is typically initiated by the condensation of palmitoyl-CoA and serine, a reaction catalyzed by the serine palmitoyltransferase (SPT). Apart
from these canonical substrates is the enzyme also using alanine and other acyl-CoAs as alternative substrates. This forms a great variety of atypical SPT products. A set of these atypical metabolites emerged in several cross sectional studies as promising novel biomarkers for cardiometabolic diseases. Here, the potential of these lipids in the prediction of cardiovascular disease and T2DM was analyzed in a prospective cohort (n = 349) which included follow up data of 8 years (VIVIT-study). From this study an atypical C20 sphingosine metabolite emerged as a significant and independent risk predictors for cardiovascular events (HR = 1.31) even after adjusting for known risk factors like age, sex, smoking, HDL, WCF, Hba1c, GFR, CRP and coronary artery stenosis. Another atypical sphingoid base (1-deoxySO) was identified as an independent predictors for the development of T2DM (OR = 2.05). Univariate logistic regression models for 1-deoxySO, Hba1c and the presence of a MetS showed similar AUCs for each variables (0.68, 0.64 and 0.64 respectively) whereas the combination of all three markers improved the AUC significantly (0.77). In conclusion we showed that atypical sphingoid bases are prognostic biomarkers for the risk prediction in cardiometabolic diseases.

Von der Systembiologie Zur Systemdiagnostik: Chancen für die Labormedizin

V23 – Talk Klawonn

Wie man gute Kombinationen von Biomarkern (nicht) findet

F. Klawonn

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High throughput technologies like microarrays, next generation sequencing and mass spectrometry enable us to measure the complete or large parts of the transcriptome, proteome or metabolome. Such data provide often more than ten thousand possible candidates for biomarkers. Unfortunately, there is seldom a single biomarker for a specific disease. A common approach is to find subsets among the biomarker candidates forming a classifier that can function as a good biomarker. But the search for suitable combinations of biomarker candidates leads to new problems. Even if only pairs of biomarker candidates are considered, this leads already to an explosion of the search space. For a data set with 10,000 biomarker candidates, there are almost 50 million pairs. Thus heuristic search strategies are needed to construct useful combinations. Feature selection and extraction techniques, as they are common in machine learning, can help to solve this problem. The danger of overfitting poses an even more serious problem. In contrast to the number of biomarker candidates and their combination, the sample size, i.e. the number of donors is usually very limited. A common mistake that leads to overfitting is to first apply feature selection on the whole data set and then evaluate classifiers on the basis of techniques like cross-validation. We present a first version of a tool that helps to find biomarker combinations and also provides techniques like permutation tests to avoid overfitting.

V24 – Talk Lavrik

Dynamics of death receptor networks in immune cells

I Lavrik

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Systems biology is an emerging field of science that combines mathematical modeling with powerful experimental methodology, providing a quantitative assessment of the signaling pathways. We apply systems biology to the analysis of death receptor networks. The members of the death receptor family control programmed cell death (PCD) and proliferative pathways. PCD is essential for regulation of homeostasis and elimination of unneeded, damaged, or infected cells in multicellular organisms. PCD deregulation contributes to cancer, as well as neurodegenerative and autoimmune diseases. Despite the fact that death receptor-mediated signaling has been studied to a high level of detail, its quantitative regulation until recently has been poorly understood. This situation has dramatically changed in the last years. Creation of mathematical models of death receptor signaling, in particular in immune cells, led to an enormous progress in the quantitative understanding of the network regulation and provided fascinating insights into the mechanisms of death receptor control. It will be discussed how systems biology studies provide new understanding of the death receptor signalling in immune cells and create a platform for the drug development in the context of diseases associated to defects in death receptor signaling pathways.
Neue Anforderungen der Akkreditierung nach DIN ISO 15189:2013

V25 – Talk Spitzenberger

DIN EN ISO 15189:2013: New requirements for quality and competence for medical laboratories

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The third edition of ISO 15189 sets substantially extended requirements for the QM-system of medical laboratories. The changes may be differentiated into three categories: editorial, structural and with regard to contents. Editorial and structural changes were made to improve the readability of the document and to enhance the transparency and the plausibility of the standard requirements for the international community. Essential criteria of the new standard edition are related to novel management and technical requirements. In the centre of the QM documentation, ISO 15189 not only requires a quality manual but a set of more than twenty mandatory “documented procedures”. The new provisions for subcontracting of referral laboratories will increase overall transparency for patients and other laboratory users. Concepts for risk management and extended review activities are newly introduced and focus on the improvement of patient safety. Key aspects of the technical requirements are related to the specification of criteria for validation and verification of examination procedures and to concepts for quality assurance. For the first time, ISO 15189 introduces incident reporting duties with regard to the malfunction of laboratory equipment. The new standard version also requires compliance with selected quality criteria for report validation and for the laboratory information management system. In Germany, DIN EN ISO 15189:2013 is used for voluntary application with or without accreditation and for formal recognition according to the medical device act.

V26 – Talk Gottschall

Anforderungen an das Personal

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The DIN EN ISO 15189 constitutes an international standard for quality and competence in medical laboratories. This standard also defines the way in which the management system of a laboratory must function, and the degree of technical competence required of laboratory staff in order to ensure valid laboratory results for patient care. Recently, the DIN EN ISO 15189:2007 standard has been completely revised in its normative sections. In Section n°5 of the new standard DIN EN ISO 15189:2013, requirements for human resource management, and its proper documentation are presented. According to this section, laboratory management is required to ensure its staff’s suitability and qualification for the various diagnostic and administrative tasks performed. Furthermore, requirements are set for continual staff training and development, as well as for the routine evaluation of staff’s competence and performance.

Qualitätssicherung Liquid Biobanking

V27 – Talk Findeisen

Decay markers for preanalytical monitoring

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Objective: Preanalytical variations have major impact on most biological assays. Specifically MS-based multiparametric proteomics analyses of blood specimens are seriously affected by limited protein stability due to high intrinsic proteolytic activity of serum and plasma. However, quality control (QC)-tools are not yet available and correspond to the ‘QC-gap’. Here, we suggest the monitoring of preanalytical quality for serum and plasma by analyzing the time dependent decay patterns of endo- and exogenous peptides with LC/MS.

Methods: Serum specimens from healthy controls and cancer patients were analyzed for a set of endogenous peptides. Proteolytic fragments were quantified with LC/MS at different preanalytical timepoints ranging from 1h to 48h. For monitoring the decay of an exogenous reporter peptide (RP) was added to serum and plasma specimens prior to LC/MS analysis.
Results: With a set of 62 endogenous decay markers we were able to characterize the time dependent changes of serum peptide profiles. For the exogenous reporter peptide a ratio of short and intermediate fragments was calculated. Classification accuracy was high with values always above 0.89 for areas under receiver operating characteristic curves (AUROC).

Conclusion: Peptides are continuously processed in blood specimens in a time dependent manner. This ‘proteomic degradation clock’ can be used to estimate the preanalytical quality of serum and plasma and might have impact on quality control procedures of biobanking repositories in the future.

V28 – Talk Oelmüller

Preanalytical factors that influence nucleic acid analysis in blood

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Objectives: The EU FP7 SPIDIA consortium project* aimed at identifying the influence of pre-analytical factors on molecular analytical tests and at developing European standards for securing good quality clinical samples.

Methods: SPIDIA performed two consecutive ring trials each for cellular RNA, genomic DNA and circulating cell free DNA testing in blood. Homogenous blood samples were shipped to participating laboratories in 28 EU countries. In ring trial 1, the laboratories followed their own sample handling and nucleic acid isolation procedures. In ring trial 2, the laboratories followed SPIDIA’s improved pre-analytical workflows. Nucleic acids isolated by the participants were intensively analyzed by SPIDIA in various analytical tests.

Results: Blood collection, storage, transport, processing and nucleic acid isolation steps can cause cellular gene up- and down regulations, nucleic acid degradation and release of genomic DNA from blood cells, all leading to nucleic acids profiles changes in the samples. This can significantly impact the reliability and validity of analytical test results. Improved standardized workflows including sample preservation can prevent or reduce these impacts. Based on this evidence, new European Technical Specification standard documents for pre-analytical workflows are in development at the CEN Technical Committee 140 “In-vitro Diagnostic Medical Devices”.

Conclusions: As pre-analytical steps can alter nucleic acid profiles in blood samples it is of utmost importance to implement workflows which prevent these changes for securing valid analytical test results. New international standard documents are needed to support this process.

* grant no. 222916 (www.spidia.eu)

Neurodegenerative Erkrankungen

V29 – Talk Lütjohann

Cholesterol within the CNS and neurodegenerative Diseases

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Objective: Nature has met the need for constant levels of cholesterol within the CNS by a highly efficient blood-brain barrier. However, there is a concentration-driven flux of 24S-hydroxycholesterol from the brain into the circulation, which is of major importance for elimination of excess CNS cholesterol. The opposite flux of 27-hydroxycholesterol from the circulation may regulate a number of key enzymes within the brain.

Methods: In vitro experiments suggest that the balance between the levels of cholesterol and its side-chain oxidized metabolites may be of importance for the generation of beta-amyloid peptides and may be the link between hypercholesterolaemia and Alzheimer disease.

Results: Within human studies the relationship between the cerebral and extracerebral cholesterol synthesis and metabolism, and the AD pathology as reflected by CSF markers was well pointed out. The possibility has been discussed that administration of inhibitors of cholesterol synthesis may reduce the prevalence of Alzheimer disease. Enhancing CYP46A1 expression rate triggers the conversion of cholesterol into 24S-hydroxycholesterol and its release via the protective blood-brain barrier and could thus slow down beta-amyloid driven AD pathology.

Conclusion: Disturbances of the cholesterol synthesis and metabolism described in Alzheimer’s disease (AD) may be both a consequence of the neurodegenerative process and a contributor to the pathogenesis.
V30 – Talk Drey

Aspects of neurodegeneration in the onset of sarcopenia

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Introduction: Age-associated muscle loss, or sarcopenia, is one of the greatest challenges in geriatrics. Due to high prevalence of sarcopenia in old people and its consequences regarding mobility, independence, morbidity and mortality, there is a high interest in understanding the pathophysiology of sarcopenia. A multifactorial concept is currently discussed. Altered hormone levels, chronic inflammation, malnutrition and reduced physical activity are involved in the genesis of sarcopenia. The role of neurodegeneration in the onset of sarcopenia is not well understood.

Method: An electrophysiological technique called Motor Unit Number Index (MUNIX) was used to investigate whether the loss of motoneurons causes sarcopenia. Further, the degeneration of the neuromuscular junction was investigated in a mouse model by overexpression of neurotrypsin reducing the acetylcholine receptor clustering activity by inactivating agrin.

Results: It could be shown that a low number of motor units is associated with sarcopenia. Using histological and functional analyses in neurotrypsin overexpressing mice, a full sarcopenia phenotype was found.

Conclusions: These findings demonstrate that neurodegenerative components play an important role in the onset of sarcopenia. More research is necessary to implement this knowledge in the diagnosis and therapy of sarcopenia.

V31 – Talk Demuth

Association between Leucocyte Telomere Length and Hematological Parameters - Data from the Berlin Aging Study II (BASE-II)

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The length of the telomeres is widely accepted as a biomarker of aging. The dynamics between telomere length and hematopoetic parameters in the normal aging process, which is of particular interest with respect to age related anemia is not well understood. Therefore we analyzed the relationship between leucocyte telomere length and hematological parameters with a focus on erythropoesis. Genomic DNA was extracted from peripheral blood leucocytes of Berlin Aging Study II participants and used to determine telomere length by a quantitative PCR protocol (Cawthon). Standard methods were used to determine blood parameters and the WHO criteria were used to identify anemic participants. Telomere length data were available for 444 (28.4 ± 3.1 years; 52% women) younger participants and 1463 (68.2 ± 3.7 years; 49 % women) older participants. We found a partial correlation between rTL (adjusted for subject’s age and BMI) and the RBC in women of the younger group (r = -0.161; p = 0.015). The rTL of older men exhibited a statistically significant positive partial correlation with MCH and MCH-C. Among all participants 6.3% met the criteria to be categorized as ‘anemic’, however, there was no association between anemia and rTL after stratification for age and sex. In the present study we have detected sporadic correlations between rTL and hematological parameters, however, in all cases rTL explained only a small part of the variation of the analyzed parameters. In disagreement with some other studies showing similar data, we interpret the association between rTL and some of the hematological parameters studied here as marginal. This applies also to the role of rTL in anemia, at least in the age groups investigated here.

V32 – Talk Buchmann

The Role of Lp(a) in Diabetes Mellitus

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Rationale: Earlier studies suggested that Lp(a) concentrations are related to type 2 Diabetes (T2D). Several studies showed increased Lp(a)-levels in T2D but recent analysis found an inverse association of Lp(a)-levels to T2D. Here we have investigated the relationship between Lp(a), and T2D in the context of the Berlin Aging Study II.
Methods: A total of 1071 subjects were analyzed (women=52%; 60-84 years old). T2D was assessed according to the ESC Guidelines. Glucose tolerance test (oGTT) was performed in subjects with no self-reported T2D. Lp(a)-levels were divided in quintiles for further analysis.

Results: The prevalence of T2D was 17.4% in men and 9.2% in women. Lp(a)-levels were lower in men and women with T2D, however, the difference reaches statistical significance only in men (15.9mg/dl in T2D, 24.3mg/dl in non-T2D; p=0.015). HOMA-IR levels as a marker for insulin resistance were significantly higher in subjects belonging to lowest quintiles of Lp(a) both in men and women.

Conclusion: Prevalence of T2D was high in subjects of the Berlin Aging Study II. Lp(a)-levels were inversely related to T2D in elderly men and women. Lp(a)-levels were also associated with insulin resistance. Whether T2D leads to a decrease of Lp(a)-levels or low Lp(a)-levels constitute a risk for T2D remains a matter of ongoing research.

Neue Entwicklungen in der klinischen Massenspektrometrie

V33 – Talk Rochat

LC-High Resolution-MS in Clinical Labs: A Step Forward for TDM and metabotyping

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Objectives: These last years, using high-resolution (HR)-MS, we have performed different analyses on various chemicals: quantifications of drugs, peptides or endogenous metabolites, studies on the fate of drugs with drug metabolite identification, metabolomics with unidentified ions for biomarker discovery and metabolotyping with identified metabolites for biochemical pathway follow-up.

Methods: Plasma extracts were injected on LC-ESI-Q-Exactive Focus and Exactive Plus-MS. Detections were performed mostly in full-scan at 35-140K resolution (m/z=200), in MS2 product ion or SIM modes. The metabolites were determined with extracted-ion-chromatograms around theoretical m/z values.

Results: Quantitative LC-MS determinations of drugs, peptides, and endogenous metabolites using HR-MS or triple-quadrupole MS were compared. It appears that the HR-MS technology we employed, showed similar precisions, accuracies and sensibilities and was at least as easy to use as triple-quadrupole MS. Moreover, qualitative or relative quantification (no calibration curves) analyses such as metabolite identification, targeted and untargeted metabolomics revealed the much higher capabilities of HR-MS over triple-quadrupole MS.

Conclusion: Our results should convince analysts working in clinical labs, to use when possible HR-MS technology for most analyses: quantitative, semi-quantitative, qualitative, routine and research. It will facilitate the analytical workflow and allow MS overlapping between research and routine requests. We think that time has come to use HR-MS for most clinical LC-MS analyses.

V34 – Talk Lehmann

Metabolomics von der Forschung zur Diagnostik - Potential, Perspektiven, Pitfalls

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Metabolomics means the investigation of thirty to hundreds (targeted metabolomics) or the profiling of thousands (non-targeted metabolomics) of metabolites. While quantitative target analysis of selected metabolites has long been applied for diagnostic purposes, the profiling approaches combining chromatographic tools and high resolution mass spectrometry is rather new. In the field of laboratory medicine metabolomics opens perspectives to shed light from a different angle on numerous topics like pre-analytical processes, diseases states, discovery of diagnostic biomarkers or the development of novel mass spectrometric strategies. In addition, functional metabolomics, conventional or stable-isotope assisted, can contribute to a better understanding of pathobiochemical mechanisms of complex multifarious diseases. Metabolomics research is full of promise and perspectives, but scientists should also be aware of current limitations and pitfalls. This talk will illustrate strengths, perspectives and critical aspects of metabolomic profiling on the way to the discovery of novel laboratory diagnostics and pathophysiological mechanisms. Furthermore, a novel metabolomic strategy complementary to conventional non-targeted analysis will be introduced, that facilitates the identification of specific metabolites, reducing the number of “unknowns” in current data bases.
Guidelines and Laboratory Diagnostic Pathways

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Translating medical guidelines into clinical pathways is not trivial. Which information must be provided by the guideline? What is the best presentation format for the pathway?

- Using the differential diagnosis of proteinuria as an example, the following standards can be recommended: Initial symptoms, clinical question (positive test strip result, suspected disease)
- Analyte or analytes to be measured, including decision limits, suitable patient materials, and preanalytical requirements (test strip, total protein, specific proteins, creatinine as a reference magnitude, second morning urine)
- Logic operations (decision rules, decision tree, scatter plot albumin vs. A1M)
- Differential diagnoses (glomerulopathy, tubulopathy, mixed forms)

If this information is available, a laboratory diagnostic pathway (LDP) can be constructed in a straightforward manner and implemented both in the laboratory information system and on the respective analytical platforms.

Decision Trees and Other Diagnostic Strategies

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Laboratory diagnostic pathways represent a workflow organization concept (reflex testing on selective analyzers) as well as a framework for effective interdisciplinary cooperation between the clinicians and the laboratory (consensus-based rules that can be implemented in an electronic order entry system). Usually, the underlying if...then...else rules are represented as graphical decision trees. This sequential paradigm for multivariate analysis is currently most popular, because it is easy to understand and very straightforward. If the tests are arranged from most sensitive to most specific, they will quickly narrow down the number of potential diagnoses with an acceptable risk of wrong decisions at each logical conjunction of the tree. However, a substantial risk for false positives exists in screening situations (hospital admission, preventive medical checkup), where the prevalence of the suspected disease is low. False negative results, on the other hand, are a serious problem if available laboratory tests are not sensitive. In these cases, parallel testing can improve the accuracy of diagnostic classification. Based on simulated and real data, we show that scores of small, carefully selected biomarker profiles can be superior to sequential reflex testing. A combined strategy is recommended. In the future, innovative analytical and bioinformatics technologies may offer “big data” approaches as a third way of diagnostic classification. Currently, however, they are just of academic interest.

Laboratory diagnostic pathways – the Good, the Bad and the Ugly

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In Laboratory Medicine diagnostic pathways (LDPs) are very useful tools to standardize processes according to the state of the art. Before implementing such pathways, all advantages, limitations and requirements should be considered. Clinicians profit from the fact, that reflex
testing and interpretation is undertaken by the laboratory, so that diagnosis and treatment can be provided in a shorter period of time and overdiagnosis is prevented. Because of the necessity of networking with the clinicians, the laboratory also benefits from LDPs, by being an equivalent partner rather than a service provider.

Major limitations are 1) potentially misleading results in patients suffering from more than one disease, 2) symptoms with many possible underlying causes of disease (e.g. abdominal pain), which would result in complex and hardly comprehensible pathways and 3) “grayzone”-results, implying the risk of erroneous yes or no decisions with substantial error propagation in pathway with many junctions.

Having all these issues in mind, one should be aware, that the development of LDPs has to be triggered by medical, rather than economical considerations, as implementation of LDPs is not necessarily associated with a decrease in laboratory costs. In any case LDPs must be developed and periodically updated according to the best available medical evidence in an interdisciplinary manner, including preanalytical issues. They should be implemented as guidelines, giving the clinician enough leeway in decision-making when to deviate.

### Bildungsforum

**V38 – Talk Kachler**

**Comparison Analysis of Health Professions in Europe (GesinE) - Implications for the Education in Biomedical Science in Germany**

*M. Kachler*

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In a study on “comparison of education in the health professions in Europe” (GesinE) were analysed 16 health professions (including Biomedical Science) in Germany, France, Great Britain, the Netherlands and Austria. The competence profiles, education and professional tasks are comparatively analyzed. In most countries, the training in Health Professions takes place on the tertiary (Bachelor, Master) level. As an important result, the analysis shows that despite the education for health professionals in Germany at the secondary level the education should not be undervalued. Nevertheless, the results indicate that the academic training in some aspects, has advantages over the existing vocational training. How the European comparison shows the academic educated professionals develop more competencies for transfer of scientific knowledge into practice and the reflection on the implementation of patient-related decision-making processes. We will discuss what are the benefits of the bachelor’s and master’s education for the process of professionalization in biomedical laboratory science in Germany and what is the effect on the patients/clients in this healthcare service?

**V39 – Talk Homberg**

**Vergleich des Arbeitshandelns zwischen MTLA in Deutschland und BMA in der Schweiz - Was können wir lernen?**

*A. Homberg*

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**Objectives:** Recent publications highlight increasing professional dissatisfaction among German biomedical scientists (BS). Changes in the socio-political context and the correlating impact on occupational identity have played a significant role. To explore these issues, this study compared the lived experience of BSs in Germany and Switzerland.

**Methods:** Problem centered interviews were conducted exploring the topics: efforts of adaption, scope of practice, job satisfaction, evaluation of future professional opportunities and social status. Participants were German BSs employed in Swiss laboratories. Data analysis was based in the qualitative approach of Grounded Theory.

**Results:** Participants reported that admission standards for training in Switzerland have increased, but in parallel professional status has not improved, leading to a loss of attractiveness of the role for BSs. Workforce shortages also had a negative impact on satisfaction. In Germany, significant BS redundancies have occurred due to automation. As a result, there have been problems finding reemployment and those employed have had far higher workloads, while also limiting their ability to work to their full scope of practice.

**Conclusion:** A higher degree alone does not generally improve individual experiences of job satisfaction due to complex workplace factors. Nevertheless, higher qualification is one step towards strengthening professional status and identity and thereby potentially enhancing the profession’s negotiating power to improve employment conditions.
Competency-based approaches are generally accepted as newly emerging conceptual frameworks for training in health professions. The CanMEDS physician competency framework is such a competency-based approach often used in medical education, not only for physicians, but also for nurses, physiotherapists and radiographers. In its original version from the royal college of physicians and surgeons of Canada, it describes knowledge, skills and abilities as roles that specialist physicians need for better patient outcomes. Seven roles are defined in the framework: medical expert, communicator, collaborator, manager, health advocate, scholar, and professional. For biomedical scientists the CanMEDS roles doesn’t exist yet. In this lecture didactical ideas for role reflection in the curriculum for biomedical laboratory science will be given, with special emphasis on the roles of scholar, communicator and collaborator. The CanMeds framework could be used in training for biomedical scientists to make the curriculum more compatible with other health professions and “fit for future”.

At the BNLD symposium in 2008 (DGKL annual meeting) the working conditions of bioscientists in Europe, especially in NL, CH and GER were discussed. An update of this report and the situation in UK as a possible model for Europe will be described: In UK the professional background of a laboratory director, usually termed consultant, can be medical or non-medical. Medical staff practice is registered by the General Medical Council (GMC), non-medical staff by the Health and Care Professions Council (HCPC). A BSc in life sciences acts as a stepping stone to registration as a healthcare science practioner. Then one may join a scientist training programme. From which one can emerge with a MSc to register as a Clinical scientist with HCPC. Clinical scientist belongs to the protected titles in this register by law. Anyone using one of these titles must be registered with HCPC. Clinical scientists are defined by HCPC as follows: They oversee specialist tests for diagnosing and managing disease. They advise doctors on tests and interpreting data. HCPC clinical scientist registration is probably lower level of competence than GMC registration. However, as a clinical scientist one can apply to join a higher scientific specialist training (HSST) programme which, if successful, allows opportunity to join the higher specialist register held by the Academy of Healthcare Science. This is the best defined route to applying for consultant positions. This registration is similar to medical staff achieving a certificate of completion of training (CCT). For detailed information and help with this presentation I like to thank Dr. Gilbert Wieringa, who is the director of Laboratory Medicine at Bolton NHS Foundation Trust, UK and chair of EFLM professional committee.

The EC4RC belongs to EFLM. Main tasks of are harmonisation of postgraduate education for all academic professionals in the field of laboratory medicine within the EU. Furthermore, EC4RC holds a Register of specialists in laboratory medicine (EurSpLabMed). Register can all who are recognised in their home country as an specialist in Clinical Chemistry or similar and whose national society, which is part of EC4RC has shown equivalence to the EC4RC standards. For Germany this is the „Klinischer Chemiker“ of the DGKL. The Directive 2005/32/EG targets on free movement and settlement for residents of Member States of the EU. It concerns recognition of professional qualification acquired in other Member States to enforce that. It gives automatic rights to several professions where education and training have been harmonised throughout the EU. For all other professions, including scientists and pharmacists trained in clinical chemistry and laboratory medicine this is not the case.
They have the right to practice throughout the EU but they must be able to demonstrate that their qualification and training is equivalent to those of the country in which they will practice. The Directive has provision for a system of “common platforms” (CP) whereby if a profession can demonstrate a collection of criteria able to bridge the substantial differences between the different Member States, particularly in a country where the profession is regulated, automatic rights to practice can be given. ECARC is very active in developing such a CP based on the Register which includes many of the conditions of the Directive and is in discussion with officials in the European Commission about using it for this.

V43 – Talk Raabe-Meyer

New aspects to the professional profile of medical science experts: What does the European framework have to offer?

G. Raabe-Meyer

Praxis für Humangenetik, Hannover, Germany

Current position of human geneticists in the EU

Due to the growing technical demands of health care in Germany, the human genetics profession has an increasingly important role to play in human genetics laboratory diagnostics, thanks to the geneticists’ specialised expertise. Whilst at a national level, the specialized qualification gained after successfully completing scientific university studies is regulated by the German Society of Human Genetics (GfH), at a European level, establishing the comparability of further education curricula amongst the individual EU member states has been problematic. This is set to change in the foreseeable future. Due to the intensive efforts of the European Society of Human Genetics (ESHG) together with the ad-hoc committee it established, a set of criteria have been developed that allow a comparison of the specialized qualifications of human genetics scientists. It is expected that upon application, the first certificates for Clinical Laboratory Geneticists (CLG) could be issued by the ESHG in the nearer future.

V44 – Talk Peters

Moderne Herausforderungen in der Klinischen Toxikologie

F. Peters

University Hospital Jena, Institute of Forensic Medicine, Germany

Clinical Toxicology is a field dedicated to diagnosis and treatment of poisonings/intoxications. Due to wide variety of toxicologically relevant compounds clinical toxicological analysis requires case-specific analytical strategies. These often consist of a so-called systematic toxicological analysis (STA) to screen for relevant compounds and complementary quantitative analyses. Gas chromatography-mass spectrometry (GC-MS) as gold standard technique for STA, has been complemented and partly replaced by liquid chromatography-mass spectrometry (LC-MS)–based methods in recent years. The latter require less sample preparation, but are associated with a number of technique-specific pitfalls, such as matrix effects or instrument-dependent differences of mass spectra, which must be considered by the analyst. Moreover, selecting the most appropriate screening approach (e.g. targeted vs. untargeted analysis) is challenging. Another problem is the ever increasing number of so-called novel psychoactive substances (NPS) that calls for continuous updates of analytical methods. However, this is difficult with reference standards only slowly becoming available and a general lack of pharmacokinetic data for these compounds. In silico tools have been proposed to overcome this problem, but their value for routine applications is yet unclear. Finally, with increasing QC requirements for quantitative analysis, the analyst must carefully assess the value of quantitation for the particular case.

Diagnostik von Autoimmunerkrankungen

V45 – Talk Thaler

Evaluation of anticardiolipin antibody assays in view of the lack of a reference method: is latent class analysis the solution?

M. Thaler

Klinikum rechts der Isar der Technischen Universität München, Institut für Klinische Chemie und Pathobiologie, München, Germany

Background: We recently developed a biosensor assay for the detection of anticardiolipin antibodies (aCL). Evaluation of the assays diagnostic performance turned out to be challenging: reference materials are controversial and an unambiguous reference method for the detection
of aCL is not available. To make things worse, common commercial aCL assays exhibit considerable discrepancies not only with respect to quantitative results but as well as to the classification aCL positive vs. aCL negative.

**Objectives:** The aim of the presented work was to evaluate the diagnostic performance of the aCL biosensor in comparison to three commercially available assays.

**Methods:** A total of 99 sera were measured with a surface plasmon resonance aCL biosensor as well as with three commercially available aCL immunoassays. Based on the cut-off information of the commercial assays, latent class analysis was applied to assign sera either to the aCL positive or to the aCL negative group. Sensitivities and specificities were calculated for all of the four performed assays.

**Results:** The diagnostic performance of the research aCL biosensor is comparable to that of the three investigated commercial aCL immunoassays.

**Conclusion:** The developed biosensor is a suitable tool and possible reference method for the detection of aCL. Furthermore, latent class analysis proves to be a valuable approach for the evaluation and comparison of antiphospholipid antibody assays.

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**V46 – Talk Roggenbuck**

Simultaneous screening and confirmation of autoantibodies associated with autoimmune vasculitis

**D. Roggenbuck**

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The novel CytoBead® technology combines autoantibody analysis by cell-based screening with the confirmation of corresponding autoantigen reactivities by microbead multiplexing using immunofluorescence technique (IFT) in one reaction environment. CytoBead® ANCA allows the simultaneous detection of anti-neutrophil cytoplasmic antibodies (ANCA) on ethanol-fixed neutrophils for screening and confirmation thereof using proteinase 3 (PR3) and myeloperoxidase (MPO) coated microbeads. Furthermore, the detection of Goodpasture antibodies is integrated by adding glomerular basement membrane (GBM) coated microbeads. Anti-GBM autoantibodies occur in 10% of rapid progressive glomerulonephritis patients together with ANCA and are required for the differential serological diagnosis in routine diagnostics. This assay format can be interpreted with a standard fluorescence microscope (having a FITC channel) for semi-quantitative analysis and with the automated interpretation system Aklides® for quantitative testing. The performance of the CytoBead® ANCA assay was investigated using sera of 666 individuals including 118 patients with ANCA-associated vasculitis, 162 healthy controls, 352 disease controls and 34 anti-GBM positive sera. Receiver operating characteristics and inter-rater agreements (kappa) were used to compare the results of novel CytoBead® ANCA assay with routine autoantibody investigation. The comparison of classical ANCA screening with ANCA screening by the novel CytoBead® ANCA assay showed good agreement (kappa=0.73-0.83). The results of anti-PR3, anti-MPO, and anti-GBM detection by this novel method compared to anti-PR3-, anti-MPO as well as anti-GBM by ELISA revealed good to very good agreement (0.72,0.78,0.87; respectively). Consequently, CytoBead® ANCA assay is an attractive alternative to classical time-consuming single parameter ANCA and anti-GBM antibody detection and is applicable for high throughput routine diagnostics.

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**V47 – Talk Diederichs**

Long non-coding RNAs in cancer

**S. Diederichs**

1German Cancer Research Center, Heidelberg, Germany

The majority of the human genome is transcribed into long non-protein-coding RNAs (lncRNAs). These outnumber the protein-coding genes in the genome, but most these tens of thousands of transcripts have not been characterized at all. Nonetheless, from the few characterized examples, it can already be concluded that virtually any physiological or pathological process can be affected by one or another lncRNA. Here, I will present our work on the role of long non-coding RNAs in lung and liver cancer.
Multiparametrische molekulargenetische Diagnostik

V48 – Talk Haferlach

Molecular markers used to diagnose leukemias

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The diagnoses of leukemias and lymphomas have changed dramatically in the last 10 years. This is fostered by new techniques as well as new discoveries. Today, more than 200 genes of interest can be studied in the field of hematology to better define the respective diagnosis of the disease, to describe molecular markers valuable for detection for minimal residual disease (MRD) as well as to follow these patients during the respective treatment from a watch-and-wait-strategy to allogeneic bone marrow transplantation. These modern techniques therefore need to investigate samples at diagnosis but more and more also for MRD. Sensitivities at diagnosis are hampered due to the respective material for the investigation. Sensitivity for MRD is influenced due to the respective laboratory tests. Thus, for a diagnosis today in many hematological diseases a specific panel of genes needs to be investigated to pick up the respective diagnosis or to exclude it. For MRD several other techniques such as melting curve analysis, fragment analysis, real time PCR and also next-generation-sequencing (NGS) are applied. All these techniques in combination today define the state of the art for using molecular markers in the diagnosis and follow up of patients with leukemias and lymphomas.

V49 – Talk Dockhorn-Dworniczak

Importance of Molecular Pathology in Cancer Diagnosis

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The rapidly growing genomic information from recent cancer genome sequencing studies has shown that virtually all cancers of every type harbor somatic genetic alterations. These alterations include single-base substitutions, insertions, deletions, and translocations. Based on these findings a new generation of biomarkers has become available and is used as diagnostic, prognostic and predictive markers in companion diagnostics a growing and emerging field in surgical pathology. As shown for a subset of cancers genetic alterations are the basis of targeted therapies. One of the first findings was that RAS mutation status in colorectal cancers affects the response to cetuximab and has treatment independent prognostic value. More and more of these driver mutations are being detected followed by the discovery and development of small molecules and antibodies that allow personalized therapy strategies for specific cancer subgroups, which may be are more effective and better tolerated than chemotherapy. Advantages in sequencing techniques provide a comprehensive analysis to detect panels of genetic alterations with high sensitivity, high through-put and short time-around even in small tumor samples or liquids. However, the success of targeted therapies is essentially based on precise histopathological classification of tumors in combination with reliable and accurate companion diagnostics to define relevant biomarkers.

Immundiagnostik

V50 – Talk Blüthner

Structural aspects in the detection of autoantibodies

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Autoantibodies serve as valuable tools in the diagnosis of a broad spectrum of inflammatory diseases. They may be associated with disease activity, or be correlated with certain clinical conditions. To improve the detection of autoantibodies it is mandatory to gain an ever more precise insight in the nature of the target structures. These structures may be categorized into different types of epitopes, which vary dramatically in complexity. The complexity level may range from a mere chain of consecutive amino acids, up to highly complex structures composed of defined regions from several subunits of a multiprotein particle. Additional levels of complexity can be contributed by post-translational modifications of the target structures, alternative spliceing mechanisms, or polymorphisms affecting the primary sites of antibody binding.
For practical purposes it is also of high interest to define the diagnostic relevance of the targeted structures, by locating major, minor or individual/personal epitopes. The location and binding characteristics of these structures are not necessarily static, but rather undergo some modulation processes during the course of the underlying disease, and throughout the personal history of the patient. The knowledge of the target structures of autoantibodies, as well as the biochemical context in which they are presented aids in the development of more specific and more sensitive diagnostic assay systems.

Autoimmundiagnostik und Allergie

V51 – Talk Müller-Ladner

Highlights from rheumatology research

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The strong dynamic in multidisciplinary basic science in rheumatology resulted in several novel pathophysiologic insights and ideas in the past years. Autoantibodies against citrullinated peptides (ACPA) have become a dominant marker for very early immune phenomena in rheumatoid arthritis and specific novel pathophysiologic roles could be attributed, e.g. the formation of neutrophil extracellular traps and the unexpected link to cytokines such as IL-12/23 in spondyloarthropathies. In addition, neuronal cells can also influence these long-term immunologic processes and -vice versa- are ACPA able to stimulate T-cells directly. These overactive mechanisms are not only stimulated by external stimuli but are based on distinct genetic alterations and an increasing number of elucidated epigenetic modulations or the effects of proinflammatory extracellular RNA. Moreover, the vascular system is severely affected by acute and chronic rheumatic diseases. Aside the known antibody-driven vasculitides, several immunocompetent cells such as B-and T-cells can act on the vasculature directly. The continuous circulation of proinflammatory mediators such as cytokines and adipokines is also an important factor in the development of inflammation-driven atherosclerosis, in some entities enhanced by novel functional anti-endothelial receptors, e.g. in systemic sclerosis, that extend the pathophysiologic spectrum to inflammation-driven fibrosis.

Das Kolonmikrobiom als therapeutisches Agens

V52 – Talk Rattei

Bioinformatik für die Mikrobiomanalyse: Methoden, Herausforderungen und Trends

T. Rattei
1Univ. Wien, Computational Systems Biology, Wien, Austria

Neues aus der Hämostaseologischen Diagnostik

V53 – Talk Ruf

Inflammation and Thrombosis

W. Ruf

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The hemostatic system and particularly the tissue factor (TF) pathway evolved as an integral part of repair mechanisms and the innate immune defense following tissue injury. TF initiates thromboembolic events in cardiovascular disease, but proteases bound to TF initiate cell signaling by proteolytic activation of the G protein-coupled protease activated receptors (PARs). TF expressed by myelo-monocytic cells requires injury signals from stressed cells or associated with complement activation to become procoagulant in a pathway dependent on thiol-disulfide exchange, coupling inflammation with cellular activation of the coagulation cascade. Emerging details of these cellular pathways provide new insights into diagnostic approaches to evaluate the cellular activation of the TF pathway in thromboembolic diseases. In addition, signaling of TF protease complexes plays a pivotal role in the regulation of innate immune responses and inflammation. The TF pathway is a critical inducer of chronic inflammation in obesity where TF expressed by adipose tissue macrophages regulates their inflammatory phenotype. Loss of TF-PAR2 signaling in the hematopoietic compartment of obese mice attenuates adipose tissue inflammation and improves the insulin resistance associated with obesity. Further insights into the control of macrophage phenotypes by the TF pathway is expected to emerge from the characterization of new genetic mouse models that selectively ablate signaling function of TF.

V54 – Talk Lindahl

Direct oral anticoagulants (DOACs): Impact on coagulation assays, and issues of treatment monitoring

T. Lindahl

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Objectives: For the new oral anticoagulants (NOACs) laboratory monitoring is not used at present, but the effects on coagulation assays are clinically valuable information.

Methods: The direct thrombin inhibitor dabigatran, or the Xa-inhibitors rivaroxaban or apixaban was added to plasma from ten healthy subjects 0 -1,000 μg/L and analysed using different reagents for activated partial thromboplastin time (APTT), prothrombin time (PT), lupus anticoagulant, fibrinogen, antithrombin, protein C and S.

Results: Dabigatran prolonged APTT at typical peak concentrations, the concentration needed to double APTT ranged between 227 and 286 μg/L. PT was much less affected. Two of four reagents underestimated fibrinogen. Dabigatran overestimated antithrombin in thrombin-based methods, Xa inhibitors in the Xa-based methods. Rivaroxaban, at peak concentration, almost invariably prolonged the APTTs. The concentration needed to double the APTT ranged 389 μg/L to 617 μg/L. Effects on PT assays varied. Apixaban displayed less effects in vitro than rivaroxaban. The concentration needed to double the APTT ranged 2,200 and 4,700 μg/L. Rivaroxaban, but not apixaban, caused false positive lupus anticoagulant results. Chromogenic anti-Xa assays displayed linear dose-response curves with both Xa-inhibitors.

Conclusions: Different coagulation reagents, even within an assay group, display variable effects at therapeutic concentrations of NOACS. Knowledge is needed for a correct interpretation of results. Dabigatran may be measured with thrombin time based methods or ecarin clotting time, Xa inhibitors with chromogenic Xa assays. There is an un-met need for point-of-care methods, but such are in development.

Präanalytik

V55 – Talk Church

e-voting session: Preanalytical interference

S. Church

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The preanalytical phase is a complex and dynamic process differing not just from one hospital environment to the next, but within the hospital as well. Preanalytical errors often cause random errors undetectable by normal quality control methods. In order to determine the cause
of these random errors, it is necessary for the laboratory professional to become a sort of “detective”. Through a series of deductions and research, we can identify the cause and put corrective actions in place, whenever possible. A number of case studies illustrate this approach, e.g., why fibrin masses were created in serum samples after overnight shipment, which led to samples requiring additional processing steps? What caused elevated potassium results, which led to a patient being admitted as an emergency but whose potassium normalized upon admission? In another case patients were admitted for bypass surgery and had postoperative complications. Although, their preoperative potassium was within the normal range, the postoperative concentration had risen to $>7.0 \text{ mmol/L}$. This led the laboratory professional to question whether this was a collection or rather a laboratory error. In all of these cases, procedures and patient treatment regimes normally outside the control of the laboratory led to preanalytical errors that have impacted on sample quality, laboratory efficiency and patient care. Recommendations should hence be issued and followed to support strategies and practical policies that laboratories can implement, to reduce the impact of the preanalytical errors, and thereby increase laboratory efficiencies and reduce the potential for inappropriate patient care.

Die Bedeutung von Referenzintervallen für die Diagnostik

V56 – Talk Haeckel

The impact of guide limits for medical diagnosis and permissible measurement uncertainty

R. Haeckel

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Results of laboratory investigations require guide limits for their interpretation (reference intervals, decision limits, action limits or therapeutic limits). Reference intervals are usually defined by a lower and upper limit of the 95% central distribution of a reference collective. The reference collective is either a collective of non-diseased persons (direct method, IFCC “gold standard”) or can be derived indirectly of a mixed collective of the particular laboratory by means of statistical decomposition procedures. The working group “guide limits” (“Richtwerte”) of the DGKL provides an Excel program on its home page. Reference intervals can also be used to estimate the empirical biological variation as a base to calculate quantity quotients (QQ) and to derive permissible measurement uncertainties. The quantity quotient standardizes measurement results in analogy to the intelligence quotient. The permissible uncertainty (permissible imprecision, permissible bias and permissible ring trial results) presumes that analytical procedures with a relatively small reference interval (small biological variation, e.g. electrolytes) require more stringent reliability criteria than procedures with a large reference interval (e.g. enzymes, hormones). Contrary to previous proposals, the working group suggests a non-linear algorithm between biological variation and measurement uncertainty. The working group also developed an Excel program for the calculation of the quantity quotient and the estimation of permissible uncertainties.

V57 – Talk Wolters

Ein Excel-basiertes Verfahren zur Abschätzung von Referenzintervallen mittels Verteilungszerlegung

B. Wolters

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Objectives: Reference limits (RL) are essential for interpretation of results. Several problems of transference must be considered if external sources are used (definition of the reference populations, lack of information on sex, age, nutrition status, drugs or ethnic homogeneity). Whether the method was modified often remains uncertain. Therefore determination and periodical review of RL by calculation intra-laboratorial RL is reasonable.

Methods: The mathematical ambitious indirect retrospective method developed by Arzideh et al. is using large data pools of routine laboratory results. Preselecting occurs by excluding e.g., pregnant women and intensive care patients. The reference interval is calculated after Cox-Box transformation and a truncation algorithm.

Results: The actually presented version of this “GuideLimitCalculator” is easy to use. Microsoft Excel® and the free statistical package R are required. The “GuideLimitCalculator” has been validated for many quantities of laboratory medicine. New implemented features are presented e.g., the calculation of confidence intervals, testing the null hypothesis and a new tool for data preparation was developed.

Perspectives: Planned new features of the tool will be presented. The “GuideLimitCalculator” will be continuously extended by the members of the working group ‘guide limits’ of the DGKL.
Age-related reference intervals demonstrated using blood cell counts

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Results of laboratory tests in children can only be interpreted in the context of age- and gender-dependent dynamics and inter-individual variation. However, the current concept of reference intervals for separate age groups derived from a population of healthy community children has ethical and practical limitations and can only approximate the age-related dynamics encountered in pediatrics. Many widely used RIs are derived from small sample numbers and are split into arbitrary discrete age intervals. Indirect methods address these issues by deriving RIs from clinical laboratory databases which contain large datasets of both healthy and pathological samples.

Methods:
A refined indirect approach was used to create continuous age-dependent RIs for blood count quantities from birth to adulthood. The dataset for each quantity consisted of 60,000 individual samples from our clinical laboratory. Patient samples were separated according to age, and a density function of the proportion of healthy samples was estimated for each age group. The resulting RIs were merged to obtain continuous RIs from birth to adulthood.

Results:
The obtained RIs were compared to RIs generated by identical laboratory instruments, and to population-specific RIs created using conventional methods. This comparison showed a high concordance of reference limits and their age-dependent dynamics.

Conclusion:
The indirect approach reported here is well suited to create continuous, intra-laboratory RIs from clinical laboratory databases and showed that the RIs generated are comparable to those created using established methods. The procedure can be transferred to other laboratory quantities and can be used as an alternative method for RI determination where conventional approaches are limited.

Automation in Microbiology: Challenges and Concepts

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Rapid evolution of healthcare- and hospital management forces clinical microbiological laboratories to undergo significant changes concerning both organization and technology. In the past, the majority of microbiological processes has been manual, slow, error prone, and often redundant and little standardized. Introduction of semi-automated work cells, e.g. blood culture automation, and instruments for identification and susceptibility testing of culture isolates have been an important, but yet insufficient step forwards. Demographic change, severe cost constraints and reimbursement issues, rapid technological progress, and a dramatic loss of skilled laboratory personal requires consolidation, integration together with workflow optimization to reduce cost and increase productivity, while improving speed and accuracy. By applying Lean Six Sigma principles we are implementing “Full Microbiology Laboratory Automation” for culture based microbiology accompanied by a pre-analytical switch towards “liquid microbiology” for roughly 80% of our specimens. A liquid matrix allows for automated streaking, and the application of novel technologies such as flow cytometry, mass spectrometry or molecular testing including new generation sequencing (NGS). It’s enabling us to timely adjust our diagnostic portfolio by adding automated molecular tests according to our customers needs while improving speed, quality and affordability. This fundamental transformation requires professional change management support preparing our workforce, both technical and medical, for their new working environment. Finally, to improve laboratory utilization communication between laboratory and clinicians should be intensified using different formats, such as electronic devices, clinical rounds, lectures and teams for establishing diagnostic and therapeutic guidelines. It should be underlined, that these novel processes are not an end unto themselves, but a necessity to provide high quality yet affordable care to our patients.
Herausforderung Metabolisches Syndrom und Diabetes mellitus - Prävention und Therapieoptionen für eine komplexe Erkrankung

V60 – Talk Peter

“Subphänotypen des Prädiabetes und individualisierte Diabetes-Prävention”

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The epidemics of Type 2 Diabetes is increasing worldwide and much effort is being undertaken to understand the pathogenesis and optimize prevention and treatment. The manifestation of Type 2 Diabetes can be the result of a variety of pathomechanisms and usually develops through an intermediary state called prediabetes, which is also heterogeneous. These pathomechanisms are already important in the intermediary state of prediabetes, as they can confer a higher risk for diabetes-associated complications. Altered organ cross talk, the accumulation of fat in the visceral cavity and particularly in the liver combined with an inflammatory process are key players in the development of insulin resistance and Type 2 Diabetes. Further phenotypes include insulin resistance of brain or skeletal muscle, as well as beta cell failure through genetic incretin resistance. The detection of prediabetes and prevention of its progression to diabetes would be desirable. Therefore, subphenotyping of prediabetic individuals and detailed knowledge about the underlying pathomechanisms are important for the development of future tailored, individualized strategies for an efficient prevention and a phenotype-oriented diabetes therapy. The development of novel, widely applicable diagnostic tools for subphenotyping is an important translational research task for clinical chemists and diabetologists.

BDL Symposium “Im Zentrum der Diagnostik”

V61 – Talk Gessner

We are not alone: Microbiota in health and disease

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Humans exist as meta-organisms comprised of both the macroscopic host and its symbiotic commensal microbiota. With approximately 100 trillion cells, bacteria outnumber host cells by at least a factor of 10 and express at least 100-fold more unique genes than their host’s genome. The tremendous enzymatic capability of the microbiome results in a plethora of metabolites found in humans which play a fundamental role in nearly all aspects of host physiology and disease development including metabolic, cardiovascular and even neuro-psychiatric illnesses. Since 2000, large-scale 16S rRNA or metagenomic studies have dramatically expanded the knowledge about diversity of the human gut microbiome. Approximately 80% of the bacteria found by molecular tools are uncultured so far, and hence can be characterized only by metagenomic studies. On the other hand, however, culture of bacteria (microbial culturomics) increased by up to 30% the microbial gut repertoire as determined by pyrosequencing, so that distinct microbiological knowledge is clearly required to analyze the human microbiome. We recently initiated the first European external quality assessment (EQAS) comparing results from different next generation sequencing (NGS) centers with special emphasis on critical preanalytic steps, nucleic acid preparation and bioinformatic data processing. Furthermore, our goal is to achieve a functional understanding of bidirectional microbe-host interactions in health and diseases, beyond largely descriptive compositional and metagenomic analyses.

V62 – Talk Reuter

Laboratory Medicine in Private Health Insurance in Germany

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Objectives:
1 Is Laboratory medicine still a medical issue and task or already an industrial enterprise?
2 What are the differences in terms of medical lab diagnostics between patients according to their status as a social or private insured in Germany?
3 What are and should be the quality requirements for the provision and the inducement of lab exams?
Results:
1. Rather only the quality control, the interpretation of the findings and the integration of the results into a clinical history are essentially doctors’ work and not the mere lab technology and processing.
2. There is apparently no medical reason for any difference of lab service provision to both groups of insures. Private insures bear five-times higher per capita costs for lab diagnostics compared to social health insured. The considerably higher fee in the private fee schedule (GOÄ) as in the social health sector (EBM) has no rationale in the difference of the service delivery.
3. All lab exam provider should be submitted to the guidelines issued by the national chamber of physicians. The implementation of the 4-eyes-principle, that the inducer of a lab exam must be different from and must not have any financial relation to the provider of the lab exam. Only the lab service provider himself should be allowed to bill his service.

Conclusions: We higher quality requirements and need less economic incentives for physicians to induce and to provide lab services for private patients.

V63 – Talk Wiegel

BDL - Professional Association of German Clinical Pathologists (GCP) - Benefits and Future

B. Wiegel
MVZ Dr. Engelschalk, Dr. Schubach, Dr. Wiegel und Kollegen, Deggendorf, Germany

Since the 1970s, the Professional Association of German Clinical Pathologists (GCP) accompanies there professional development. Was the past marked by changes in fee schedules, training regulations and professional codes, so today one of the main topics is the professional access of scientists, medical technologists, physicians and pharmacists who run laboratory testing beside the core-professionals like CPs or CMs. From the beginning the BDL stood for sustainable remuneration of laboratory testing done under same basic structural conditions, which include above all the execution and control according to the Directive of the German Medical Association for quality assurance of medical laboratory investigations (RiLiBÄK). Unfortunately this monitoring including the physical inspection of the Medical laboratories of all kinds shows large gaps depending on the province where the laboratory is located. This is especially to be emphasized for laboratories run by physicians in the self-referral manner (= means the opportunity to present themselves to subcontract the provision of laboratory services). On an international basis this German phenomenon is outlawed since the 1990s. The provision of laboratory services exclusively on the order paths as in France can be rightly regarded as a lighthouse and is strictly demanded by the professional association of CPs for Germany. The inherent 4-eyes-principle guarantees best patient and consumer protection, also from financial overload. All of this will shape the future work of the Professional Association of GCPs.

Advances in clinical proteomics

V64 – Talk Borchers

Immuno-MALDI for Accurate and Precise Clinical Protein Quantitation

C. Borchers
UVic Protein Centre, Victoria, Canada

Immuno-MALDI (iMALDI) technology which combines the sensitivity of immunoaffinity capture with the specificity of MS detection. We have now taken a multifaceted approach for translating our iMALDI technology into clinical laboratories for routine protein quantification. First, we have automated the sample preparation using the Agilent Bravo liquid handling robot for improved sample throughput. Secondly, we have optimized iMALDI assays for the Bruker Microflex MALDI-TOF, a bench-top instrument that is already widely used in regulated healthcare environments. Thirdly, we have written custom software that automates the entire data analysis pipeline, making it suitable for clinical laboratory technicians without MS training. Here we demonstrate iMALDI technology for the clinical measurement of plasma renin activity (PRA), an established biomarker for primary aldosteronism. The current method automates 192 iMALDI captures and analysis requires only seconds per sample. The inter-day and intra-day precision in reflector mode is <10 %CV, satisfying FDA guidelines for clinical assays. Initial validation indicates a strong correlation between iMALDI-PRA values and an established clinical LC-MS/MS assay (n = 188, $R^2 > 0.94$). This iMALDI approach to protein quantification can be multiplexed and is applicable to a wide array of clinical peptide and protein biomarker targets.
Molekulargenetische Diagnostik

V65 – Talk von Kalle

Molekulare Diagnostik in der Onkologie

C. von Kalle

1Nationales Centrum für Tumorerkrankungen (NCT) Heidelberg und Abteilung Translationale Onkologie, Deutsches Krebsforschungszentrum (DKFZ) Heidelberg, Nationales Centrum für Tumorerkrankungen (NCT) Heidelberg, Heidelberg, Germany

NCT has implemented the NCT Precision Oncology Program (NCT POP) as a center-wide master strategy that, together with NCT’s dedicated Clinical Cancer Programs, coordinates all translational activities and focuses resources towards individualized cancer medicine, including patient-oriented strategies in genomics, proteomics, immunology, radiooncology, prevention, and early clinical development. For this purpose, the center-wide NCT MASTER (Molecularly Aided Stratification for Tumor Eradication) umbrella protocol has been created to streamline the entire diagnostic trial workflow. NCT MASTER makes it possible to perform and evaluate molecular diagnostics on materials from all consenting NCT patients, with the explicit purpose of stratifying each patient for the best treatment or trial strategy. By funding high-throughput analyses in selected, hypothesis-driven clinical studies, the Heidelberg Center for Personalized Oncology (DKFZ-HIPO) and the NCT Precision Oncology Program (NCT POP) enable clinical investigators to rapidly implement novel molecular analyses in the patient context. DKFZ-HIPO, the DKFZ Sequencing Core Facility, and bioinformatics groups on campus streamline data acquisition and analysis in close collaboration with NCT POP. In order to best integrate and exploit the data produced in all NCT programs, a central NCT DataThereHouse contains a working copy of every patient-related dataset from all IT sources to enable efficient retrieval, aggregation, and evaluation of molecular and clinical data for clinical decision making and translational research. The ultimate goal of this multidisciplinary center-wide effort in precision oncology is to provide a validated workflow for trials to infer rational recommendations for mechanism-based therapeutic interventions in advanced malignancies and improve patients care by integrating systematic molecular data.

ÖGLMKC-Symposium Moderne Medikamentenanalytik und Pharmacogenomics

V66 – Talk Sunder-Plassmann

Therapie nach Maß - Vision oder Realität?

R. Sunder-Plassmann

1AKH Wien, Klinisches Institut für Labormedizin, Wien, Austria

For optimal modern personalized therapy it is necessary to consider every information on the patient that can be obtained including various biomarker signatures. In the majority of cases however, this information will not be available due to high costs of the required analyses, the lack of general recommendations for dosing or clinical practice guidelines according to the individual genotype, lack of information on available tests, lack of genetic/genomic understanding and last but not least due to ethical barriers. A few patient subgroups that can be identified by a specific genetic or metabolic signature, however, already benefit from individually tailored therapy: in oncology genetically defined patient populations are routinely distinguished - due to severe side effects of anticancer drugs or due to the design of the prescribed drug that targets a specific molecule (or a variant of this molecule) that may or may not be expressed in the patient’s tumor cells. Thus, specific subgroups of patients suffering from leukemia, breast cancer, lung and gastrointestinal cancer, or melanoma already receive therapy that optimally fits their genetic makeup. Outside oncology the number of IVD approved pharmacogenetic tests is very low and usually cover only the most frequent variants of a specific gene. In the near future, however, pharmacogenetic testing may become more complete and part of routine point of care testing, if it is facilitated by new sequencing technologies including nanopore sequencing and the development of devices that can be handled by nurses or technicians without extensive training for genetic analyses.
V67 – Talk Stimpfl

Mass spectrometry for routine clinical applications

T. Stimpfl
1AKH Wien, Klinisches Institut für Labormedizin, Bereichsleiter Medikamentenanalytik und Toxikologie, Wien, Austria

Objectives: Immunoassays are available as complete solutions (including IVD-CE certification, automation, data processing, integration into the LIMS...). These assays are easy to use and therefore popular for routine clinical applications. However, the drawbacks and limitations of immunoassays (i.e. lack of specificity for the target compound) are often overlooked. Whether hyphenated techniques based on mass spectrometry are a good alternative to immunoassays for routine clinical applications is discussed, using immunosuppressive therapy as an example.

Discussion: The major advantages of hyphenated mass spectrometric techniques (such as LC-MS/MS) are their high sensitivity and specificity. Targeted analytes are unequivocally identified and quantification can be achieved, unimpaired by metabolites and matrix compounds. Simultaneous analysis of several compounds per sample run can be performed, allowing for a short turn-around time. Sample preparation is minimized. Full automation reduces costs and increases robustness of the analytical procedure.

Conclusion: In order to establish hyphenated mass spectrometric techniques as a superior alternative to immunoassays in routine clinical applications, complete solutions must become available. These should be easy to use, even by less-skilled technicians, and must include full software and LIMS integration and fully automated sample preparation (based on IVD-CE certified kits).

POCT Management Point-of-Care Diagnostik

V68 – Talk Kachler

Legal certainty of Point of Care Testing (POCT)

M. Kachler
1Fachhochschule Kärnten, Biomedizinische Analytik, Klagenfurt, Austria

Starting from the question of what is point of care diagnostic, the talk will address the legal and organizational implications and thus address the question: Who is allowed because even perform of POCT and how it looks with the liability issues? Which organizational design options for the implementation of POCT are possible taking into account the legal framework?

V69 – Talk Gottschall

Management POCT Point-of-Care Diagnostics - the function and role of POCT Managers

K. Gottschall
1Instit. für Klin Chemie und Lab Medizin, Rostock, Germany

Point-of-Care-Diagnostics include medical single-sample measurements performed outside of the central laboratory, typically by ward staff without specialized trained. A key criteron of POC diagnostics the direct derivation of therapeutic decisions and consequences. If Point-of-Care Testing (POCT) within a hospital is supervised by the central laboratory, it is responsible for monitoring the implementation of internal quality assurance measures in the individual organizational units, in accordance with the legal requirements set by the “RiLiBÄK”. Consequently, overlapping processes and responsibilities arise between the central laboratory and the hospital departments implementing POCT. POCT-managers are responsible for the coordination of all activities related to the operation of POCT systems. These managers communicate and monitor compliance to the quality assurance measures set by the German Medical Association. They mediate between the central laboratory, the POCT-Commission, POCT users and other service areas, providing guidance and assistance in the development, implementation, documentation, and maintenance of the processes required for quality assurance. These tasks can only be carried out if the POCT officers’ responsibilities and authority are defined relating to their specific institution.
Lipidstoffwechselstörungen und kardiovaskuläre Erkrankungen

V70 – Talk von Eckardstein

Stand der Kunst, offene Probleme und neue Entwicklungen bei der Lipidstoffwechsel-Diagnostik

A. von Eckardstein

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Rechtliche Aspekte im Labormanagement

V71 – Talk Spitzenberger

Laboratory analysis for clinical studies – Regulatory requirements for testing of medicinal products and medical devices according to national and European legislation

F. Spitzenberger

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Objectives: Laboratories that perform the analysis of human samples collected as part of clinical studies play a central role in the evaluation of medicinal products and medical devices with regard to the quality, safety and efficacy or performance of the products. There is currently no comprehensive laboratory guidance for testing in the context of clinical studies that would cover the different types of studies and the different products in question. This survey lecture compares the major regulatory documents and their requirements for laboratories involved in testing for clinical studies.

Methods: The major regulatory documents such as German laws and regulations, European and international health legislation, standards and guidelines are reviewed and compared with regard to their relevance for laboratories performing analysis of human samples in the context of clinical studies.

Results and Conclusion: Laboratory analyses for clinical studies shall be conducted in accordance with relevant EU directives and regulations, applicable guidance and the Declaration of Helsinki. Laboratories shall establish and maintain a quality management system based on essential quality criteria such as the 8 QM principles according to ISO 9000ff or the 12 CLSI quality principles. Additional regulatory requirements depend upon the product as defined by European or national legislation. Medicinal product testing should be performed according to good clinical laboratory practice as defined by the European Medicines Agency and through the relevant ICH documents. Testing of medical devices including performance evaluation of in vitro diagnostic medical devices should follow European standards that provide the consumption of conformity with EU directives. Additional national requirements apply for both clinical testing of medicinal products and medical devices.
V72 – Talk Benzler

Mandatory notification of laboratory evidence of pathogens in Germany

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Reliable and timely reporting of diseases and pathogens is the backbone of infectious disease surveillance and thus of the prevention of infectious diseases and the detection and control of outbreaks. Laboratory personal and managers have a crucial role in this process. The German Act on the Prevention and Control of Infectious Diseases in Man (Protection against Infection Act, Infektionsschutzgesetz, IfSG) acknowledges this by stipulating the mandatory notification of direct or indirect (serological) evidence of a number of relevant pathogens to the competent local health authority or - for selected pathogens - to the Robert Koch Institute (RKI), respectively. Local health authorities use this information - including the identity and address of the patient - for immediate intervention, where appropriate. Furthermore, it is forwarded in anonymised form to state health authorities and from there to the RKI, where it is used for the detection and control of trends and dispersed outbreaks as well as for statistical purposes, feedback to data providers and information of decision makers, experts and the general public.

RKI is aware that mandatory notification puts an additional burden on those concerned by it and is working on solutions that reduce this burden, create added value, and improve compliance. The presentation gives an overview of notifiable pathogens, notification pathways and deadlines, processing of notification data within the public health system, and its dissemination through publications and websites. It also highlights some common misunderstandings, e.g. regarding the application of case definitions.

V73 – Talk Kachler

Validation of Laboratory Test Results

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Almost all diagnoses and therapeutic decisions depend on laboratory results. Biomedical Laboratory Scientists (BMA) play an important role in our health care system by providing quality laboratory services. The process of laboratory analysis consists of three phases pre-analysis, analysis and post-analysis. The analytical process is autonomous task of BMA; the indication and interpretation of laboratory results is the task of the clinical doctors working. BMA are therefore responsible in the analytic process in addition to the implementation of the quality assurance and plausibility check, ie they validate the results before getting the therapist to make decisions. The terms of the technical and medical validation are not consensually clearly defined and are often misinterpreted in practice. Therefore, it is often debatable, who can validate laboratory diagnostic data and what is the subject of this task. Referring to the legal framework of international standards and empirical results of the research is introduced and explained the concept of biomedical validation for clear linguistic distinction between medical expertise and BMA expertise.

Schilddrüse

V74 – Talk Zingler

Labordiagnostische Aspekte von Schilddrüsenerkrankungen mit Fallbeispielen

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Thyroid gland diseases are among the most common endocrine disorders. The basal determination of Thyroid-stimulating hormone (TSH, Thyrotropin) increasingly serves as the initial laboratory test in thyroid diagnostics. Measurements of the serum TSH concentration are valuable in the diagnosis and management of hypothyroidism and thyrotoxicosis. In addition, there is a close relationship between the hypothryal release of TSH and the biological activity of thyroid hormones. Therefore, free triiodothyronine (FT3) - and free thyroxine (FT4) - measurements support the investigation of the thyroid state. In past decades other hormone parameters such as total triiodothyronine (T3), total thyroxine (T4), and thyroxine-binding globulin (TBG) were determined. In the first part of the lecture, basic principles of lab diagnostics regarding primary and secondary thyroid dysfunctions are introduced. The relevance of thyroid function tests and the determination of thyroid autoantibodies, such as antibodies against the TSH receptor (TRAbs), thyroid peroxidase (TPOAb), and against thyroglobulin (TgAb), are explained pertaining to the differential diagnosis of thyroid hyper- and hypofunction. In the second part of the lecture, the use of several
different lab parameters, and potential causes of misinterpretation are discussed. The optimized diagnostic work-up is explained on the basis of numerous case studies.

Infektionsserologie

V75 – Talk Zeichhardt

Qualität der immunologischen und molekularen Virusdiagnostik - neue internationale virologische INSTAND-Ringversuche

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Die hohen Gesamterfolgsquoten einzelner Untersuchungen in den Ringversuchen (häufig 95-100% richtige Ergebnisse) zeigen, dass die Testdurchführung in den Labors und die Leistungsfähigkeit der Methoden i.Allg. von hoher Qualität sind. Eine Qualitätsverbesserung ist möglich, wenn bei aufgedeckten Abweichungen im Ringversuch (i) laborbedingte Fehler durch Training direkt behoben und (ii) Probleme einzelner kommerzieller Methoden in Kooperation zwischen INSTAND e.V. und den o.g. Kooperationspartnern gemeinsam mit dem Hersteller gelöst werden.

V76 – Talk Schmidt

International proficiency panels for virus detection of the Reference Institute for Bioanalytic – the next generation

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Background: Laboratories have to demonstrate the compliance with diagnostic standards by successful participation in external quality assurance programs. Usually all samples within an interlaboratory comparison test are labeled with identical labels (e.g. A-D or 1-4) for all participants. Exchange of test-data between laboratories cannot be excluded and would thwart the intention of external quality assurance.

The Reference Institute for Bioanalytics started in 2013 individually bar-coded samples for eight viruses which are relevant for blood banks as well as for laboratory medicine.

Study protocol and Methods: The proficiency panel includes HCMV, HBV, HIV-1, HIV-2, HAV, WNV and B19. All viruses were included in two different concentrations and distributed among four samples. All four samples contained four different viruses for multiplex analysis. In the next step all samples were labeled with barcodes. The study period was defined to last for 3 months. Within this period laboratories could participate at any time. After submission of test results the certificates for qualitative analysis were sent out within three business days. The certificates for quantitative analysis were sent out to the participants at the end of the study period.

Results: The diagnostic specificity was 100% for all eight viruses. The analytical sensitivity was 100% except for WNV (low virus concentration 1,000 copies/ml, analytical sensitivity was 83.4%). The overall acceptance was excellent.

Conclusion: The proficiency panel with individual barcodes demonstrates that screening in blood transfusion services as well as in diagnostic laboratories is done on a very high quality level. The panel incorporates new screening systems that have already implemented a multiplex multicolor analysis. Therefore the new format demonstrates a well-accepted high standard of external quality assurance to enable quantitative and qualitative analysis and a genuine interlaboratory comparison without any bias. The RfB will continue with this concept for all RiliBÄK relevant viruses of section B3 in 2015.
V77 – Talk Ghebremedhin

Complete implementation of the Quality Assurance Guidelines of German Federal Medical Council (RiliBÄK) with Quality Assessment by the Reference Institute for Bioanalytics (RfB) for the sections B2 and B3

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Objectives: Over the past few decades quality management has become mandatory for all German medical laboratories. Hence, they must do internal and external quality control (QC), or peer comparison testing for any analytes that they test according to the RiliBÄK. This reflects the greater effort in Germany to establish reference methods for external QC.

Methods: Approximately 120 laboratories participated in the External Quality Assessment pilot events (April 2014 Ring Trials Section B2: bacterial, viral and parasitic serological analysis) of the RfB. The different commercially available ELISA test kits were utilized. The data were analyzed in July 2014.

Results: The majority of the evaluated participants achieved success rates either >90% (e.g. B. burgdorferi IgG, T. pallidum IgG, T. gondii IgG) or <39% (e.g. Schistosoma IgG, Echinococcus IgG). There is significant deviation between the test kits of the different manufacturers. Few analytes (esp. IgM) did not achieve desired status and may require more rigorous QC practices.

Conclusion: Majority of the serological kits function at high-class specifications. These data should help the laboratories to choose which analytes to focus on for method improvement. The RfB will be nominated by the chamber of health (Bundesärztekammer) for all parts of the RiliBÄK (B1 – B5) and will continue with Pilot Ring Trials for section B3 (direct detection and analysis of pathogens) in 2015.

FREIE VORTRÄGE / FREE TALK

Klinisches Biobanking in der Laboratoriumsmedizin

FV1 – Talk Nauck

Biobanking concept of the National Cohort

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The National cohort is a prospective epidemiological study which will recruit 200,000 probands in 18 study centers during the next 4 years. It aims at the collection of medical data and biomaterials for investigation of common diseases. Besides phenotyping of for example Cardiovascular Diseases, Diabetes mellitus or Cancer, liquid biobanking represents an elementary part on which about 20 % of the total grant is spent. Two thirds of the planned 20 Million aliquots will be stored centrally at the Helmholtz Centre (Munich). Highly standardized processes across all study centers are necessary to ensure high and homogeneous quality of the samples. Primary blood and urine samples (Becton Dickinson) are not pooled prior to aliquoting with pipetting robots (Hamilton) at each study center. The pipetting robot can process different biomaterials at the same time including serum, EDTA-plasma, urine and buffy coat. Cya vials (FluidX) with working volumes of 250 μl or 500 μl are labeled with a specific 2-D-barcode at the bottom as well as on the side of the jackets. Samples will be stored at the study centers at least at -80°C and transported to the central storage twice a month. The -180°C central storage will be fully automated and operated in the gas phase of liquid nitrogen. It will consist of 16 tanks with a 2.5 m outer diameter. Sample processing e.g. transport time or automated aliquoting will be run by and documented in CentraXX (Kairos).

FV2 – Talk Zander

Effect of biobanking conditions on stability of antibiotics in human serum

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Background: Therapeutic drug monitoring (TDM) of beta-lactam antibiotics, chinolons and oxazolidinones may play an increasing role in individualized medicine. However, only few data are available regarding stability of antibiotics. We therefore analysed the effect of several storage conditions on stability of different antibiotics.
Methods: Stability of piperacillin, tazobactam, meropenem, cefepime, ciprofloxacin and linezolid in serum samples was tested after storage at room temperature (RT, 19-24°C) for 2, 4 and 12 hours, at 4°C for 6, 12, 24 hours and 7 days, and at -20°C and -80°C for 1, 7, 30, 60 and 90 days. Furthermore, stability during three freeze-thaw cycles was evaluated. Antibiotic concentrations were determined by 2D-UHPLC-MS/MS.

Results: Biobanking at -80°C did not lead to substantial changes (defined as >15%, p<0.01) for any antibiotic studied. In contrast, storage at -20°C and 4°C for ≥7 days, and at RT for ≥4 hours led to substantial changes for piperacillin, tazobactam, cefepime, and meropenem in serum. Indeed, after storage at -20°C for 90 days, antibiotic concentrations declined by >88% for piperacillin, >98% for tazobactam, >75% for cefepime and >97% for meropenem. Freeze-thaw cycles did not have any effect on antibiotic concentrations.

Conclusions: There is a high instability of several antibiotics in serum when stored at ≥-20°C. This may have important implications both for analytical method development and TDM.

FV3 – Talk Ahmad-Nejad

External Quality Assessment Scheme for Biomaterial banks: Assessing Quality and Functionality of DNA-Isolations from FFPE-Tissues

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Background: Biobanks are becoming important for assessment of disease risk and predisposition, identification and validation of new diagnostic biomarkers and druggable targets. The validity of data obtained is critically limited by the quality of the biological samples. Yet, external quality assessment (EQA) programs suitable to comprehensively measure the biomaterial quality in archived materials are lacking. We report quantitative assay designs for the analysis of both structural and functional integrity of DNAs and used them in a first pilot EQA within a priority cancer biobanking program.

Methods: Participating biobanks isolated DNAs from a standardized set of samples comprising sections of 4 different formalin-fixed paraffin-embedded tissues using their standard procedures. Isolated DNAs and analytical results were analyzed centrally for nucleic acids yield, purity, fragmentation and amplificability at a quantitative level.

Results: The recovery of DNA greatly varied in isolates ranging between 1.53 and 25.78 μg/ml. Quantification of DNA fragmentation and amplificability allowed to highlight considerable discrepancies in DNA quality. Amplicons yielded from the isolates of these identical EQA samples ranged from 411–105 bp.

Conclusions: Recovery of bioanalytes from biobanks is diverse even for stable biomolecules like DNA isolated with highly standardized methods. EQAs are appropriate tools to uncover strengths and weaknesses in a systematic fashion. Biomaterial integrity is insufficiently reflected by standard methods, but needs to be assessed. Finally, our results also point towards the scope of the problem of measuring the quality of more delicate biomolecules like proteins or metabolites.

Entwicklung und Klinische Anwendung des Next-Generation Sequencing

FV4 – Talk Sollfrank

Implementing NGS- based CF testing for routine diagnostics

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Objectives: 62 patients (typical CF, atypical CF, CF conductor) carrying 41 different CF- causing mutations (substitutions, deletions, insertions, large CNVs), being pre-characterized by Sanger- or Pyrosequencing (some followed by MLPA) and 3 external controls were subjected to NGS.

Methods: 37 amplicons out of 2 multiplexes were used for targeted resequencing of all 27 CFTR exons including splice sites and intronic ROI. NGS was performed working with 2 different platforms (GS Junior/ Roche; MiSeq/ Illumina) through 4 sequencing runs (2 454 runs-15 samples each, 1 Standard Flow Cell run- 65 samples, 2x 250 cycles and 1 Nano Flow Cell run- 23 samples, 2x 250 cycles). Variant calling and CNV analysis was performed applying NextGENe®. CNV analysis was additionally carried out using an in- house excel- based analysis tool.
**Results:** NGS data averages in 77% (MiSeq) and 75% (454) aligned reads. Within the regions covered by both, 454- and MiSeq-reads, all anticipated variants have been detected. The allelic state of all expected variants was correct. TG-T haplotyping in IVS8 is enabled using 454-as well as MiSeq-data. CNV analysis has to be carried out with specific setting with special regard to exons covered by more than 1 amplicon.

**Conclusion:** Rising sequencing capacity and falling prices generate excellent preconditions for NGS in routine diagnostics of classic genetic diseases as CF. Mutation specific therapy options for CF cause good reasons to rethink genetic testing in CFTR analysis.

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**Hämostaseologie**

**FV5 – Talk Mayer**

The role of platelets in hematopoietic stem cell mobilization

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**Background:** Hematopoietic stem cell (HSC) mobilization is of major importance for therapeutic bone marrow (BM) and HSC transplantation. Recent investigations indicate an important role of platelet derived microparticles in the course of HSC mobilization as well as in HSC engraftment. In this study we investigated the role of platelets in HSC homeostasis and mobilization in an acute but transient platelet deple- tion mouse model.

**Methods & Results:** Platelet depletion was first assessed under “steady-state” conditions. For this purpose male wild-type mice were injected with platelet-depleting or control serum on days 0 and 4. Mice were sacrificed on day 7 and femora as well as PB were collected. BM was extracted and PB samples were prepared for FACS-analysis and examined with antibodies specific for murine (long-term) hematopoietic stem and progenitor cells including CD48, CD 150, CD34, Sca-1, c-Kit, and a Lin cocktail. Platelet depletion for 7 days caused a highly significant increase of HSCs within the BM (P < 0.01). To assess the contribution of platelets in the course of HSC mobilization, platelet-depleted mice were injected with G-CSF subcutaneously once a day on six consecutive days. Platelet depletion in the course of HSC mobilization induced a significant increase of HSCs in the BM as well as is in the PB (P < 0.01).

**Conclusion:** The results from this ongoing study indicate that platelet depletion causes a strong proliferative stimulus to HSCs within the BM and potently increases the G-CSF induced mobilization of HSCs from bone marrow to peripheral blood. These findings might have significant clinical implications for patients with platelet disorders who are considered for HSC mobilization.

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**FV6 – Talk Brandenburger**

PlateletWeb: A tool to investigate signaling networks in patients with platelet disorders

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Understanding the cellular mechanisms of platelet activation and their pharmacological modulation is of major interest for basic and clinical research as they play a major role in haemostasis after vascular injury. Therefore we developed a comprehensive platform (PlateletWeb) for the systems biological analysis of platelets in the functional context of integrated networks exploiting information on the platelet transcriptome, proteome and phosphoproteome. Together with integrated protein–protein interactions and site-specific phosphorylation network, this provides a first blueprint of platelet signaling on a global scale thus the PlateletWeb offers the investigation of signal transduction in platelets on the basis of various information sources, eg to separate platelet activatory and inhibitory signaling. Moreover, including functional data, drug and pathway associations we offer a first systemic insight into the multifaceted aspects of platelet functionality and potential options for pharmacological regulation. To investigate pathways associated with functional platelet disorders in more detail we analyse functional data from patients with platelet disorders including platelet function tests (aggregometry, PFA) and biochemical and haemostatic laboratory findings. The combination of systems biological resources and clinical data offers the possibility to obtain a more detailed picture of the complex cellular processes involved in the diverse defects of platelet disorders.
Molekulargenetische Diagnostik

FV7 – Talk Böckeler

Stability of epigenetic biomarkers and their suitability in a prospective melanoma study

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Circulating cell-free DNA (cfDNA) is used as template for the detection of potential biomarkers in human blood samples. Applications of cfDNA analysis include prenatal diagnostics and non-invasive detection of cancer. CfDNA associated markers comprise genetic polymorphisms and aberrant epigenetic patterns. Here we report on pre-analytical aspects of epigenetic biomarkers and their suitability for non-invasive oncological tumor profiling. Definition of pre-analytical standards is crucial for the generation of standard operating procedures (SOPs) and subsequently for the implementation of epigenetic biomarkers into clinical routine diagnostics. To examine the stability of hypermethylated and hypomethylated gene segments in DNA aging experiments we used the MethyLight qPCR approach. Methylated sequences are thought to be covered with distinct chromatin packaging protecting these DNA-segments from breakdown. Based on this hypothesis we prepared protein-bound DNA from human melanoma cell lines which display typical hypermethylation profiles including hypermethylated COL1A2, CYP1B1, RASSF1A and RAR-β. We then compared methylated to non-methylated DNA after incubation in plasma at different temperatures and for various time intervals. Our results show that during blood clearance at physiological temperature, hypermethylated sequences are longer detectable than their hypomethylated correlates confirming the stabilizing effect of DNA-methylation. To validate these in-vitro results, we also tested blood samples of melanoma patients for their distinct methylation profiles depending on sample age.

Lipidstoffwechselstörungen und kardiovaskuläre Erkrankungen

FV8 – Talk Grammer

CoroPredict: A novel multimarker-based algorithm for determining the risk of mortality in clinically stable coronary artery disease

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Introduction: Patients suffering from coronary artery disease (CAD) are in general thought to have very high risk for future cardiovascular events and mortality. Available cardiovascular risk scores have limited discriminatory power and cannot be applied to patients with previous vascular events. The discrimination between CAD patients with low, moderate and high risk must be improved as a basis for optimal and cost-effective therapy.

Methods: More than 200 biomarkers were measured in clinically stable CAD patients enrolled in the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study. Risk scores were derived to predict the ten years incidence rates of all-cause and cardiovascular mortality. Markers were selected by univariate analysis and bootstrapping of the most predictive markers in Cox models. Further, biomarker selection considered the involvement of particular markers in different pathophysiological pathways and their availability in a routine laboratory.

Results: All models contained age and sex as predictors. Eight laboratory parameters were chosen in the final models, among them NT-proBNP, troponin T, cystatin C, hemoglobin A1c, C-reactive protein and cotinin. For patients with established CAD the areas under the curve (AUC) in predicting total mortality and CVD mortality were 0.81 and 0.78, respectively. An established score for determining long-term prognosis in stable CAD, the Marschner-Score, yielded an AUC of 0.64.

Discussion: In the present study we showed that a score based on age, sex and several novel biomarkers is highly predictive of future cardiovascular events and mortality. The diagnostic performance and practicability of our scoring system may be superior to previous algorithms.
FV9 – Talk Sprott

Role of Autophagy in Pathological Angiogenesis

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Autophagy is a catabolic process that enables cells to degrade cytosolic proteins and organelles. It is crucial for cellular homeostasis and has been implicated in many pathologies. The objective of this study is to elucidate the connection between autophagy and pathological angiogenesis. We applied an oxygen induced retinopathy model (OIR) in mice, which displays hyperproliferative neovascular vessels. By immunofluorescence, we found induction of autophagy in the vascular endothelium in retinae of mice subjected to OIR, but not in endothelial cells of physiologically developing retinae. Next, we used mice with endothelial-specific deletion of autophagy related gene (Atg) 5 (EC-Atg5KO). OIR retinae of EC-Atg5KO mice displayed less pathologic neovascularization and endothelial cell proliferation and increased endothelial apoptosis. VEGF treatment of isolated endothelial cells from EC-Atg5KO mice showed reduced VEGF receptor 2 and AKT phosphorylation and decreased total VEGF receptor 2 presence, while apoptotic markers were upregulated. Finally, suppression of Atg5 by siRNA in human umbilical vein endothelial cells (HUVEC) diminished sprouting in a 3D spheroid sprouting assay. Here we report that autophagy may promote pathologic angiogenesis. We suggest that impairment of autophagy results in reduced VEGF receptor signalling leading to increased endothelial apoptosis and decreased vascular density. However, endothelial-specific Atg5 deletion did not alter physiological angiogenesis. We currently perform mechanistic studies to understand the role of autophagy in pathologic angiogenesis.

POSTER

Immunology, Autoimmunity, Allergy

P001

CytoBead® ANA - a novel immunoassay for fast screening and confirmation of anti-nuclear antibodies

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Introduction: The detection of anti-nuclear antibodies (ANA) by indirect immunofluorescence (IIF) on HEp-2 cells has been evolved as the standard screening assay and enzyme-immunoassays are used to confirm HEp-2 IIF findings. Here, a novel immunoassay for the serological diagnosis of systemic autoimmune diseases was developed that combines screening for ANA using HEp-2 cells and the confirmation of ANA testing via IIF simultaneously in one reaction environment (CytoBead ANA®).

Methods: Screening for ANA is done on HEp-2 cells in the middle part with confirmative testing using microparticles coated each with Ro60, Ro52, La, CENP-B, RNP-Sm, Sm, dsDNA and Scl-70 which are clockwise immobilized in four compartments around the middle part. For assessment of the novel assay, clinical reference sera (n = 438) and healthy controls (n=104) were tested for ANA by IIF using CytoBead® ANA and automated analyzed with AKLIDES®.

Results: Inter-rater agreement statistics showed a very good agreement of ELISA and CytoBead® ANA testing for the integrated parameters CENP-B, SS-B, Sm, RNP-Sm, Scl-70, SS-A/Ro60 and SS-A/Ro52 (Cohen’s kappa χ = 0.84 – 0.95) and a good agreement for dsDNA (Cohen’s kappa χ = 0.76). The relative sensitivity was 87%–100%, and relative specificity 94%-99%.

Conclusions: The new test system can replace the two-stage analysis by combining IIF screening with multiplex confirmative testing. The test is suitable for automation with AKLIDES® as well as for manual ANA screening.

P002

Simultaneous screening and confirmation of ANCAs and detection of anti-GBM antibodies in case of emergency

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Introduction: The detection of anti-neutrophil cytoplasmic antibodies (ANCA) by indirect immunofluorescence (IIF) on myeloblasts has been the method of choice for clinical diagnosis of ANCA-associated vasculitides. Here, a novel immunoassay for the serological diagnosis of ANCA was developed that combines screening for ANCA using myeloblasts and the confirmation of ANCA testing via IIF simultaneously in one reaction environment (CytoBead® ANCA®).

Methods: Screening for ANCA is done on myeloblasts in the middle part with confirmative testing using microparticles coated each with Myeloperoxidase (MPO) and Proteinase 3 (PR3) which are clockwise immobilized in two compartments around the middle part. For assessment of the novel assay, clinical reference sera (n = 354) and healthy controls (n=104) were tested for ANCA by IIF using CytoBead® ANCA and automated analyzed with AKLIDES®.

Results: Inter-rater agreement statistics showed a very good agreement of ELISA and CytoBead® ANCA testing for the integrated parameters MPO and PR3 (Cohen’s kappa χ = 0.84 – 0.95) and a good agreement for dsDNA (Cohen’s kappa χ = 0.76). The relative sensitivity was 87%–100%, and relative specificity 94%-99%.

Conclusions: The new test system can replace the two-stage analysis by combining IIF screening with multiplex confirmative testing. The test is suitable for automation with AKLIDES® as well as for manual ANA screening.
The novel CytoBead® technology combines autoantibody analysis by cell-based screening with corresponding confirmation by multiplex microbead technology using immunofluorescence technique in one well. CytoBead® ANCA allows the simultaneous screening and confirmation of anti-neutrophil cytoplasmic antibodies (ANCA) on neutrophils, proteinase 3 (PR3) and myeloperoxidase (MPO) coated microbeads. Furthermore, the detection of anti-GBM antibodies for emergency diagnostic of rapid progressive glomerulonephritis is integrated. This assay is interpretable with a standard fluorescence microscope (FITC channel) for semi-quantitative and with Aklides® system for quantitative analysis. Performance of the CytoBead® ANCA assay was investigated using 666 human sera including 118 patients with ANCA-associated vasculitis, 162 healthy controls, 352 disease controls and 34 anti-GBM positive sera. Receiver operating characteristics and inter-rater agreements were used to compare the results of novel CytoBead® ANCA assay with routine autoantibody investigation. The comparison of ANCA screening showed very good agreement for pANCA and cANCA patterns (kappa=0.862, 0.868; respectively) and the results of anti-PR3, anti-MPO, and anti-GBM detection revealed good to very good agreement (0.78, 0.72, 0.87; respectively). Consequently, CytoBead® ANCA assay is an alternative to classical time-consuming single parameter diagnostic and is therefore a clinical diagnostic tool for emergency situations.

P003

Simple detection of celiac-disease specific antibodies and total IgA in one reaction environment

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The novel CytoBead CeliAK assay allows the simultaneous analysis of endomysial IgA-antibodies (EmA), anti-tissue transglutaminase enzyme (tTG)- and anti-deamidated gliadin peptide (DGP) IgA-antibodies as well as the determination of total IgA in one well. The assay runs on glass slides with tripartite wells, left: two different microbeads (MP) coated with tTG or DGP, middle: monkey esophagus, and right: MP coated with anti-IgA. The assay was assessed visually and by Aklides®. Overall, sera of 377 patients and controls (155 celiac disease (CD), 5 IgA-deficient, 127 with other diseases, 90 blood donors) were run. Each CD patient was positive for at least one of anti-tTG, anti-DGP or EmA resulting in diagnostic sensitivity of 100%. All IgA-deficient patients were IgA negative. Diagnostic specificity for anti-tTG, anti-DGP, and EmA was 100%, 93.3%, and 100%, respectively.

By automated evaluation, the diagnostic sensitivity for anti-tTG and anti-DGP was 97.4% and 88.3%, respectively and the diagnostic specificity of anti-neutrophil cytoplasmic antibodies (ANCA) on neutrophils, proteinase 3 (PR3) and myeloperoxidase (MPO) coated microbeads. For both assays results were close to each other.

P004

Exome Sequencing Pilot Study in Children with Carbamazepine-induced Serious Skin Reactions

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Stevens-Johnson syndrome (SJS) and drug-induced hypersensitivity syndrome (HSS) are life-threatening drug hypersensitivity reactions. Previous studies on genetic predictors for SJS and HSS were focused on immune-related genes and on known, frequent polymorphisms in the genome. For the anticonvulsant carbamazepine (CBZ), genetic markers for SJS and HSS have been identified in the HLA region (HLA-B*15:02, HLA-A*31:01). However, many patients carrying HLA-B*15:02 or HLA-A*31:01 tolerate CBZ, resulting in a low positive predictive value of a genetic test for these markers alone. In this pilot study, we aimed to identify novel genetic variants potentially associated with CBZ-induced SJS and HSS using whole exome sequencing in seven Canadian children with CBZ-SJS and five children with CBZ-HSS. A DNA pool from 95 CBZ-tolerant children was sequenced to estimate variant frequencies for comparison with hypersensitivity cases. All study participants were recruited through the Canadian Pharmacogenomics Network for Drug Safety. No individual rare single nucleotide variant (SNV) was observed.
in all SJS or HSS cases, respectively. Single variant and gene-based analyses revealed several candidate genes and variants overrepresented in hypersensitivity patients, including immune regulatory and cell adhesion genes. More candidates were identified when analyzing SJS and HSS separately, suggesting different underlying mechanisms of these hypersensitivity reactions. With a hypothesis-free, comprehensive genetic screening approach, we identified new candidate genes and variants, which require follow-up in additional patients to further investigate their potential involvement in the development of CBZ-induced SJS or HSS.

P005

Generation of Tools and Assays to Study Human Mast Cell Tryptases

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Objectives: Tryptases are mast cell-specific proteases that are involved in innate and adaptive immune responses as well as disorders such as allergy and asthma. In man, this group of serine proteases comprises four distinct enzymes (α–δ). Among them so far only the closely related tryptases a and b have been characterized. In contrast, the (patho-)physiological functions of tryptases γ and δ and their usefulness as biomarkers of mast cell activation remain to be determined.

Methods: For structural and functional comparison we have expressed tryptase α, β, δ and soluble forms of γ as well as their zymogens in Pichia pastoris. Mutations were introduced to increase the solubility and yields of the recombinant proteins.

Results: Functional studies showed that tryptase β and γ are enzymatically active with trypsin-like substrate specificity whereas α and δ as well as the zymogens have minute if any activity. The active tryptases differ, however, considerably in their pH optimum, substrate and inhibitor profiles, quaternary structure and regulation. The recombinant proteins are currently utilized to generate and characterize specific antibodies and immunoassays that discriminate between the four closely related tryptases.

Conclusion: These assays will be useful to analyze the cellular localization and tissue distribution of the tryptases and their role in health and disease.

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P006

CD63 and CD203c expression during specific immunotherapy (SIT) for wasp venom allergy using Basophile Activation Test (BAT): preliminary 2 years follow-up results

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Objectives: SIT is an established therapy for wasp venom allergy. The aim of our work is to investigate the progression of surface antigen CD63 and CD203c expression during SIT using BAT.

Methods: We included 71 patients in our study (61 wasp and 10 honey bee allergy; 15 aborted). Blood samples were collected before and repeatedly every 6 months after SIT start until 3 years. Here we report on adult patients (n=31) at 1 year (1y) and 2 years (2y) follow-up. For all samples we determined CD63 and CD203c expression using BAT after stimulation with four wasp venom concentrations (c1:284.0/c2:56.8/c3:11.0/c4:2.3 μg/l). We evaluated the relative proportion of activated basophilic granulocytes (a2) at c2 and the calculated concentration to stimulate 50% of total activatable basophile granulocytes (c50). The study was approved by the institutional ethical review board.

Results: (1) CD63 expression (and inversely c50) at 1y/2y (CD63 nonresponder: 4) decreased in 18 and increased in 5 patients, while it was constant in 4 cases. Median changes to baseline at 1y/2y were a2=−28% (p<0.01)/−58% (p<0.01) and c50=230% (p<0.01)/500% (p<0.01). (2) CD203c expression (and inversely c50) at 1y/2y (no CD203c nonresponder) decreased in 11/3, increased in 11/10 and did not change in 9/8 patients. Median changes to baseline at 1y/2y were a2=−4% (p=0.50)/−15% (p=0.06) and c50=186% (p=0.06)/199% (p<0.01).

Conclusion: Expression of CD63 and CD203c in BAT show individual progression in time. Statistically significant differences to baseline stimulation can be found already at one year follow-up. However, further work is required to gain more detailed inside into long-term stimulation behaviour in BAT and correlation with sting challenge.
P007

Sensitization profile against PR10, profilin, non-specific lipid transfer proteins (nsLTP) and storage proteins (SP) in nut and pollen allergy

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Introduction: We investigated the sensitization pattern of recombinant allergen components of PR10, profilin, nsLTP and SP groups in IgE pollen positive samples.

Materials and Methods: In 117 pollen positive samples we determined IgE against PR10 (Bet v1, Gly m4, Api g1, Pru p1, Ara h8, Cor a1), profilin (Bet v2, Phl p 12, Pru p4), nsLTP (Pru p3, Ara h9, Cor a8), SP (Ara h1, Ara h2, Ara h3, Cor a9, Cor a14), peanut (f13) and hazelnut (f17) using CAP. Results were considered positive at IgE > 0.1 kU/l.

Results: We observed a marked fraction of double-positive IgE within allergen groups: 86-100% (PR10), 83-100% (profilin), 63-100% (nsLTP) and 63-90% (SP). In IgE (peanut/hazelnut)-positive samples 89%/90% were also positive for PR10, while only 36%/28% were also positive for SP. Pairwise correlations within recombinant allergen groups yielded (R²): (a) PR10: Bet v1/Gly m4 0.77, Bet v1/Ara h8 0.83, Bet v1/Cor a1 0.93, Ara h8/Cor a1 0.81; (b) profilin: Bet v2/Pru p4 0.85; (c) nsLTP: Pru p3/Ara h9 0.60, Pru p3/Cor a8 0.71, Ara h9/Cor a8 0.61; (d) SP: Ara h1/Ara h2 0.79, Ara h1/Ara h3 0.76, Ara h1/Cor a9 0.57, Ara h1/Cor a14 0.27, Ara h2/Ara h3 0.60, Ara h2/Cor a9 0.54, Ara h2/Cor a14 0.27, Ara h3/Cor a9 0.67, Ara h3/Cor a14 0.32, Cor a9/Cor a14 0.64. Fraction of double-positive IgE findings for native f13/f17 and recombinant allergen components were 78-89% (PR10), 27-31% (profilin), 22-35% (nsLTP) and 27-32% (SP) for f13 and 78-92% (PR10), 26-31% (profilin), 26-35% (nsLTP) and 26-35% (SP) for f17.

Conclusion: In order to better estimate the clinical relevance of a positive IgE finding for native peanut and/or hazelnut allergens it is always indicated to determine SP and nsLTP. In vitro sensitization pattern will help to identify patients at risks for systemic reaction.

P008

Melting the Iceberg with novel diagnostic markers for Celiac Disease

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Aim: Research into the underlying causes of celiac disease (CD), revealed a novel powerful diagnostic marker: a covalently formed complex between transglutaminase from Streptoverticillum mobaraense (mTg) and gliadin.

Methods: First, complexes of mTg and gliadin were formed, resulting in mTg-gliadin complexes (mTg-neo-epitopes). The purified mTg-neo-epitopes were used as antigen in an ELISA. An in-house cohort of 82 patients with confirmed CD diagnosis and 85 blood donors were analysed using the following ELISAs: tTg “New generation”, Tg2, and DGP (AESKULISA(R)) as well as ELISAs against the mTg and mTg-neo-epitope, all for determining both IgG and IgA values.

Results: Purified mTg-neo-epitopes show an increased immunoreactivity in ELISA compared to single antigens and similar sensitivity (mTg-neo-epitope IgA 78.0 % / IgG 84.1 % and tTg-neo-epitope IgA 89.0 % / IgG 74.4 %) and specificity (mTg-neo-epitope IgA 95.3 % / IgG 92.9 % and tTg-neo-epitope IgA 92.9 % / IgG 96.0 %) values like the tTg-neo-epitope ELISA (AESKULISA® tTg “New generation”). Further studies revealed that mTg- and tTg-neo-epitopes have identical outcomes regarding the level of the ELISA results.

Discussion: Both neo-epitopes show comparable performance in the ELISAs despite the absence of sequence or 3D similarity that could come along with immunopotency. Even without overall homology, mTg and tTg display the same type of catalytical activity. If an antibody-paratope exists, it could be in a way that an anti-mTg-neo-epitope antibody shows cross reactivity and binds to a tTg-neo-epitope. Molecular mimicry and epitope-spreading of the mTg-neo-epitopes could be a risk factor in genetically predisposed individuals.

P009

Comparison of the AESKU HELIOS IFA system with another ANA Screening Method

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Background: This study compares the AESKUSLIDES® HEp-2 cell line processed on the fully automated HELIOS IFA analyzer to the NOVA Lite® HEp-2 cell line processed on the PhD system.
Methods: 82 de-identified serum samples were tested on the HELIOS at AESKU, Oakland and processed on the PhD system at University of Pittsburgh Medical Center. Base dilutions were performed at 1:80 and titrated to an end-point dilution of 1:1280. Several discrepant positive samples were subsequently run in the BioPlex® 2200 system using the BioPlex 2200 ANA screen. Two independent clinical laboratory scientists evaluated the results at both locations.

Results: Qualitatively, 80/82 (97.6%) HELIOS results were concordant with the PhD system. Titration results also correlated well: 19 samples were double negative, 10 were borderline, 41 within 1 titer, 7 within 2 titers, 4 within 3 titers, and 1 within 4 titers. Borderline outcomes were treated as comparatively equal when paired results were 1:80 and negative. 5 out of 7 samples within two titers of the PhD/INOVA results had higher HELIOS end-point titrations. 3 out of 4 samples with a distance of 3 titers had higher HELIOS end-point titrations. Clinical data and BioPlex results of these 3 samples indicated autoimmune hepatitis or Sjogrens syndrome and an SSA or Centromere result >8. Two additional samples with no clinical history of connective tissue diseases had a BioPlex result of negative and SSA/SSB >8 respectively.

Conclusion: Many laboratories allow a comparative titer discrepancy of 1 dilution as a diagnostic convention when determining precision. Therefore, the combination of AESKUSLIDES and HELIOS analyzer has a higher sensitivity and specificity than the INOVA/PhD system.

P010

Comparison of Luminometric Immunoassays

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Introduction: Quantitative luminometric immunoassays are used for the determination of Ferritin, Cortisol, Parathormone (PTH), Cancer Antigen 125 (CA125), Follicle Stimulating hormone (FSH), Luteinizing hormone (LH), Prolactine and Alpha-Fetoproteine (AFP) were compared with an Architect i2000SR (ABBOTT GmbH & Co.) and a LIAISON XL (DiaSorin Deutschland GmbH).

Materials: The following parameters were tested: Ferritin, Cortisol, Parathormone (PTH), Cancer Antigen 125 (CA125), Follicle Stimulating hormone (FSH), Luteinizing hormone (LH), Prolactine and Alpha-Fetoproteine (AFP). Commercially available luminometric Immunoassays were used as per the manufacturer’s instructions. To compare the methods, first comparative measurements were carried out on both devices. To express the results for the evaluation mathematically Pearson’s Correlation Coefficient and the Passing Bablok regression were determined.

Results: Passing-Bablok-Regression and Pearsons Correlation Coefficient (R) were calculated for the following methods: Ferritin $Y = 0.84x + 53.12$, Cortisol $Y = 1.16x - 45.65$, PTH $Y = 0.51x + 10.96$, CA125 $Y = 1.14x + 2.11$, FSH $Y = 1.27x + 1.23$, LH $Y = 1.20x + 0.68$, Prolactin $Y = 0.70x + 1.56$, AFP $Y = 1.10x + 0.21$. The correlation coefficients lie very near 1. With CA125 and AFP these are even more than 0.99. Only FSH has a correlation coefficient smaller than 0.9 (0.8874). The following pictures show the Passing Bablok-regression straight lines of the single Assays. 20 patient tests were measured for every Assay in parallel on the same day.

Conclusions: The results showed a high degree of correlation and regression. The immunoassays at LIAISON XL can be accurately measured using the automated method, which can be recommended.

P011

Putative low IL-10 producer genotypes are associated with a favourable long-term response to etanercept treatment in patients with rheumatoid arthritis

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Objective: Outcome predictors of treatment with TNF inhibitors in patients with rheumatoid arthritis (RA) are needed since not all patients show a favourable response and therapy increases the risk of serious infections. We studied whether IL-10 promoter polymorphisms with potential relevance for IL-10 production capacity in patients with RA were associated with response to etanercept.

Methods: RA patients with a good (n=25), moderate (n=17) or no response (n=8) to etanercept therapy (median 36 weeks, range 4-52) according to EULAR criteria and 160 matched controls were genotyped by sequencing for the IL-10 promoter SNPs -2849G>A, -1082G>T and -819C>T. Haplotypes were reconstructed via mathematical model and tested for associations with disease susceptibility and therapy response.

Results: Four predominant haplotypes with almost equal distribution were identified (AGCC, GATA, GGCC, GACC). Patients with the -2849A allele or the haplotypes AGCC and GATA that have a putative low IL-10 production capacity were overrepresented in the group with a good response (RR 2.1 and 4.0, respectively; 95% CI 1.14-4.0 and 1.14-8.4). In contrast, carriage of the GGCC haplotype with a putative high IL-10 production capacity had an increased risk of a moderate or no response (RR 1.9; 95% CI 1.14-8.4). IL-10 promoter alleles or haplotypes were not associated with disease susceptibility.
Conclusion: Genetically determined low IL-10 production may favour the response to etanercept in RA patients. The iatrogenic blockade of TNF may reveal proinflammatory effects of its endogenous antagonist IL-10. Further studies are required to correlate the genetic findings with functional data, eg. cytokine levels in vivo.

P012

Real Time PCR Quantification of Mast Cell Tryptases

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Tryptases are mast cell-specific serine proteinases that are involved in innate and acquired immunity as well as mast cell-related inflammatory disorders such as allergies and asthma. Thus far, four human tryptases (gene symbols TPSAB1, TPSB2, TPSD1 and TPSG1) have been identified that differ in expression, cellular localisation, enzymatic activity, and presumably function. Due to a high degree of sequence similarity (up to 99.5% on the RNA level) the quantification of individual tryptases by means other that restriction analysis or sequencing has been difficult up until this point.

For expression analyses of the individual tryptases we have now established and validated real-time quantitative PCR assays. Hence all 4 tryptases were cloned and verified by sequencing. Primer pairs were designed and authenticated for qPCR assays according to MIQE guidelines in respect to efficiency, sensitivity and specificity. In addition melting curves and agarose gel electrophoresis were analysed to study matrix effects in cDNAs from cell lines. After optimisation, all qPCR assays have a high sensitivity (< 10 copies), specificity (≥ 106-fold discrimination between the tryptases) and amplification efficiency (≥ 90%). Analyses using tryptase-positive and -negative cells suggest that these assays can detect a single mast cells within >10⁵ fibroblasts.

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P013

Allergen-specific immunotherapy induces immunosuppressive sialylated IgG antibodies

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Allergen-specific immunotherapy (SIT; hyposensibilization) is associated with the development of regulatory T cells and allergen-specific IgG serum antibodies (Abs). However, the function of SIT-induced IgG Abs is still unclear. It becomes more and more clear that the Fc glycosylation pattern determines the pro- or anti-inflammatory effector functions of IgG Abs, whereby sialylated IgG Abs are anti-inflammatory (Kaneko Y et al, 2006 Science; Collin M and Ehlers M, 2013 Exp. Dermatol.). However, the IgG Fc glycosylation pattern induced by SIT is unknown. We sought to examine the Fc glycosylation and anti-inflammatory quality of IgG Abs formed upon antigen-specific tolerance induction. Stimulation with protein antigens under pro-inflammatory conditions induced low-sialylated IgG Abs. In contrast, tolerance induced immunosuppressive sialylated IgG Abs that were sufficient to block antigen-specific T and B cell responses, DC maturation and allergic airway inflammation (Oefner C et al, 2012 JACI). Importantly, successful SIT in allergic patients also induced sialylated allergen-specific IgG Abs. Our data show a novel antigen-specific immunoregulatory mechanism mediated by anti-inflammatory sialylated IgG Abs that are formed upon tolerance induction. These findings may help to understand and to develop novel allergen-specific therapies for the treatment of allergy.

P014

In vivo enzymatic modulation of IgG antibodies prevents immune complex-dependent injury in the skin

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IgG antibodies are potent inducers of proinflammatory responses by cross-linking Fc receptors on innate immune effector cells resulting in tissue injury. The recently discovered enzymes endoglycosidase S (EndoS) and IgG-degrading enzyme (IdeS) of Streptococcus pyogenes are able to modulate the interaction between IgG antibodies and the Fc receptors, by hydrolysis of the glycan associated with the heavy chain of the IgG molecule (EndoS), or cleavage in the hinge region of the heavy IgG chain (IdeS). In this work, we investigated their ability to inhibit damage mediated by skin-bound antibodies in vivo in two different experimental models, the Arthus reaction, and epidermolysis
bullosa acquisita, an autoimmune blistering skin disease associated with autoantibodies against type VII collagen. We demonstrate that both enzymes efficiently interfere with IgG-mediated proinflammatory processes, offering a great asset to specifically target pathological IgG antibodies in the skin and holding great promise for future applications in human therapy.

P015

Miniaturized fluidic system for detection of arthritis associated antibodies

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Miniaturisation is a helpful tool in diagnostic device development. Small volumes are thought to reduce fees for reagents and incubation times should be reduced due to close proximity of the reaction partners. Aim of project RHEUMA-CHIP is to develop an automated and miniaturized fluidic system for detection of arthritis associated antibodies. In the experimental set-up, a sequence of small channels and reaction chambers is realized as an injection molded plastic disposable. This microfluidic unit builds the core unit of the test set up. Fluidic transport needed for buffer dilution, supply with reactive components and sample flow is directed by program-driven valves and pumps. The operation unit is composed by standard electrochemical touch screen input. Following serum uptake the fluidic structure guides the sample into a serial dilution area. The diluted sample is conducted through the fluidic network, thus passing the binding cavities. Enhancement of binding efficiency is achieved using capturing molecules embedded in a three dimensional gel matrix. Detection of bound antibodies is obtained using an immuno assay with precipitating dyes. Immuno assay procedures were successfully transformed into a variant fitting the fluidic requirements. Patient sera were used to define the usefulness of antigenic structures, antigen preparations, printing techniques and plastic surface influences. To value its gain in an application with clinical aspect, the prototype was tested to detect and discriminate between rheumatoid and post infectious arthritis.

Quality Assurance

P016

Alkaline Phosphatase, change of paediatric reference values with new IFCC-method

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Introduction: In 2011 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) described a reference measurement procedure for alkaline phosphatase ALP, based on the old AP-method from 1983.

Method: We conducted from 27/11/2013 to 02/06/2014 (N=217) parallel measurements on our Siemens Dimension EXL analyser with the old AP- and new ALP-method, provided sufficient remaining serum was available.

Results: Enzyme activities of 100 U/l in the old AP-method were measured at about 80 U/l ALP, whereas the quotient ALP / AP rose to about 0.9 above 600 U/l AP. Infants have a higher conversion factor than teenagers. Linear regression analysis with age or AP-activity on the x-axis versus ALP/AP on y-axis showed little correlation (R² <0.12). 6 sera were measured in parallel using the new IFCC ALP-method on a Roche cobas c 6000 Analyser, with excellent agreement (R² = 0.9995).

Discussion: We lowered our paediatric reference ranges for the ALP in new IFCC method (e.g. in first month of life AP 140 - 400 U/l, now ALP 110 - 340 U/l, AP in children >6 y formerly 130 - 525 U/l, now ALP 100 - 460 U/l). Different enzyme activity- or age-dependent conversion factors could be due to different proportions of AP isoenzymes.

P017

Interlaboratory reproducibility of 34 routine clinical chemistry parameters on the Beckman Coulter AU5800, Roche Cobas 8000, Roche Cobas 6000, Roche Modular PE, or Siemens ADVIA 2400 analytical systems

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Introduction: A lack of reproducibility of results obtained in different medical laboratories frequently hinders a (cost-)efficient patient care. We therefore performed a comparison of results of routine biochemical parameters between 5 different laboratories using different analytical systems.
Methods: 2040 anonymized residues of sera submitted to our laboratory were frozen in aliquots, then analyzed in parallel in five different laboratories (23193 individual analyses), using either the Beckman Coulter AU5800, Roche Cobas 8000, Cobas 6000, and Modular PE, or Siemens ADVIA 2400 analyzers and results statistically evaluated.

Results: We found a very good agreement between the Roche Systems themselves, closely followed by the Roche Modular PE or Cobas 6000 systems and the AU5800, however. The comparison of Modular PE and AU5800 demonstrated the least variance of all calculated parameter-specific coefficients of correlation, indicating a largely method- and parameter-independent reproducibility of results between these systems, being generally best in enzymatic photometry and worst in immunoturbidimetry. Worst results were obtained when comparing the Siemens ADVIA 2400 with all other analyzers, but also with the Beckman Coulter AU5800.

Conclusion: In conclusion, the Beckman Coulter AU5800 demonstrated the best reproducibility with all other systems and, different to the other systems tested, this reproducibility was largely independent from the tested parameter and method used.

P018

Optimising the preanalytical phase with a targeted approach using BD Laboratory Consulting Services

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Introduction: The majority of errors that can lead to incorrect laboratory results are generated in the preanalytical phase, before the sample reaches the laboratory. However, the preanalytical phase is complex and difficult to control. We used BD Laboratory Consulting Services (BD LCS) to improve the quality of blood samples.

Methods: In 2009, we analysed the blood collection and sample processing practices using a BD Preanalytical Review. Fifty-two blood collections were observed, additionally, 351 samples were visually inspected. After the review, an improvement plan was developed including: dedicated phlebotomists on selected wards, tailored training for all persons involved in the preanalytical phase & the introduction of lower draw volume tubes. In 2013, the review process was repeated, 29 blood collections were observed and 254 samples visually inspected.

Results: There was a significant improvement in practices and sample quality over the period. Disinfection errors decreased from 50% to 0%. The introduction of lower draw serum tubes together with training resulted in a decrease in underfilled tubes from 39% to 8%. Adherence to sample mixing increased from 18% of the tubes to 62%, which led to a decrease in fibrin formation in serum specimens from 18% to 1%.

Conclusions: By using BD LCS, it is possible to improve practices and sample quality and consequently, patient care.

P019

Frequency and magnitude of extreme differences of results demonstrated in ten assays by up to 72,000 duplicate measurements

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Introduction: Internal quality controls do not detect occasional drop-outs or extreme differences. Duplicate measurements have been used to improve precision, but may also offer a way to demonstrate the frequency and magnitude of differences between the results provided many samples are studied.

Methods: Concentrations of Calcium, Triglycerides, Cholesterol, TSH, CRP, Lactate, ALT, AST, Creatinine and Troponin I were measured using three Dimension Vista 1500. During 12 months between 2,700 (Lactate) and 72,000 (Creatinine) duplicates were measured. Evaluation was based on regression and difference analyses. A variable zone of acceptance (A-zone) around the equal line allowed quantification and comparison of extreme differences.

Results: The frequency of extreme differences varied between the assays. When all results within the measurement interval were included, an A-zone of 5% left 0.7% (Calcium) and 33% (Creatinine) of the results outside the A-zone; if only those within ±20% of the clinical decision limits were included the number was 0.7% and 28% for Calcium and Creatinine, respectively.

Conclusion: Differences between duplicates occurred with considerably different frequencies and magnitudes. The percentage outside a specific width of the A-zone indicated a possible classification of assays into three major groups with Calcium, TSH and Troponin I representing the groups. It would be reasonable to include frequency of extreme differences in the description of measuring systems.
P020

Establishment of the first collaborative trial for the detection of bacterial contamination in platelet concentrates with rapid and cultural detection methods: field report of one year experiences

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Background: Bacterial contamination of platelet concentrates (PCs) is the most frequent infectious complication in transfusion therapy. Different bacterial detection methods are available, but the evaluation of these methods is a complicated process. Since some detection principles required bacterial vitality and bacteria are capable to grow in PCs, variable titers prevent an objective comparison. In this context, a proficiency panel (RfB) with stabilized sample material was established and currently three independent collaborative trials were performed.

Methods: Three different modules were available: 1) rapid methods, 2) culture methods, 3) bacterial identification. Currently, three independent trials were performed, each trial included six to eight samples which were analyzed with three different rapid methods (BactiFlow, 16S rDNA NAT, Q-MAP Bakt) and culture methods.

Results: The setting of the collaborative trial proved sufficient for stabilization of bacterial titers. The results of the three collaborative trials showed that samples spiked with bacteria in the range of 100,000 CFU/ml obtained positive results with all methods. Samples spiked with 1000 CFU/ml showed a lower number of correctly identified positive results, the diagnostic sensitivities ranged from 100% (n=3) for BactiFlow and culture methods, followed by NAT (89–100 %, n=3) and Attomol (33.4 %, n=1). All participants identified bacteria correctly.

Conclusion: The collaborative trial proved successful for the three offered modules. This proficiency panel enables the verification of the analytical sensitivity of bacterial detection methods under controlled routine conditions and represents an important contribution for blood safety.

P021

Evaluation of Technopath Controls According to RiliBÄK Rules

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Introduction: For quantitative measurement of analytes in whole blood, serum and urine specific quality control requirements are set by the German Medical Association (RiLIBÄK). New consolidated multiconstituent controls (MCC) from Technopath were evaluated to check adherence of internal quality to these guidelines.

Methods. The Technopath MultiChem S Plus and MultiChem IA Plus controls were tested on the ARCHITECT instruments (c8000, i2000sr and i3000sr) with ARCHITECT assay reagents for a minimum of thirty days. Data from 22 clinical chemistry and 13 immunosassay parameters listed in RiliBÄK Table B1 were collected and analyzed. Means, standard deviations, relative root mean square deviations were calculated for one control period. Preliminary target values were used to check for RiliBÄK compliance over the quality control period.

Results. For the clinical chemistry assays, the %CVs ranged from 0.6 (chloride) to 6.9% (lipase), with the majority of control results (93.8%) with CVs less than 5%. For the immunosassay the %CVs ranged from 2.38 (AFP) to 11.95% (cTnI low control) with CV less than 10% for the majority of control results (92.6%). With increasing analyte concentration lower %CVs were seen. Relative root mean square deviations were well within acceptable range (Table B1, column 3) except for CA 19-9.

Conclusions. The new consolidated Technopath MCC allow effective quality control management fulfilling RILIABÄK requirements.

P022

Traceability of cell concentration determination: challenge and impact

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Accurate cell counting – including the correct identification of cell subpopulations and enumeration – is essential for a wide range of clinical applications. To ensure comparability and reliability of measurement results traceability to SI units is needed. However, in cell concentration
measurements traceability has not been established yet, in part because of the difficulties to unambiguously identify target cells in complex biological matrices. Primary methods for the determination of concentrations of selected target cells are developed to establish traceability according to ISO17511. Implementation of traceability includes reliable cell identification and enumeration techniques combined with high accuracy volume measurements. Procedures for cell enumeration based on flow cytometry and microscopy which are potentially suited as primary methods are being evaluated. To enable manufacturers and end users to easily assess the accuracy of their measurement results, a secondary reference method and a selected method, both based on relative concentration measurements with respect to traceable calibrators, are being investigated. The methods are evaluated using the model systems of CD4 positive lymphocytes and circulating endothelial cells. The research receives funding from the EMRP project SIB-54 Bio-SITrace. The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

P023

Cyclosporin A: Consensus values versus Reference Measurement Values in the EQA Scheme of RfB-DGKL

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Target values used by DGKL-RfB in the EQA scheme for Cyclosporin A (CSA) are consensus values (CONS), since an accepted reference measurement procedure (RMP) does not exist. Recently we have published a candidate RMP for CSA. Respective reference measurement values (RMV) were displayed in addition and compared to CONS of the surveys from 2012 and 2013. Here we evaluate the participants’ survival rates with regard to CONS and RMV. For all 20 EQA samples CONS were overestimated compared to the respective RMV (mean: +3 %). Based on CONS, in most cases the survival rate of participants using mass spectrometry (group MS) was slightly higher (mean: 95.1 %) than for all participants (mean: 92.5 %). Based on RMV as the target, the survival rate of group MS (mean: 96.2 %) was higher in any case compared to all participants (mean: 91.6 %). If comparing RMV related evaluations to those of CONS, slightly higher survival rates were found for RMV in case of group MS (mean: 96.2 %) vs. CONS (mean: 95.1 %), in case of subgroup “MS, Chromsystems” (mean: 96.7 %) vs. CONS (mean: 96.0 %), and in case of subgroup “MS, others” (mean: 96.0 %) vs. CONS (mean: 94.7 %). Survival rates using RMV were slightly lower for all participants (mean: 91.6 %) vs. CONS (mean: 92.5 %).

The results indicate, that switching from CONS to RMV as target values in the EQA of RfB-DGKL would not lead to distinct clashes regarding participants’ survival.

P024

Is the outcome of the presently used internal quality control systems comparable?

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Background: The quality of measurements in the laboratory is continuously monitored by internal quality control (IQC) systems based on regular measurements of control materials. We compared the outcome according to the German RiLi-BAEK and the Westgard 1:2s and 1:3s rules referring to the 95% and 99% variation of control results respectively, for some typical assays.

Methods: Four groups of assays were recognized: 1) High precision and high trueness 2) Low precision and high trueness 3) High precision and low trueness, and 4) Low precision and low trueness. Examples for each of the 4 groups were retrospectively identified from established IQC data. Target values and variances of these analytes were first established and then used during the evaluation period of 30 days. Performance criteria were the number of results violating the limits according to RiLi-BAEK and the two Westgard rules.

Results: The imprecision in both periods was comparable. The RiLi-BAEK limits were violated between 0 and 7 times. The Westgard 1:2s and 1:3s rules were violated between 2 and 17 times and 0 and 2 times, respectively.

Conclusion: The RiLi-BAEK requires that laboratories meet nationally defined goals and thus provides a potency to improve harmonization of results. Our results indicate that some RiLi-BAEK acceptance limits may need reconsideration. The Westgard 1:2s and 1:3s rules depend on limits established in local laboratories and the external quality assessment for harmonization of results.
P025

Retrospectively estimated intra-laboratory reference limits applied to coagulation assays

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Introduction: Manufacturers of coagulation assays report reference limits but also recommend that the transferability needs still to be examined by each medical laboratory. The classical CLSI approach cannot be performed by routine laboratories. An indirect retrospective method of Arzideh et al. is now available in the GuideLimitCalculator. In this study, we evaluated the applicability of this method to global coagulation assays for the first time.

Results: Two manufacturers of instruments/reagents measuring aPTT and PT (Quick) were evaluated (Siemens BCS/Pathrombin SL/Innovin and Thrombolyzer XR/TrimiCLOT aPTT HS/PT Excel). The GuideLimitCalculator was able to estimate reference limits and 95% confidence intervals for all measurands. The upper reference limits estimated using aPTT-assays were less than 1 second lower than the limits reported by the manufacturers. Lower reference limit of the PT was estimated 7.7 percentage points higher than 70% indicated by the manufacturer when using the Thrombolyzer XR while, when using the Siemens assay it was found to be merely 2.5 percentage points higher than the recommended 82%.

Discussion: The GuideLimitCalculator can be applied to aPTT and PT resulting in satisfactory accordance with reference limits indicated by the manufacturers. For practical purposes the tool is suitable and easy to use, helping laboratories to verify their own reference limits and consequently, to increase the diagnostic validity of the results.

P026

Audits of 120 routine blood collections

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Objective: About 46%-68% of erroneous laboratory results are caused in the preanalytical phase and have the potential to affect patient safety. Audits can identify deviations from standard operating procedures (SOP) in the routine blood collections.

Methods: We audited 126 blood collections. These were performed by 58 professionals and students on 23 wards of the University Medicine of Greifswald from October 2011 to February 2013. We used an audit-questionnaire with approximately 150 items.

Results: Medical students in their last year (47%) and nursing staff (43%) performed most blood collections. They had received training by skilled colleagues (50%) or in courses of the laboratory (45%). Vacutainers® were labeled before sampling in 94% of all cases. Patients were identified by addressing them with their name (63%) or by leading (13%) and open (2%) questions, respectively. 22% were not identified verbally. Patient positioning was adequate in 89% of all cases, and in 60% tourniquet time was below 60 s. Disinfection time was sufficient in 38%. Supportive measures like closure of fist (43%) and tapping of veins (6%) were observed. In 99% puncture sites were treated with sterile swabs. Gloves (75%) and needle security mechanisms (86%) were used.

Conclusion: Deviations from SOPs were observed at a considerable rate. Our findings are in line with data from other hospitals indicating that continuous training programs should be taken into account to improve patient safety.

P027

Software-supported quality management – requirements and function algorithm

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The necessity of a quality management (QM) in clinical chemistry is beyond the debate. Like other processes in the lab the QM should be standardized and automatized. This can be realized by a specialized software (QM monitor). We present the requirements and the function algorithm.

QM relevant changes must be sent to the QM monitor which informs the QM representative (QMR). The QMR instructs the responsible persons. The responsible persons initiate the laboratory as well as the administrative implementation. Laboratory implementation: The function
volume depends on the task (changing a reference range in data base is completed faster compared to the implementation of a new analytical machine). Each step should be documented in the QM monitor informing the QMR.

Administrative implementation (esp. here is a specialized software very helpful and can simplify QM): The software should administrate QM sectors which are basic areas in the field of QM like the directory of services, standard operating procedures (SOP). These sectors have field functions also administrated by the software. The method of operation should be explained by the change of a reference value of an analyte. The software should duplicate the SOP, the reference value should be adapted as well as the version number of the SOP. Furthermore the software should actualize all documents containing the reference value including the interface to other labs. Then the SOPs should be sent to the QMR to confirm him. The precursor SOPs should be archived and all persons concerned should be automatically informed.

P028

Long term stability of whole blood glucose for 96 hours using Terumo VENOSAFE™ tubes

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Introduction: Long sample transportation times can occur in rural areas, due to centralization of laboratories or in epidemiological multi-center studies with core laboratories. Measuring glucose concentration is known to be critical due to unsatisfactory glycolysis inhibition. Terumo VENOSAFE™ tubes proved to be superior to other anticoagulant systems, especially according to the complete inhibition of hexokinase and phosphofructokinase-1 enzymes at low pH levels, which is not given by the conventional NaFl approach. We investigated long term stability for Terumo tubes for up to 96 hours at room temperature, conditions which may occur during long sample transport.

Methods: Venous blood samples were collected from 40 volunteers using one Terumo. Samples were stored at room temperature and mixed continuously for the whole storage period, to imitate mechanical transportation. Samples were (re)centrifuged at 0, 24, 36, 48, 72 and 96 h. Plasma glucose was determined after each centrifugation using the Glucose Hexokinase method on the Dimension Vista® 1500 System (Siemens Healthcare Diagnostics, Eschborn, Germany). Glucose concentration measured at 0 hours were the initial glucose concentration to which all other concentrations were compared.

Results and Discussion: The mean glucose concentration at 24 hours was 100.42 % ± 2.26 %; at 48 hours 100.71 % ± 2.6 %; at 72 hours 101.14 % ± 2.26 % and at 96 hours 100.55 % ± 2.3 %. We conclude that Terumo tubes can effectively stabilize glucose in whole blood samples kept at room temperature over a 96 h time period and therefore are suitable material for long sample transport.

New Methods and Parameters

P029

Elevated serum concentrations of cysteine-rich angiogenic inducer 61 (CYR61) in patients with liver cirrhosis and hepatocellular carcinoma

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Introduction: Previously, it was shown that the circulating concentration of CCN2, i.e. the serum level of CTGF (connective tissue growth factor), is elevated in chronic liver diseases. However, it is unknown so far, whether the serum levels of other CCN-members, e.g. cysteine-rich angiogenic inducer 61 (CYR61), are affected similarly.

Methods: Using a newly developed commercial assay for the measurement of CYR61 in body fluids, CYR61 concentrations were assayed in 580 serum samples of patients with hepatocellular carcinoma (HCC), in 165 samples of patients with LC and in 162 samples of healthy controls. The relationship between serum concentrations and clinical features was evaluated.

Results: Serum concentrations of CYR61 were significantly higher in patients with liver cirrhosis than in patients with HCC and in healthy control samples. In HCC patients, CYR61 concentrations increased depending on the tumor stage, with significantly higher concentration in TNM stage III-IV than in TNM stage I-II. Also, serum concentrations of CYR61 in patients with HCC correlated markedly positive with clinical-pathological features. For example, CYR61 concentrations in patients with tumors \( =10 \text{cm} \) were significantly higher than in patients with tumors of less than 5cm diameter. Also, serum concentrations of CYR61 in patients with tumors of 5-10 cm diameter were significantly higher than in those with tumors <5cm of diameter.

Conclusion: CYR61 serum concentrations are indicators of hepatocellular carcinoma and fibrosis and correlate with recurrence and metastasis of HCC.
New Methods and Parameters

P030

Connective Tissue Growth Factor (CTGF/CCN2) in Serum is an Indicator of Fibrogenic Progression of Chronic Liver Diseases

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Introduction: Pathophysiological reason and previous studies suggest connective tissue growth factor (CTGF/CCN2), an important downstream mediator of profibrogenic TGF-β, as a potentially valuable, single serum biomarker of fibrogenesis to monitor progression of chronic liver diseases.

Aim: To investigate serum concentrations of CTGF/CCN2 in chronic liver diseases including liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) and to evaluate the relationship between the serum level of CTGF and metastasis formation of HCC.

METHODS: Using a newly developed commercial assay for CTGF in body fluids we investigated serum CTGF in a cohort (n = 222) of patients with histologically differentiated stages of fibrosis, cirrhosis and primary HCC compared with an age- and gender-matched control population.

Results: Compared to normal subjects and early stages of fibrosis mean CTGF concentration was significantly elevated in S3/S4 stages of fibrosis (p = 0.001), cirrhotic (p = 0.004) and HCC-patients (p = 0.001) but individual values scattered. Importantly, a small subgroup of HCC-patients displayed CTGF-levels similar to healthy control subjects. Calculation of ROC-curves displayed an AUC of 0.78 for S3/S4 fibrosis, a positive predictive value of 85% and a sensitivity around 60% depending on the cut-off values selected. Slightly worse criteria were obtained for the population of cirrhosis and S3/S4 fibrosis.

Conclusion: The data point to CTGF in serum of patients with chronic liver diseases as a valuable biomarker of the ongoing process of connective tissue formation, i.e. active fibrogenesis, rather than established, fully developed cirrhosis. In HCC elevations of serum CTGF is likely to indicate accompanying fibrogenesis.

P031

Fetal molecular blood group RhD determination from maternal plasma for decision making on Rh prophylaxis in D-negative pregnant women

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Objective: Non-invasive prenatal testing of the fetal blood group RhD (NIPT RhD) has been introduced for decision making on Rh prophylaxis in D-negative pregnant women by several European countries and NIPT RhD is an emerging laboratory parameter in Germany. In this study a control reaction for confirming the presence of fetal DNA in the blood sample was validated to minimize the rate of false negative results.

Methods: Cell-free fetal DNA was automatically isolated from 1 ml EDTA-plasma (Chemagic viral DNA/RNA kit, Perkin Elmer Chemagen). Hypermethylation in the promoter region of RASSFIA has been published previously as a reliable marker indicating the presence of fetal DNA. A novel home-brew single-tube methylation sensitive restriction enzyme based real-time PCR detecting hypermethylated RASSFIA promoter was evaluated.

Results: In a group of 20 D-negative pregnant women with a D-positive fetus and average concentrations of fetal DNA the ct-values of the novel control reaction ranged from 34.1 to 37.0. In a second group of 20 pregnant women selected because of known low concentrations of fetal DNA ct-values ranged from 36.2 to 45.6. In a third group of 20 non-pregnant individuals the lowest ct-value was 40.2 (median 45.3). When the cut-off ct-value of 40 was applied, 3 samples from pregnant women were identified to contain not enough cell-free fetal DNA for reliable determination of the fetal blood group RhD status whereas no false positive reactions were observed in non-pregnant control samples.

Conclusion: Even in a large-scale fetal RhD blood group screening laboratory which might be introduced in Germany in the near future the presence of fetal DNA can be tested by a rapid single-tube real-time PCR method.
P032

Symmetrical Dimethylarginine in acute kidney injury – indicator of renal function and potential predictor of mortality

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Symmetric Dimethylarginine (SDMA) and Asymmetric Dimethylarginine (ADMA) are two endogenous derivatives of the amino acid L-arginine which is the exclusive substrate of NO-synthases for NO production. Recently, we have demonstrated that SDMA and ADMA play important roles in the regulation of NO-generation in an animal model of acute kidney injury (AKI). Here, we hypothesized that SDMA and ADMA are characteristically regulated in AKI and are discriminators of outcome in humans. 114 patients with AKI (mean age 65 years, 62% male) were enrolled in an open label, single centre, prospective cohort trial. The follow up after AKI captures hospitalisation and mortality data up to 6 months. SDMA, ADMA and other relevant parameters were determined in patient serum samples during AKI and after amelioration of renal function (drop of creatinine >0.03 mg/dl, n=69). Creatinine decreased from 3.66 to 1.71 mg/dl and ADMA and SDMA dropped, while L-arginine levels were unchanged. SDMA strongly correlated with creatinine and urea during AKI. SDMA was associated with length of hospitalisation in a univariate linear regression analysis. Overall mortality rate was 10.5% during follow up. We determined SDMA but not ADMA nor renal function (creatinine / urea) to be associated with death independently of classical risk factors (age, hypertension). For the first time, this study confirms experimental findings that SDMA strongly correlates with renal function in AKI. SDMA is associated with length of hospitalisation and overall-mortality after AKI, while creatinine was not. These findings highlight the potential clinical value of SDMA and points to a pathogenic role of elevated SDMA in AKI.

P033

Short evaluation of preeclampsia markers Thermo Scientific BRAHMS PlGF and sFlt-1 Kryptor compared to Roche Elecsys

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Background: Preeclampsia may be caused by an imbalance of angiogenic factors. It has been demonstrated that high serum levels of sFlt-1, an anti-angiogenic protein, and low levels of PlGF, a pro-angiogenic protein, predict subsequent development of preeclampsia. The sFlt-1/PlGF ratio was markedly elevated before the onset of clinical preeclampsia.

Methods: Samples of 4/4 pregnant women which developed a preeclampsia and 33 healthy women at different weeks of gestation were analyzed for PlGF and sFlt-1 with the new Thermo Scientific BRAHMS Kryptor assays and compared to the Roche Elecsys system.

Results: Method comparison studies of the novel assays resulted in good correlation to the corresponding Roche assays. The Pearson coefficient of correlation was 0.979 \([\text{Elecsys} = 5.1 + 1.071 \text{Kryptor}]\) for PlGF and 0.914 for sFlt-1\([\text{Roche} = 364 + 0.879 \text{Kryptor}]\), respectively. A significant difference was found for the comparison of the PlGF/sFlt-1 ratio \((r = 0.779, p = 0.07)\).

Conclusions: In spite of good correlation data between the Kryptor and the Elecsys preeclampsia assays, the cut-off values evaluated with the Elecsys preeclampsia assays, the cut-off values evaluated with the Elecsys preeclampsia assays for the support of the diagnosis of an early or late onset preeclampsia cannot be transferred to the newly developed Kryptor assays.

P034

Implementation and verification of sonication, a method to improve pathogen detection at infected endoprosthesis

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Infections of endoprostheses occur rarely, but are very serious. Detection of bacteria from prosthesis surface with conventional microbiological methods is usually difficult because of their aggregation in a biofilm. To break up this film the ultrasonic bath BactoSonic can be used. Present work was performed to introduce the method in our lab accredited to DIN/ISO 15189.
Design qualification:
- choice of suitable ultrasonic bathes
- account costs
- transport logistic of implants
- comprehensive risk analysis (showed open issues, which couldn't answered by manufacturer = parameters for method verification)

Installation qualification:
- installation of device
- creating SOP

Operational qualification and verification (carried out together):
- have change in sonication time an influence on test result (timer can displaced easily)
- bacterial concentration
- species
- material of sonication tube
- temperature of the bath liquid

In absence of original implants bacterial suspensions were used for the investigations. The selected bacteria are most commonly associated with implant infections. The results showed that the recommended sonication time of one minute has to be met. At this time there are also obtained the highest bacterial yield, independent of concentration, species, temperature, or material of sonication tube. Thus, the verification was completed with an assessment of the risk analysis. Performance qualification will be done during a test phase with real implants.

P035

**Xylosyltransferase II is the predominant isoenzyme which is responsible for the steady-state level of xylosyltransferase activity in human serum**

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In mammals, two active xylosyltransferase isoenzymes exist. Both xylosyltransferases I and II (XT-I and XT-II) catalyze the transfer of xylose from UDP-xylose to select serine residues in the proteoglycan core protein. Altered XT activity in human serum was found to correlate directly with various diseases such as osteoarthritis, systemic sclerosis, and pseudoxanthoma elasticum. To interpret the significance of the enzyme activity alteration observed in disease states it is important to know which isoenzyme is responsible for the XT activity in serum. Until now it was impossible for a specific measurement of XT-I or XT-II activity, respectively, because of the absence of a suitable enzyme substrate. This issue has now been solved and our experimental study demonstrates for the first time, via the enzyme activity that XT-II is the predominant isoenzyme responsible for XT activity in human serum. The proof was performed using natural UDP-xylose as the xylose donor, as well as the artificial compound UDP-4-azido-4-deoxyxylose (UDP-XylAz), which is a specific xylose donor only for XT-I. Furthermore, recombinant XT-I and XT-II were used as specific XT sources in the experimental analysis.

P036

**Whole blood levels of choline, betaine, and dimethylglycine and their relations to plasma levels**

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Objectives: Although increased intracellular choline levels were reported in disorders such as malignancies and acute coronary syndromes, the relationship between choline and its metabolites betaine and dimethylglycine (DMG) in plasma and whole blood has not been sufficiently studied. We aimed to investigate this relation.
Methods: Plasma and WB concentrations of choline, betaine, and DMG were quantified using UPLC-MS/MS in 61 elderly individuals (median age: 82 y), which were not supplemented with choline or B-vitamins.

Results: The median concentrations of blood choline, betaine, and DMG were 11.3, 27.8, and 5.9 μmol/L in plasma and 66.6, 164.7, and 13.7 μmol/L in WB (adjusted for hematocrit), respectively. There were positive correlations between WB and plasma choline (r=0.42), betaine (r=0.56), and DMG (r=0.56) (all p≤0.001). Compared to men, women had lower concentrations of plasma betaine (median: 36.5 vs 26.5 μmol/L, p=0.002) and WB betaine (228.1 vs 154.9 μmol/L, p=0.034). Concentrations of choline and DMG did not differ significantly according to sex. Age showed no significant correlations to any of the metabolites.

Conclusion: Concentrations of WB choline, betaine and that of DMG were higher (2.3-6 fold) than in plasma, and seem to reflect intracellular concentrations of the metabolites. WB betaine reflected the expected sex differences. The values of WB choline, betaine and DMG has the potential to be used as long-term markers for the status of these metabolites.

P037

Evaluation of proteotypic peptides for the quantification of TAFI by LC-MS/MS

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Introduction: Thrombin activatable fibrinolysis inhibitor (TAFI) is known as an important link between coagulation and fibrinolysis. Different assays have been developed based on antigen determination and measurement of TAFI activity after quantitative conversion of the proenzyme. Mass spectrometry has the potential to overcome some of observed assay limitations. In the case of proteins, a specific tryptic peptide can be selected as a stoichiometric representative of the protein from which it is cleaved.

Method: Our aim was to use LC-MS/MS to quantify TAFI concentration in human plasma. We evaluated different candidate peptides and chromatographic conditions and compared the LC-MS/MS assay with a TAFI-activity assay and a proTAFI immunoassay.

Results: Several signature peptides predicted through modeling and proteomic data were selected and validated. Especially five peptides show good results through all our performed experiments. Preceding measurements identified DTGTYGFLLPER as the peptide currently detectable with the highest intensity. There is an excellent correlation between the proTAFI (r=0.96) and TAFIa (r=0.95) values measured on the BCS coagulation analyzer and the concentration of the peptide measured with LC-MS/MS.

Conclusion: An MS-assay for the absolute quantification of TAFI will be very useful in future research into the identification of pathological conditions where TAFI is generated and would be very helpful for standardization.

P038

Effects of Preanalytical Conditions and DNA Isolation Procedures on Telomere Length Quantification

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Objective: Changes in telomere length (TL) in peripheral blood leukocytes are associated with age-related disorders such as heart disease and bladder cancer. The aim of the present study was to evaluate effects of preanalytical conditions and DNA isolation methods on TL.

Methods: Whole blood pools from male and female individuals (n=4/4) were incubated with and without actinomycin D (actD) to induce apoptosis. DNA was either directly isolated or after freezing at -80°C. DNA was isolated with kits from 5 different suppliers (5prime, Invisorb, Invitrogen, Macherey-Nagel, Qiagen) and a published isopropanol precipitation protocol (IPP). A multiplex qRT-PCR assay was established to simultaneously quantify TL in relation to a single copy reference gene.

Results: The new multiplex qRT-PCR was robust and superior with respect to time- and cost-effectiveness. Notably, DNA isolation methods significantly affected TL length (e.g. IPP vs. Sprime: 2.1-fold change in LT). Apoptosis-related shortening of TL was detected in all samples except where DNA was isolated with the Sprime or Strattec kits. Preanalytical storage conditions did not affect TL with exception of the 5prime kit.

Conclusion: The choice of DNA isolation method as well as preanalytic conditions such as degradation, but not sample freezing, had a major impact on the measurement of TL. Findings of the current study highlight the need for standardization of sample processing especially in multi-center studies.
conCLIP: A novel technique to illuminate the role of RNA-binding proteins in pathophysiological processes

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Each cell carries information in form of DNA. Still the readout of this information differs from cell to cell, leading to formation of different cell types and tissues with various functions. This diversity is achieved by tight regulation of gene expression on many levels in the cell, amongst which posttranscriptional regulation plays a crucial role. Importantly the fate of the transcriptome is regulated by numerous RNA-binding proteins (RBPs). Mutations in some of them are known to be coupled with diseases (i.e. PABPN1 mutation leads to oculopharyngeal muscular dystrophy (OPMD)). Yet the exact mechanism of their contribution on post-transcriptional gene regulation in most cases remains unclear.

We have established a new technique to study RNA-protein interactions in vivo. It implements UV-crosslinking to preserve RNA-protein interactions, formed in vivo, in combination with a New Generation Sequencing (NGS) approach, and gives the opportunity to study RNA-protein interactions transcriptome-wide. The improved protocol eliminates problems with low input material and increases library complexity. Using this protocol we can visualize binding of RBPs to their cognate binding site and thereby resolve their contribution to the dynamic regulation of gene expression. Further this protocol can be used to unravel the contribution of RBPs to various disease processes.

Molecular Diagnostics/Oncology

Human xylosyltransferase-I mediates arthrofibrotic remodeling

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Objectives: Arthrofibrosis is defined as painful impairment of joint flexibility due to fibrotic tissue remodeling after joint trauma or surgery. Until today, neither a differential diagnostic biomarker nor a therapeutic strategy has been established. Thus, the aim of this study was to evaluate the putative association of human xylosyltransferase-I (XT-I), a fibrotic mediator catalyzing proteoglycan glycosylation, with the arthrofibrotic outcome after total knee replacement surgery.

Methods: Primary cultures of human control and arthrofibrotic synovial fibroblasts were exposed to transforming growth factor-β1 or mechanical strain. Relative mRNA transcript levels were monitored by quantitative real-time PCR. XT activity was determined by a radioactive enzyme assay or by an HPLC-ESI-MS method in cell culture supernatants or serum of control and arthrofibrosis patients. Serum levels of galectin-3 and growth differentiation factor-15 were quantified by enzyme-linked immunosorbent assay.

Results: Treatment of synovial fibroblasts with profibrotic stimuli revealed a stronger increase in XT activity as well as XYLT1 mRNA expression and accumulation of extracellular matrix in arthrofibrotic than control fibroblasts. Contrarily, serum XT activity and other common fibrotic serum markers were not influenced by arthrofibrotic remodeling.

Conclusion: Our data indicate XT-I as a cellular key mediator of arthrofibrosis. However, we suggest that molecular changes based on arthrofibrosis are, due to local restriction of the affected joint by the blood-synovial-barrier, not detectable in human serum. Hence, future studies to monitor arthrofibrotic remodeling should rather rely on local than systemic parameters.
Introducing thallium autometallography as an in vivo indicator for the establishment of novel biomarkers for degenerative neuronal diseases

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Reliable biomarkers for degenerative neuronal diseases, such as Alzheimer’s disease (AD) are still lacking. Mechanistically, it is increasingly recognized that neuronal network dysfunction may be a mechanism underlying early disease-related cognitive decline. In AD, pathogenic beta amyloid assemblies have been shown to induce multifaceted neuronal impairments, including alteration of neuronal signaling pathways, structural and functional synaptic disruption and changes in neuronal morphology and plasticity. All these beta amyloid-mediated neuronal alterations likely cause dysfunction of neuronal network activity and may be reflected by pathological changes of signaling molecules in blood or CSF. However, tools to monitor neuronal network dysfunction in vivo and thus to identify suitable biomarkers have been lacking hitherto. We investigated the Alzheimer mouse model 5xFAD by analysing patterns of neuronal activity during normal behaviour with single-cell resolution mapping of neuronal activity by thallium autometallography. We observed significant layer specific changes of neuronal activity within the AD-affected cortex. As AD lacks definite diagnostic approaches at the present, these findings of early pathology manifesting itself in particular cortical layers have a potential for early diagnosis with laminar resolution brain imaging in suspected AD cases. We are now using this technique to screen for suitable biomarkers and to gain new insight into related pathomechanisms.

Head-to-head comparison of three NAT assays for the identification of cytomegalovirus-positive blood donors

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Background: CMV infection in immunocompromised patients, a major group of transfusion recipients, represents a significant risk for a serious morbidity. CMV break-through infections persist with 1-3 % of transfused patients, not least possibly due to the window-phase donations during acute primary CMV-infection. . We implemented a routine CMV screening procedure for the identification of CMV DNA positive donors and evaluated the sensitivity and performance of different CMV DNA amplification systems.

Methods: Plasma samples of 54,451 blood donations (18,405 donors) were screened for CMV DNA. The analytical sensitivity and the precision of three different commercial assays were compared using a twofold dilution series of plasma inoculated with the first WHO international Standard for CMV . The presence of anti-CMV IgA, IgM and IgG antibodies was determined using different immunological assays.

Results: Five CMV DNA positive donors (0.03%) were identified by CMV screening, with DNA concentrations ranging from 435–4.30E+03 IU/ml. Four donors already showed reactive IgA, IgM and/or IgG antibody titers (IgA+/IgM+/IgG-, IgA+/IgM+/IgG+), whereas one donor showed no presence of anti-CMV specific antibodies. Comparison of NAT assays showed analytical sensitivities ranging from 10.23–11.14 IU/ml with variation coefficients <5%.

Conclusion: The clinical relevance of transfusion-associated CMV infection still requires further investigations, and the evaluated methods present powerful basic tools providing sensitive possibilities for viral testing. The application of CMV NAT facilitated the identification of one donor with a window-phase donation during acute primary CMV infection.

Direct identification of Streptococcus pneumoniae from blood culture vials and phylogenetic analysis of different serotypes of S. pneumoniae using MALDI-TOF mass spectrometry

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Background: The rapid and accurate identification of alpha-hemolytic (viridans) streptococci is very significant in hospital setting for understanding their relevant pathogenicity. However, an extremely high level of similarity within the mitis group (Streptococcus pneumoniae,
**P044**

Quality assurance of rapid near-patient nucleic acid tests – metrological view

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Nucleic acid tests (NAT) are an excellent tool for specific and sensitive detection of infectious agents. Typically, NATs require extensive lab equipment, complex sample preparation, and hence have a long turnaround time. To overcome these drawbacks, upcoming technologies like cartridge based rapid PCR or isothermal nucleic acid amplification techniques appear to be well suited, in particular for near patient approaches or point of care tests (POCT). Consequently, such technologies are being developed by various companies. Aside from simple and quick handling of “rapid” NATs, adequate quality assurance is essential. From metrological point of view, quality assurance of tests utilizing nucleic acid amplification requires standardisation of materials used and procedures applied. The challenge for metrology is the assignment of DNA/RNA concentration values to the control materials, needed for tests aiming at quantification of DNA or RNA. For qualitative tests the working range has to be controlled, particularly an appropriate procedure to establish the limit of detection or sensitivity is necessary. To this end, in the Infect-Met consortium corresponding protocols are being developed and will be proved. The research within this EURAMET JRP receives funding from the European Communitys 7th Framework Program, EMRP project HLT08 Infect-Met. The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

**P045**

Detection of HLA Risk Alleles in Type I Diabetes mellitus using two Single Nucleotide Polymorphisms

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**Purpose:** The human leukocyte antigen (HLA) genes play an important role in many autoimmune disorders such as type I diabetes. Individuals with the heterozygous HLA genotype DR3-DQ2.5 and DR4-DQ8 are at the highest risk of developing type I diabetes. Testing for HLA risk molecules by sequence-based typing techniques are time consuming and expensive. Earlier investigations have shown the wide spread possibility of predicting HLA alleles from single nucleotide polymorphisms (SNPs). In our study, we used two tagging SNPs to predict HLA DQ2.5 (rs2187668) and DQ8 (rs7654108) by real time PCR.

**Methods:** DNA extracted from EDTA blood specimens of 35 individuals was analysed both by sequence-specific-primer (SSP) and sequencing-based-typing (SBT) techniques for DQB1 locus high resolution typing as gold standard. SNPs rs2187668 and rs7654108 were detected by real-time PCR and melting curve analysis in comparison.

**Results:** The gold standard HLA typing techniques and the two real-time PCR’s showed complete concordance in detecting the HLA alleles DQ2.5 and DQ8, respectively.

**Conclusions:** Using the tagging SNPs rs2187668 and rs7654108, it is possible to predict an individual genetic HLA risk for type I diabetes. Two simple SNP real-time PCRs can identify the genotype associated with the highest risk for type I diabetes in a reliable but time saving and cost effective manner.
P046

A new microarray-based HPV test system for the simultaneous detection and typing of 30 relevant high- and low-risk anogenital HPV has been developed

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Background: An accurate and reliable microarray-based HPV test system for the simultaneous detection and typing of 30 relevant high- and low-risk anogenital HPV has been developed.

Methods: For the PCR amplification an individual specific primer system for each HPV subtype was designed. The PCR systems address directly the pathogenic genetic element E6/E7 responsible for oncogenic cell transformation, with all PCR primer systems being integrated in a single multiplex-PCR reaction. For analysis of the PCR, the products are hybridized to a microarray containing specific probes for each HPV subtype. A microarray scanner and software evaluate and report results automatically.

Results: For all HPV the test revealed an adequate sensitivity for primary screening (LoD between 50 - 1000 DNA copies). HPV subtypes can be reliably detected and discriminated also in presence of all other 30 anogenital HPV. No cross reactivity was observed even at high copy number (2mn DNA copies). The test was shown not to be influenced by other non anogenital HPV subtypes or organisms of the cervical flora.

Conclusions: This new test-system is easy and robust to use and therefore optimally suited for routine analysis of patient samples. The individual typing of the HPV subtypes provides insight into the progression of an HPV infection and may help to differentiate between new and persistent infections. The test system has been developed primarily as a diagnostic tool for the identification and monitoring of a (multiple-) cervical HPV infection also in an early stage of the disease. However, it is suitable to address a multitude of scientific questions, for example HPV screening in conjunction with head and neck tumor screening.

P047

A glycan microarray for the detection of carbohydrate specific antibodies in human serum

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Background: Carbohydrate microarrays have a substantial potential for the application in diagnostics, because carbohydrate specific antibodies play an important role in human disease. Antibodies against glycan epitopes are for example formed during bacterial infections, in autoimmune diseases, and heparin-induced thrombocytopenia. Our aim was the development of a glycan microarray platform, which enables the detection of carbohydrate specific antibodies.

Methods: A library of naturally occurring and synthesized glycans was printed on N-hydroxysuccinimide activated Codelink glass slides and incubated with human serum samples. Anti-human-IgG-Cy3, anti-human-IgM-Cy5 and anti-human-IgA-Alexa Fluor 594 secondary antibodies were used for fluorescence based detection.

Results and conclusions: We were able to detect carbohydrate specific antibodies against dextran, which is being used in iron formulations to treat iron-deficiency anemia. Sera of patients with heparin-induced thrombocytopenia contained antibodies against heparin. The patients’ blood group was reflected in the bound blood group antigen specific antibodies. The knowledge gained from this project can be used to establish more glycan-based microarrays for in vitro diagnostics in the future.

P048

Silibinin Down-regulates Expression of Secreted Phospholipase A2 Enzymes in Cancer Cells

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Secreted phospholipase A₂ (sPLA₂) enzymes, especially those of group IIA (bHGLA) play an important role in inflammation and carcinogenesis. In this study we examined the effect of silibinin, a flavonoid with anti-inflammatory and cancer-preventive activities on the expression of
sPLA₂ enzymes. For analysis of sPLA₂ expression in human HepG2 hepatoma and PC-3 prostate cancer cells quantitative reverse transcription-polymerase chain reaction and enzyme linked immunosorbent assay were used. We found that silibinin treatment significantly decreased mRNA and protein levels of sPLA₂-IIA in unstimulated and cytokine-primed HepG2 and PC-3 cells. Silibinin also inhibited the mRNA expression of sPLA₂ group IB, III and V. Concerning the examined signaling pathways our results reveal that nuclear factor- κB, but not specificity protein 1 is involved in the silibinin-mediated down-regulation of hGIIA. In conclusion silibinin has inhibitory effects on basal and cytokine-induced expression of sPLA₂ in cancer cells and therefore, may be able to counteract up-regulation of sPLA₂-IIA and other sPLA₂ isoforms during inflammation and cancer.

**P049**

**Determination of the M-protein by serum protein gel electrophoresis (SPE) and by capillary electrophoresis (CE)**

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**Objectives:** For monitoring the therapy of patients with a multiple myeloma the quantification of the M-gradient in SPE is recommended. Monoclonal IgA (mIgA) often shifts into the beta fraction, making a reliable densitometric estimation impossible. The aim of the study was to compare SPE and CE for the quantification of mIgA.

**Methods:** Sera from 88 subjects with mIgA were included in this study (51 IgA type kappa, 36 IgA type lambda and one IgA type kappa/lambda). In these sera the M-protein was determined by quantification of the M-gradient in SPE and CE (Sebia, Lisses, France). IgA kappa and IgA lambda (HLC, Hevylite™) and free light chains (FLC, Freelite™) were quantified (The Binding Site, Schwetzingen, Germany).

**Results:** In 27 sera no M-gradient could be detected by SPE, because the mIgA shifted into the beta fraction. Therefore a quantification using SPE was only possible for 61 samples. Using CE the mIgA could be quantified in 77 sera, by a separation of the beta fraction into a beta1 and a beta2 subfraction. Eighty-two of the 88 sera showed a pathological HLC ratio, but only 60 sera showed a pathological FLC ratio (93% and 68%, respectively). The combination of SPE or CE with the HLC ratio showed a sensitivity of 97.7% and 96.5% for the identification of the mIgA.

**Conclusion:** The CE is better suited for the quantification of mIgA with beta-mobility than the SPE. The HLC ratio might become important for the detection of monoclonal gammopathies.

**P050**

**miR181b mediates the anti-tumoral activity of the plant-derived polyphenol Curcumin**

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We have previously reported that the chemopreventive polyphenol Curcumin inhibits the expression of the pro-inflammatory cytokines CXCL-1 and -2 leading to a diminished formation of breast and prostate cancer metastases. In the present study we have analyzed the effects of Curcumin on miRNA expression. Microarray miRNA analyses show that Curcumin modulates the expression of a series of miRNAs, including miR181b, in metastatic breast cancer cells. Interestingly, miR181b down-modulates CXCL-1 and -2 through a direct binding to their 3’-UTR. Overexpression or inhibition of miR181b in metastatic breast cancer cells has a significant impact on CXCL-1 and -2, and is required for the effect of Curcumin on these two cytokines. miR181b also mediates the effects of Curcumin on inhibition of proliferation and invasion as well as induction of apoptosis. Importantly, overexpression of miR181b in metastatic breast cancer cells inhibits metastasis formation in vivo in immunodeficient mice. Finally, Curcumin up-regulates miR181b and down-regulates CXCL-1 and -2 in cells isolated from several primary human breast cancers. These results suggest that miR181b mediates the anti-tumoral activity of Curcumin and may be useful as a biomarker for its efficacy.
P051

In-vitro functional assay of dihydropyrimidine dehydrogenase variants related to 5-fluorouracil sensitivity

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5-fluorouracil (5FU) is a commonly used chemotherapeutic agent. Genetic variations in DPYD, which encodes the rate-limiting catabolic enzyme dihydropyrimidine dehydrogenase (DPD), have been studied extensively, and known deleterious DPYD variants explain up to 30% of severe fluoropyrimidine toxicities. Yet, clinical studies show contradicting results regarding the influence of other non-synonymous DPYD variants (e.g. c.85T>C, c.496A>G) on 5FU toxicity. Thus, further study of their impact on enzyme activity is essential, particularly for compound heterozygous genotypes carrying mutations either on the same (in cis) or on different chromosomes (in trans). The aim of this study was to implement a cellular assay for functional assessment of DPYD variants, and in particular for the investigation of c.85T>C and c.496A>G either in cis- or trans- chromosomal position. HEK293T cells were transfected with plasmids containing the DPYD variants c.85T>C, c.496A>G and c.85T>C/c.496A>G in cis and trans position. After 48 hours, cells were harvested and cell lysates were incubated with 5FU for 30 minutes, proteins were precipitated, and the 5-fluoro-dihydrouracil/5-fluorouracil ratio was determined by LC-MS/MS to estimate enzyme activity. Initial results suggest a protective effect for the DPYD mutations c.85T>C and c.496A>G when in cis, whereas DPD activity was similar to that of the wild-type enzyme if both mutations were in trans-formation. The method used is a robust functional assay for the investigation of DPYD missense mutations and will provide important clarification on the combined impact of multiple DPYD risk variants.

P052

Association of a 3'UTR HDAC3 single nucleotide polymorphism with colorectal hyperplasia

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Epigenetic marks, e.g. acetylation or methylation of histone proteins, are considered crucial for the maintenance of the chromatin structure. Deregulation of histone deacetylase (HDAC) expression, enzymes necessary for regulation of the chromatin structure, is thought to be a major contributor to tumor progression and cancer. Pivotal changes observed in colon tissue lesions are the reduction of apoptosis and reduced differentiation. In in-vitro cell culture models, HDAC inhibition acts as a strong inducer of differentiation and cell death. Moreover, in tissue sections of hyperplastic polyps and colon adenomas HDAC3 was found to be overexpressed but the cause for this overexpression is still unclear. We hypothesize that individuals with hyperplastic polyps harbor genetic alterations within the HDAC3 gene that could cause the deregulation of HDAC3 expression. For HDAC3, several single nucleotide polymorphisms are reported and we investigated a possible association between genetic polymorphisms and the occurrence of hyperplastic polyps. Using a custom DNA chip assay for the genotyping of 1536 SNPs on samples of 272 endoscopy patients harboring hyperplastic colorectal polyps (HP) and for 512 sex and aged-matched controls, rs10476823 (HDAC3) together with 7 other SNPs showed significant association (combined P<0.01) with the occurrence of hyperplastic polyps. Functional testing for the 3'UTR SNP rs10476823 (HDAC3) revealed use of a cryptic polyadenylation signal in the 3UTR of HDAC3 mRNA and a longer mRNA half-life in a cell line heterozygous for rs10476823.

P053

GLYCOV, a glycan-based biomarker for the diagnosis of early-stage epithelial ovarian cancer

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Changes in cellular glycosylation are observed during the development of cancer, indicating that alterations of the serum glycome could therefore be used as tumor biomarkers. Current diagnostic methods of OC show only a moderate sensitivity especially at an early-stage of the disease. Therefore, better biomarkers are needed to improve the diagnosis of OC. The study comprised a cohort early stage primary serous EOC, benign ovarian diseases and age-matched healthy controls. N-glycans were released from total serum proteins, permethylated and
measured by MALDI-TOF-MS. The areas of the glycan structures that were significantly up- or downregulated were combined as a score named GLYCOV that was compared with CA125. Median GLYCOV was 3.65 for early stage EOC, 0.38 for benign ovarian diseases and 0.13 for healthy controls. For early-stage patients, the ROC curve of GLYCOV was more accurate (AUC=0.99) than for CA125 (AUC=0.88). GLYCOV (AUC=0.97) was much more accurate than CA125 (AUC=0.68) to discriminate between benign ovarian diseases and healthy controls.

**Diagnostics of non-blood based Specimens (Urine, CSF, others) / POCT / Therapeutic Drug Monitoring - Toxicology**

**P054**

Profiling urinary exosomes in patients with Diabetes mellitus type 2 using the novel Nanosight Tracking Analysis (NTA) technique

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Exosomes are small vesicles (20-100nm in diameter) that are released from all sections of the nephron into urine. They contain protein and RNA derived from their cell of origin and hence may be informative regarding the pathophysiology within the kidney. Current methods for the analysis of urinary exosomes are time consuming and only semi-quantitative. A novel technology, nanoparticle-tracking analysis (NTA) enables a rapid and robust quantification of urinary exosomes: It counts and sizes particles by measuring their Brownian motion in solution. Employing NTA in a pilot study, we determined that there is a significant correlation between total urinary exosome concentration and albumin to creatinine ratio (r=0.344) in patients with type 2 diabetes mellitus. In addition there is a significant increase in the total urinary exosome concentration in diabetic patients with reduced eGFR (n=34) compared with those with maintained renal function (n=16). For further characterization, we used fluorescently-labelled antibodies against aquaporin 2 (principal cell of collecting duct) and CD24 (pan-exosome marker). The ratio of urinary exosomes expressing aquaporin 2 compared with total CD24+ exosomes was very strongly inversely correlated with eGFR (r = -0.41, p < 0.01, Spearman correlation), suggesting increased excretion of aquaporin 2+ exosomes from the principal cells into the collecting ducts from patients with impaired renal function. Our preliminary data indicates that quantification of urinary exosomes by using the new NTA technology may be a useful tool in the assessment of renal injury in patients with diabetic CKD and represent a reservoir for biomarker discovery.

**P055**

High-fluorescent cells in cerebrospinal fluid detected by the Sysmex XE-5000: diagnostic significance and cellular origin

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Introduction: Hematology analyzers like the Sysmex XE-5000 are able to differentiate cells of diverse body fluids into polymorphonuclear, mononuclear, and high-fluorescent cells (HFC). Until today, the cellular identity of HFC in cerebrospinal fluid (CSF) has been unclear. Due to their high nucleic acid content HFC might represent intrathecal tumor cells. In this study, we evaluated the cellular origin and the diagnostic significance of HFC in CSF samples.

Methods: Sixty-five CSF samples with and 126 CSF samples without tumor cells were analyzed using the XE-5000 and manual microscopy of cytopsin preparations.

Results: The Sysmex XE-5000 detected HFC in 51 of 65 tumor cell-positive and in 33 of 126 tumor cell-negative CSF samples (sensitivity: 78.5%, specificity: 73.8%). The percentages of HFC and tumor cells in CSF samples correlated (r²=0.41, p<0.0001). Tumor cells escaped detection by the XE-5000 hematology analyzer especially in CSF samples with a low percentage of tumor cells.

Conclusion: We were able to identify tumor cells as the predominant correlate of HFC in CSF. Nevertheless, measuring HFC is not an appropriate diagnostic tool for the detection of intrathecal tumor cells. However, if HFC are incidentally detected in CSF samples using the XE-5000, further evaluation by CSF microscopy is mandatory.
P056

Evaluation of the IRIS iQ200 automated urine sediment analyser in routine laboratory operation

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Objective. To compare and evaluate the impact of iQ200 (Beckman Coulter) urine sediment analyser in routine laboratory analysis through comparison with manual microscopy and urine cultures.

Methods. A total of 1000 uncentrifuged samples positive from dipstick testing were analysed using the IRIS iQ200 over a period of six weeks. Results obtained from the analyser were compared against test strip results microscopy performed according to laboratory SOP. Particles that were evaluated included erythrocytes, leukocytes, bacteria, non-squamous epithelial cells and casts.

Results. Analytically the iQ was superior to that of the microscope being highly sensitive in the detection of erythrocytes and leukocytes with considerably more samples being found positive for cells through automation with specificity levels of 59 and 69% respectively. Both methods correlated well for erythrocytes (r=0.80) and leukocytes (r=0.83). Detection of bacteria by the iQ200 was not optimal with a sensitivity of 70% and correlation coefficient of 0.65. The APR software often failed in correctly classifying hyaline and pathological casts, however user re-classification of images resulted in relative sensitivities of 40 and 63% moderately correlated with microscopy with an average coefficient of 0.53.

Conclusion. The iQ200 should not directly replace microscopy but does allow for a significant decrease in the number of samples that required manual analysis. Implementation of an automated urinalysis system in routine operation improves precision, reduces the degree of variation in results and also through eliminating pre-analytic steps allows for more rapid turnaround times for samples.

P058

Evaluation of 6 Emergency Laboratory Tests on the PATHFAST® Analyzer for Point-of-Care Testing

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Background: The PATHFAST® system consists of an automated analyzer that uses single cartridges containing ready to use reagents for quantitative measurement in human whole blood (WB), serum or plasma within 16 min. We evaluated PATHFAST® cardiac troponin I (cTnI), hsCRP, myoglobin (Myo), CK-MB, NT-proBNP, and D-Dimer in comparison with Roche E 170 and Roche COBAS Integra 800.

Methods: Intra- and inter-assay imprecision were evaluated using BioRadLiquicheK Cardiac Markers Control, patient plasma and patient WB samples. Linearity, analytical and functional sensitivity, limit of blank (LoB) were determined by using predefined samples and zero calibrators. The method comparison was performed using patient samples comprising the whole measurement range.

Results: CVs of intra- and inter-assay imprecision ranged between 3.3% and 8.0%. All assays showed recovery between 91% and 105% and complete linearity across the total range. The LoB was determined by measurement of 10 replicates of the zero calibrator and of the lowest non-zero calibrator in parallel. Sample matrix evaluation was performed using WB and plasma samples. All assays showed high comparability between WB and plasma. The method comparison with Roche E 170 and Cobas Integra 800 revealed high concordance rates.

Conclusion: The evaluation of the PATHFAST cTnI, hsCRP, myoglobin, CK-MB, NT-proBNP, and D-Dimer revealed high concordance with the Roche E 170 and Cobas Integra 800 analyzer. Point-of-care testing on the PATHFAST® analyzer allows measurement of whole blood samples within 16 min after blood drawing in the point-of-care setting providing comparable results with the central laboratory.

P059

Influence of semi-automated sample mixing on hemoglobin concentration measurements at the Point of Care

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Objectives: Pre-analytical requirements for measuring hemoglobin (Hb) concentration include thorough mixing of samples. In core-laboratory instruments sample mixing is commonly automated whereas most point of care (POCT) devices leave sample mixing to the user. We compared Hb measurements using two sampling systems: one relying on manual mixing only and one offering a semi-automated mixing to the user under routine conditions of an emergency room.
Materials and Methods: We compared the Sarstedt Blutgas-Monovette® (B-M; manual mixing) to the Radiometer safe PICO® (S-P; semi-automated mixing). Each sampling system was used for 3 months in the emergency room as the routine sampling system. Hb measurements in the study periods were included if the core-laboratory Hb-result was released within 2 hours after the POCT Hb-result. Hb measurements were carried out on the Sysmex XE 5000 (core-laboratory) and the Radiometer ABL 90 Flex analyzer (POCT). Differences of more than 10% between these 2 measurements were evaluated.

Results: We included 3786 measurements, 1922 for B-M and 1864 for S-P. The correlation coefficient was 0.846 for B-M and 0.953 for S-P. 6.0% of the 2 Hb-results deviated more than 10% using the B-M system and 3.4% using the S-P system.

Conclusions: Semi-automated sample preparation can improve the pre-analytical phase for POCT Hb measurements. Important clinical decisions, e.g. blood transfusion, still should be based on fully automated sample preparation for Hb measurements.

P060

Ionized Calcium Concentration Measurements in Citrate Dialysis Samples

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Objectives: Ionized calcium (Ca²⁺) is often available at the point of care through blood gas analysis instruments. Due to recommendations of the manufacturer of citrate dialysis (Fresenius) these instruments are not only used for measuring Ca²⁺ in arterial and venous blood samples but also for monitoring and adjusting citrate dialysis by analyzing post-filter samples with high amounts of citrate and therefore extremely low Ca²⁺ concentrations (target range: 0.25-0.34 mmol/L). The available point of care Ca²⁺ methods are not designed nor evaluated for this purpose.

Materials and Methods: Five common blood gas instruments were used to measure Ca²⁺ concentrations in spare material from routine citrate dialysis samples. 100 samples from 20 different patients were included to calculate medians, regression functions and difference plots.

Results: For low concentrations Ca²⁺ medians of all investigated instruments ranged from 0.2 to 0.5 mmol/L but the differences between methods were constant. For Ca²⁺ concentrations above 1.0 mmol/L all methods correlated well without systematic differences.

Conclusion: For post-filter samples systematic differences in Ca²⁺ concentrations were larger than the delta of the recommended target range (0.09 mmol/L) or steps of the adaptation scheme for citrate flow (0.04 mmol/L). Recommendations of the citrate dialysis manufacturer need to be revised to ensure patient safety.

P061

Determination of the immunosuppressant Mycophenolic acid in human serum by immunoassay in comparison to HPLC

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Mycophenolate mofetil (MMF) is the morpholinoethyl ester of mycophenolic acid (MPA), which acts as an immunosuppressant mainly by inhibition of inosine monophosphate dehydrogenase, an enzyme that is necessary for the de-novo synthesis of guanosine nucleotides. Therapeutic drug monitoring, which is necessary due to the high inter-individual variability in the pharmacokinetics of MPA, can be performed with two methods: enzyme-multiplied immunoassay technique (EMIT) and high performance liquid chromatography (HPLC). From a theoretical methodology point of view, EMIT would be suspected to be less specific for the determination of MPA blood levels due to cross-reactivity with glucuronide metabolites of MPA, which is not the case for HPLC methods. In the presented study, we compared the immunoassay and two commercially available HPLC methods for quantitative measurement of MPA. MPA plasma concentrations were determined with the three different tests in 54 patients samples sent for quantitation of this drug to our institute. We found good correlations between MPA concentrations determined with the immunoassay method and each of the HPLC methods (adjusted R²=0.908 and 0.912, respectively). Even better correlation was found between MPA concentrations measured with the two HPLC tests (R²=0.99). MPA values measured with the immunoassay were higher compared to HPLC results in the same probes, likely due to cross reactivity with drug metabolites. In summary, we found the immunoassay to be a method, which enables quick measurement of MPA concentrations at good sensitivity. The HPLC method has been identified to be more time consuming and labor intensive, but to be more specific and accurate for therapeutic drug monitoring of MPA.
P062

Simultaneous quantification of 6 antibiotics in serum - Development and validation of a multi-analyte isotope dilution 2D-UHPLC-MS/MS method

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Background: Recent studies have demonstrated highly variable blood concentrations of piperacillin, tazobactam, cefepime, meropenem, ciprofloxacin and linezolid in critically ill patients with a high incidence of sub-therapeutic levels. Consequently, therapeutic drug monitoring (TDM) of these antibiotics has to be considered, requiring robust and reliable routine analytical methods. The aim of this work was to develop and to validate a multi-analyte UHPLC-MS/MS method for simultaneous quantification of the antibiotics mentioned above.

Methods: Sample preparation included a manual protein precipitation step followed by 2D-UHPLC. Stable isotope labelled internal standards were used for all analytes except for tazobactam. The injected sample volume was 7 μl, the run-time was 5.0 min.

Results: Inaccuracy was ≤ 8% and imprecision (CV) was < 9% for all analytes. Only minor matrix effects and negligible carry-over was observed. The method was found to be robust during the validation period.

Conclusions: We were able to develop a reliable UHPLC-MS/MS method addressing analytes with very heterogeneous physico-chemical properties. The novel assay may be an efficient tool for TDM of important antibiotics.

P063

Characterization of 3-dimensional organoid liver structures generated in vitro using differentiated human cells

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3D structures resembling embryonic liver buds have recently been described using stem cell-derived cell types (Takebe et al., 2013). Such structures can be transplanted into animals/patients but may also be useful for applications like ex vivo drug toxicity tests. We here used differentiated, upcyte® cells which are derived from primary human cells that underwent targeted genetic modification (upcyte® process) in order to transiently induce or extend proliferation resulting in mortal, but expendable, differentiated cells. upcyte® cells can undergo up to 40 population doublings, and when contact-inhibited they differentiate into functional cells while maintaining most of their specific characteristics.

We used a defined combination of human upcyte® cells (hepatocytes, liver sinusoidal endothelial cells (LSECs) and mesenchymal stem cells (MSCs)) that spontaneously formed organoids in vitro which were then cultured in a matrigel-coated bioreactor (kirkstall® quasi vivo system). These self-organized, liver-like structures harbor healthy, living cells showing typical functional characteristics of liver parenchyme, including basal as well as drug-induced activity of Cyp3A4. By immunohistochemistry we found that the cells formed a typical liver-like architecture. We hereby demonstrate that 3D functional liver structures can be generated in vitro using upcyte® cells and that these “mini-livers” are useful models to study human liver functions ex vivo for acute and long-term studies.

P064

Variability of linezolid concentrations after standard dosing in critically ill patients: a prospective observational study

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Background: Severe infections in ICU patients show high mortality rates. An adequate and effective antimicrobial therapy is therefore crucial for these patients. Here we aimed to evaluate whether standard dosing of linezolid leads to therapeutic serum concentrations in critically ill patients.
Methods: In this prospective observational study, 30 adult ICU patients with suspected infections received standard dosing of 600 mg linezolid i.v. twice a day. Over 4 days, multiple serum samples were obtained from each patient for determination of linezolid concentrations by LC-MS/MS.

Results: A high inter-patient variability of serum linezolid concentrations was observed (range of area under the linezolid concentration time curve over 24 h (AUC_{24}) 50.1-454 mg/L, median 143 mg*h/L; range of trough concentrations (C_{min}) <0.13-14.5 mg/L, median 2.06 mg/L). Furthermore, potentially subtherapeutic linezolid concentrations over 24 h and at single time points (defined according to literature and based on minimal inhibitory concentrations of relevant bacteria as AUC_{24} <200 mg*h/L and C_{min} <2 mg/L) were observed in 63% and 50% of the patients respectively. Potentially toxic levels (defined as AUC_{24} >400 mg*h/L and C_{min} >10 mg/L) were observed in 7% of the patients.

Conclusions: These findings suggest that therapeutic drug monitoring of linezolid for critically ill patients might be important for individualisation of linezolid dosing, leading to optimized antimicrobial therapy for these patients.

P065

Improved agomelatine therapeutic drug monitoring by inclusion of the metabolite hydroxy-agomelatine

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Objectives: Agomelatine is an antidepressant drug with a novel melatonergic mechanism of action. Agomelatine has a short plasma half-live (1.2h), is primarily metabolised by CYP1A2 and about 80% of the drug is excreted in the urine as metabolites (1). Monitoring of compliance is hampered by non-detectable day-time blood levels after once-daily bedtime dosing. We reasoned that monitoring of metabolites can significantly extend the detection window.

Methods: Agomelatine was measured after glucuronidase-incubation, addition of methabenzthiazurone as internal standard (IS), ether extraction and chromatographic separation on a C18 column by mass spectrometry (API 4000). The main metabolite OH-agomelatine was identified by its molecular mass with a mass shift of 16 Da. A peak area ratio OH-agomelatine/IS <0.01 was considered negative.

Results: Before metabolite monitoring was introduced only 393/1409 samples (27.9%) had results above the LOQ (>0.1 μg/L), median 0.3 μg/L, 5.-95. percentile 0.1-9.9 μg/L. A significantly higher (p<0.001) proportion of samples (640/851 [75.2%]) had positively confirmed drug exposure after introduction of the OH-agomelatine metabolite monitoring.

Conclusion: Medication compliance was proven in 75% of patients after introduction of agomelatine metabolite monitoring compared to 28% before. Inclusion of metabolites in LC/MS analytical runs is of particular value for drugs with short half-live.


P066

A spectral library based LC-MSn approach for comprehensive drugs of abuse screening in urine

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We present an ion trap LC-MSn method using full scan MS, MS2 and MS3 spectra from positive and negative polarity including retention time matching for a broad automated research screening based on spectral libraries (ca. 900 drugs) in comparison to an immunoassay/triple quad workflow.

For the evaluation of the method 4 calibrator, 2 controls and 5 urine samples were analyzed. Calibrator and controls show that drugs with concentrations ≥10 ng/ml were typically detected; one exception is amphetamine that requires a concentration of 50 ng/ml. For the urine samples the combined immunoassay/LC-QqQ approach led to the identification of 1-4 drugs per sample whereas the LC-MSn research screening revealed 4-12 more drug identifications (overall 7-20 IDs/sample). Among the compounds that were not detected by the immunoassays forensically relevant drugs like doxepin and tramadol could be identified. Furthermore, metabolites such as ecgoninemethylester or desmethyl-citalopram were detected which give an additional level of confidence for the identification of the corresponding drugs. In summary, the LC-MSn research screening provides a more complete picture of DOAs in urine samples compared to the IA/LC triple-quad workflow. It offers fast and flexible routine identification and confirmatory analysis in one run. Moreover, the open library concept simplifies the addition of new compounds for fast method adjustment which is particularly important in the field of designer drugs.
Cardiovascular Disease

P067

Presepsin and ST2 as Prognostic Markers in Acute Heart Failure

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Background: Presepsin (sCD14-ST) is released after conversion of CD14++ monocytes into CD14+/16+ monocytes after M-CSF (macrophage colony stimulating factor) activation and can be detected in the blood. Presepsin has proven as sepsis marker and may play a role in heart failure as monocyte TLR4 expression has been shown to be increased in this condition.

Objective: ST2 is a member of the interleukin-1 receptor family. Plasma concentrations of ST2 are increased in acute heart failure. Aim of our study was to evaluate the diagnostic and prognostic value of presepsin in patients with acute heart failure in comparison to ST2.

Methods: ST2 and presepsin concentrations were measured in base-line plasma samples obtained from 60 patients (50 to 90 years old, median 77 years; 26 females, 34 males) with acute heart failure attending the emergency department (ED). Outcome measure was mortality at 2 years. ST2 was measured using the Presage ST2 assay (Critical Diagnostics, San Diego, CA, USA). Presepsin was determined using the PATHFAST assay (Mitsubishi). NT-proBNP, hscTnT and CRP were measured using the ELECSYS tests (Roche Diagnostics).

Results: During the 2years follow up 25 patients (41.7%) died. The marker levels differed significantly between the groups. ROC analysis revealed AUCs of 0.83, 0.67, 0.65, 0.64, and 0.61 for presepsin, ST2, hscTnT, CRP, and NT-proBNP, respectively.

Conclusion: Presepsin was found to be the best prognostic marker in acute heart failure. ST2 and hscTnT provided also prognostic information. The data provide new information on the pathogenesis of acute heart failure and may improve therapeutic approaches in the future.

P068

Development of a fully automated assay to quantify Lp-PLA2 activity in biological samples

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Background: Inflammation is a key event in pathogenesis of atherosclerosis. Lp-PLA2 contributes to atherosclerotic plaque instability by promoting inflammatory processes. A meta-analysis of around 80,000 individuals from 32 prospective studies showed that Lp-PLA2 activity levels are closely linked to increased risk of coronary heart disease, stroke, and vascular death. DiaSys presents a liquid-stable reagent to determine Lp-PLA2 activity on automated systems. A specific substrate for Lp-PLA2 is hydrolyzed in the reaction and subsequently converted to 4-nitrophenol, quantified at 415 nm.

Methods: Results presented are based on the photometric measurement of the vascular specific inflammatory enzyme Lp-PLA2. Analytical performance was evaluated on a fully automated clinical chemistry analyzer according to the CLSI protocol. Reagents, calibrators and controls were from DiaSys GmbH.

Results: Performance data were determined for serum and plasma. Method comparison, performed against competitor activity test on Hitachi 917 (range: 203–1553 U/L, n=97), demonstrated highly correlated results (y=0.909x–4.28 U/L; r=0.999). Data have been evaluated by using regression analysis according to Passing and Bablok. The test covers a wide measuring range and shows outstanding intra-assay precision with a CV of <0.72%.

Conclusions: DiaSys Lp-PLA2 FS shows excellent performance characteristics for recommended sample material. The test correlates well to competitor assays. The new reagent provides fast, precise and convenient measuring of Lp-PLA2 on any clinical chemistry analyzer. Determination of Lp-PLA2, facilitates reliable identification of patients at increased risk for cardiovascular events.

P069

Modulation of atherogenesis by adaptive immunity in BALB/c mice

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In our study, immunogenic genesis of atherosclerosis was studied in immunodeficient transgenic mice of the Th2-prone BALB/c background, which represent a suitable model for the generation of humanized mice. BALB/c-Ldlr<sup>−/−</sup> (C-Ldlr<sup>−/−</sup>) mice were used as controls. We generated two immunodeficient strains, referred to as C-Ldlr<sup>−/−</sup> Rag1<sup>−/−</sup> and C-Ldlr<sup>−/−</sup> Rag1<sup>−/−</sup> Ifngγ<sup>−/−</sup>, which show severe combined B- and T-cell immunodeficiency. The second strain additionally has a complete loss of NK cells because of γ-chain inactivation. All mice were fed an atherogenic Western type diet (WTD) for 12 or 24 weeks, respectively. Serum cholesterol of both immunodeficient mice was significantly increased compared to controls, respectively. The atherosclerotic lesion development and composition were quantitatively analysed. Double (73861±49358 µm²) and triple (56698±3167 µm²) mutants developed significantly more atherosclerosis after 24 weeks on WTD as indicated by plaque area of aortic sinus, compared to controls (21829±14820 µm², p<0.01). Triple mutants show significant increase of lesional macrophages ((Mø 40,1±6,4%, p<0.01) and a decrease of smooth muscle cells (SMCs 9,6±4,7%, p<0.01) compared to controls (Mø 31,6±6,7%, SMCs 17,7±7,4%) and double mutants (Mø 31,1±8,8%, SMCs 16,1±7,0%). Summery, a combined B and T cell immunodeficiency in a Th2-prone BALB/c background significantly accelerates atherosclerotic lesion progression. Furthermore, combined immune and γ-chain defects have the potential to change atherosclerotic cellularity to a more unstable phenotype.

P070

Determination of an European 99th Percentile Upper Reference Limit with ARCHITECT STAT high sensitive Troponin-I Assay

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Introduction: The Third Universal definition of myocardial infarction (MI) recommends the use of the 99th percentile upper reference limit (URL) of troponin as the medical decision point. The concentration at this decision point is dependent upon the troponin assay as well as the population measured. The ARCHITECT STAT high sensitive Troponin-I assay was recently introduced to the European market and the goal of this study was to determine the URL based on healthy populations from five European countries.

Methods: This study was approved by local Ethics Committees/Investigation Review Boards in Belgium, France, Germany, Norway and Poland. Subjects completed health questionnaires and blood samples were collected for troponin-I testing; in addition, some subjects also had BNP, HbA1c and/or creatinine results available. These additional biomarkers were used The 99th percentile URL were calculated by the non-parametric method.

Results: The study population was divided into “apparently healthy” (n=1368) based on health questionnaires only and “healthy” (n=636) based on additional biomarker results. The overall 99th percentile of the apparently healthy population was 23.7 pg/mL and 11.2 pg/mL for the apparently healthy population. Additional analysis revealed both and age and gender effect, with males demonstrating higher values than females and older subjects (over 60 years of age) having higher values.

Conclusion: 99th percentile URLs were determined for a European population with the new ARCHITECT STAT high sensitive troponin-I assay that were dependent on gender and age and also differed based on the qualification of the subjects.

P071

Plasminogen activator inhibitor-1 (PAI-1) - a possible vascular risk indicator in obesity

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Among obese men, the vascular risk varies widely. Cardiovascular morbidity and mortality increase in cases with metabolic syndrome. Besides its role in control of fibrinolytic activity, PAI-1 is an important regulator of extracellular matrix turnover, tissue remodeling and fibrosis. Visceral fat has been identified as an important source of PAI-1 in humans. We hypothesized that PAI-1 plasma activity might be a possible indicator for the differentiation between metabolic inert and high risk obesity as driving force in metabolic syndrome resulting in progressive cardio-vascular disease. PAI-1 activity (chromogenic assay) and fasting concentrations of triglycerides, glucose, uric acid and HDL-cholesterol (ROCHE Hitachi 717) were measured in 615 obese men (BMI > 25 kg/cm²; mean age 46.6 ± 10.1 years) from DRECAN study. According to PAI-1 activity individuals were divided into tertiles. The PAI-1 activity in the lower tertile was < 3.08 and in the upper tertile > 5.22 U/mL. As compared
to the lowest tertile men in the upper tertile showed significantly higher values for BMI, fasting triglycerides, HDL-C, uric acid (p < 0.0001), diastolic (p < 0.001) and systolic blood pressures and fasting glucose (p < 0.05). Among men with BMI > 30 kg/cm² established differences could be confirmed at a lower level of significance for BMI, HDL-C and fasting triglycerides only. Conclusion: PAI-1 seems to be a possible marker for calculation of the vascular risk in mild obesity.

P072
Validation of fatty acid-binding protein 4 (FABP4) as prognostic and diagnostic biomarker for coronary artery disease

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Measurement of blood biomarkers as a direct estimate of atherosclerotic burden has great potential to improve clinical management of coronary artery disease (CAD). By integrating data from transcriptomic analysis of coronary thrombi and from proteomic analysis of secretomes derived from atherosclerotic plaques (Cooksley-Decasper et al. 2012), we identified fatty acid-binding protein 4 (FABP4) as a potential biomarker candidate for CAD. The FABP4 expression in atherosclerotic plaques was previously associated with the occurrence of adverse secondary cardiovascular events (Peeters et al. 2011). Circulating FABP4 levels were measured in an asymptomatic population-based cohort (N=414) and in two clinical cohorts of patients with stable CAD or acute coronary syndrome (ACS; N=820 and N=200) to validate its potential as a prognostic and diagnostic biomarker.

The FABP4 levels showed no association with cardiovascular events in the prospective population cohort with five years of follow-up. In the clinical cohort, circulating FABP4 levels were higher in patients with myocardial infarction as compared to the asymptomatic controls. However, FABP4 levels considerably overlapped between these groups. Moreover, FABP4 was strongly influenced by many confounders like sex, age, body mass index, or diabetes mellitus. Interestingly, substantially elevated FABP4 levels were associated independently of age, sex, and BMI with incidence of cardiac death during 30 days of follow-up after primary ACS event. In summary, FABP4 appears to have very limited potential as diagnostic biomarker for ACS or predictive risk factor in the asymptomatic population. However, FABP4 may serve as prognostic biomarker of cardiac death after primary ACS.

P073
Neutrophil gelatinase-associated lipocalin (NGAL) in heart transplant recipients after conversion to everolimus therapy

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Background: Due to lack of nephrotoxic activity proliferation signal inhibitors (PSI) such as everolimus are recommended for the immunosuppressive therapy after heart transplantation, but the assessment of renal function in patients receiving PSI led to conflicting results. We here evaluated kidney integrity and function using conventional markers (urine albumin and α₂-microglobulin (α₂M), plasma creatinine and eGFR) along with neutrophil gelatinase-associated lipocalin (NGAL) in heart transplanted patients, who underwent conversion to everolimus or were maintained on therapy with calcineurin inhibitors (CNI).

Methods: 44 patients on everolimus and 77 patients on CNI were included in the study. Renal parameters were determined in plasma and urine using standard enzymatic or immunochromatographic methods.

Results: Significantly lower NGAL concentrations were found in plasma and urine from heart transplant recipients on everolimus. By contrast, no significant differences were seen between everolimus- and CNI-treated groups with regard to creatinine and eGFR as well as urine albumin and α₂M. Significant correlations were noted between plasma NGAL and creatinine, eGFR, NT-proBNP and chronic inflammation indicators (sCD40L, lipoprotein-associated phospholipase A₂ (Lp-PLA₂)) and between urinary NGAL and α₂M. Creatinine and Lp-PLA₂ predicted plasma NGAL upon multiple regression analysis.

Conclusion: The present study documents reduced plasma and urinary NGAL levels in absence of differences in conventional renal parameters in course of CNI-free immunosuppressive therapy with everolimus. These results are consistent with favorable sub-clinical effects of everolimus on renal integrity in heart transplant recipients.
P074

Acvr1c is a Novel Candidate Gene for Atherosclerosis and Adipose Tissue Mass on Mouse Chr2

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Objectives: The aim of the present study was to identify causal genetic variants for atherosclerotic lesion size and white adipose tissue (WAT) mass at a quantitative trait locus (QTL) on chromosome 2 previously identified in an F2-intercross of atherosclerosis-susceptible C57BL/6 (B6) and atherosclerosis-resistant BALB/cByJ (BALB) mice on the LDL-receptor deficient background.

Methods: QTLs for both phenotypes were validated in an independent intercross of F2 mice (n=245). Genetic differences between strains were identified by next generation sequencing. Expression QTL mapping of candidate genes was performed in F2 WAT (n=242) and validated in F0 mice, followed by functional analysis of mechanisms of atherogenesis using RNAi.

Results: A nonsense mutation in Acvr1c was identified in BALB mice co-segregating with QTLs of atherosclerosis, WAT mass and differential expression of Acvr1c in WAT of F2 mice. Tissue expression profiling in parental F0 mice validated significantly decreased Acvr1c expression in BALB mice and suggested a potential role of Acvr1c-mediated signaling in WAT, brown adipose tissue and aorta. Truncated Acvr1c affected Smad-signaling and trans regulated lipases in WAT.

Conclusion: Acvr1c was identified as plausible candidate gene of atherosclerosis-susceptibility and WAT mass on mouse Chr2 likely through Smad-mediated trans regulation of pro-atherogenic gene expression. Results of the current study provide a molecular link between atherosclerosis and obesity.

P075

Targeting Protein Tyrosine Phosphatase-1 (PTP1B) improves cerebral arteriogenesis in the hypoperfused rat brain

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Collateral growth, arteriogenesis, refers to rapid proliferation of preexisting arterioles to fully functional arteries, thereby compensating circulatory deficits. Thus, improving arteriogenesis is considered a promising treatment approach in arterial occlusive disease. In a rat model of adaptive cerebral arteriogenesis (occlusion of one carotid and both vertebral arteries; three-vessel occlusion, 3-VO) the impact of inhibition of protein tyrosine phosphatases (PTPs) on collateral growth and function were assessed, focusing on PTP1B. 3-VO was followed by increased cerebral arteriogenesis, with most significant changes in the ipsilateral posterior cerebral artery (PCA), with proliferative markers being time-dependently increased. The cerebrovascular reserve capacity (CVRC) was lost in the early phase after 3-VO and partially recovered after 21 days. In addition, Sprague Dawley rats underwent 3-VO surgery and were treated with the pan-PTP inhibitor BMOV , or a PTP1B inhibitor. PTP inhibition and PTP1B antagonism enhanced both the growth of the PCA and the CVRC. Furthermore, hyperphosphorylation of the platelet-derived growth factor (PDGF)-β receptor in the vascular wall in situ was detected by a single recognition approach (proximity ligation assay), suggesting a major impact of PTP1B inhibition on receptor tyrosine kinase phosphorylation and signaling. Finally, in vitro tyrosine-phosphorylated PDGF-β receptor was reduced by recombinant PTP1B. Taken together, PTP1B represents a negative regulator in cerebral arteriogenesis. Furthermore, PTP1B inhibition leads to enhanced collateral growth and blood perfusion, suggesting PTP1B as novel target ameliorating ischemic vascular disease.

P076

ABCC6- a new player in cellular cholesterol and lipoprotein metabolism?

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Background: Alterations of lipid metabolism and soft tissue calcification are linked pathologies. Pseudoxanthoma elasticum (PXE) is a rare, autosomal-recessive disease and proposed as a model for soft tissue calcification disorders, e.g. arterial calcification.

Methods: We analyzed the gene expression profile, enzyme activity and protein expression of selected targets involved in cholesterol and lipoprotein metabolism in dermal fibroblasts derived from patients and healthy controls.
**Results:** Twelve genes (ANGPTL3, APOD, APOLI, CEL, CYP39A1, HDLBP, LRPB1, PCSK9, TM7SF2, TRERF1) were induced, whereas mRNA expression of six genes (APOE, APOF, APOL5, CXCL16, NPC1LI, ORLI) was decreased, comparing controls and PXE patients (with at least 2-fold changes). Quantitative real-time PCR verified most of the results obtained by array analysis, most notably in the cholesterol biosynthesis pathway (HMGCR, FDP5, LSS, TM7SF2, DHCR7) and in lipoprotein metabolism (APOE, APOD, APOLI). We detected significantly increased HMGCR activity in PXE fibroblasts. Elevated PCSK9 transcript levels were confirmed similarly on PCSK9 protein level. Interestingly, transcript levels of ABCC6 were strongly increased under culture conditions known to induce cholesterol biosynthesis (serum starvation and lipoprotein depletion).

**Summary:** Conclusively, an aberrant cholesterol and lipoprotein metabolism is suspected to enforce soft tissue calcification in PXE. Therefore, we suppose a functional role for ABCC6 in human lipoprotein and cholesterol homeostasis. Uncovering the physiological function of ABCC6 will help to understand the molecular mechanisms underlying more common disorders characterized by soft tissue calcification.

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**Inflammation**

**P077**

**Del-1 regulates murine pathological angiogenesis and revascularization processes**

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Progenitor cells (PC) and inflammatory cells (IC) are recruited to ischemic tissues and improve neovascularization (NV). Beta2-integrins are essential for adhesion and homing of PC to ischemic tissues. Developmental Endothelial Locus-1 (Del-1), an extracellular matrix protein that binds beta2-integrins, inhibits homing of IC as shown previously (Choi et al., Science 2008). We here investigated the role of Del-1 in pathological angiogenesis, in the model of retinopathy of prematurity (ROP) and in revascularization of ischemic muscles. In the ROP model, we observed increased pathological NV due to Del-1-deficiency, associated with increased expression of the macrophage marker F4/80. In addition, Del-1−/− mice showed an increased angiogenic response in ischemic muscles compared to wild type (WT) mice in the model of hind limb ischemia, which was associated with higher infiltration of CD45+ cells. To further study the role of Del-1 for in vivo homing of PC, we iv injected murine fluorescence-labeled WT Lin− bone marrow (BM) PC in WT and Del-1−/− mice 2 days after the induction of hind limb ischemia. Interestingly, the homing of injected Lin− cells to ischemic muscles was increased in Del-1−/− compared to WT mice. Moreover, we found that soluble Del-1 inhibited the adhesion of PC to endothelial monolayers and to the major beta2-integrin ligand, ICAM-1. In addition, we observed that WT murine BM mononuclear cells displayed higher adhesion rates on Del-1-deficient murine lung endothelial cells (LEC) than on WT LEC. In summary, Del-1 blocks beta2-integrin-dependent adhesion and homing of PC and IC to ischemic tissues, resulting in a reduced ischemia-induced NV.

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**P078**

**DHEA: an endogenous regulator of inflammatory cell recruitment**

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Dehydroepiandrosterone (DHEA) is the most abundant circulating steroid hormone in humans and its concentration declines with age. DHEA has been implicated as a regulator of immunity. In the present study we investigated the potential role of DHEA in leukocyte recruitment. To examine the direct effect of the DHEA rather than of its derivatives (androgens, estrogens), we used a non-metabolized analogue of DHEA, the synthetic steroid BNN27. To investigate the role of DHEA on leukocyte recruitment in vivo, we engaged the LPS induced acute lung inflammation model in mice and performed intravital microscopy by using the cremaster model. The in vitro effect of DHEA on the expression of adhesion molecules in endothelial cells as well as on β2 integrin–dependent leukocyte adhesion was also evaluated. DHEA pre-stimulation led to impaired LPS-induced leukocyte adhesion efficiency but increased rolling velocity as well as prevented the LPS-induced drop of peripheral white blood cells. In the lung inflammation model, pretreatment with DHEA or BNN27 displayed lower accumulation of neutrophils in the inflamed lung. All these indicate that DHEA influences integrin dependent leukocyte adhesion and their consequent extravasation. In vivo experiments revealed that DHEA can inhibit leukocyte adhesion, β2 integrin activation and endothelial ICAM-1 expression. Our results show the anti-inflammatory effect of DHEA and BNN27 through inhibition of leukocyte adhesion. We also propose that the synthetic steroid BNN27 is a potential novel anti-inflammatory therapeutic agent.
P079

Hif2alpha in myeloid cells regulates angiogenesis in proliferative retinopathy

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Retinal hypoxia is the main trigger for pathological neovascularization (NV) in proliferative retinopathies (PR). Synergisms between hypoxia, angiogenesis and innate immune response have been suggested, which could be integrated at the level of the hypoxia-inducible factors (HIF). HIF2α is expressed highly in endothelial and myeloid cells. Thus we studied the role of myeloid cell HIF2α for pathologic NV in the course of PR. We engaged myeloid specific HIF2α ko mice in the model of Oxygen Induced Retinopathy (OIR), which mimics the PR in diabetes. In the OIR model we observed significantly reduced NV due to myeloid HIF2α deletion, whereas physiological retinal vascular development was not altered. Immunohistochemistry and flow cytometry analysis revealed higher numbers of myeloid cells/microglia in myeloid-specific HIF2α ko mice in the course of the OIR model, which was due to increased proliferation rate of HIF2α deficient microglia cells in the retina. Moreover, proangiogenic M2 polarization of primary mouse microglia and of the BV2 microglia cell line was reduced by HIF2α deficiency. Whereas endothelial cell (EC) proliferation in the retina of OIR mice was not affected by HIF2α deficiency in myeloid cells, we observed increased EC apoptosis. This result could account for the reduced NV in the OIR in myeloid HIF2α ko mice, and ongoing experiments aim at clarifying the molecular mechanism leading to EC apoptosis. Our study indicates that myeloid HIF2α regulates myeloid cell polarization and thereby pathological NV in PR.

P080

MPA modulate tight junction through influencing MLCK and MLC-2 promoter activity

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**Introduction:** Mycophenolic acid (MPA) is an important immunosuppressive drug that prevents graft rejection, but is also associated with serious adverse event like sporadic gastrointestinal (GI) disturbances in the transplanted patients. Recently, we reported a MPA-mediated increased in expression of MLC2 and MLCK at both transcriptional and protein levels together with re-localization of TJ proteins after MPA treatment. Here we investigated the promoter activities of MLCK and MLC-2 genes after MPA treatment.

**Methods:** Caco-2 cells were grown to establish TJ and treated with MPA and DMSO (Control). Transepithelial electrical resistance (TEER) and FD4 influx were measured to confirm the TJ formation and regulation by MPA and DMSO. In parallel, chromatin immunoprecipitation (ChIP) was performed and precipitated DNA was amplified by real time PCR using primers, designed against the promoter region of the genes related to TJ.

**Results:** The MPA treatment decrease TEER in a time dependent manner and produced 2.6 fold increase in FD4 influx across the 16-21 days Caco-2 monolayer as compared to the control. ChIP analysis showed an increase activation of 2.87 and 1.13 fold of MLCK and MLC-2 gene promoter respectively in MPA treated Caco-2 cells. Decrease in repression 1.78 and 1.05 fold of MLCK and MLC-2 gene promoter respectively was also observed in the MPA treated cell as compare with untreated Caco-2 cell monolayer.

**Conclusion:** Results indicate an activation of MLCK and MLC-2 genes in the MPA treated cell which may consequently disrupt the integrity of Caco-2 monolayer by redistributing ZO-1 and Occludin proteins of TJ. Further investigation can help to design the appropriate interventions to save the patients from unwanted adverse effects of MPA.

P081

Elevated serum aldolase activity in combination with normal serum creatine kinase activity

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The most useful enzymes in diagnosis and monitoring of inflammatory disorders of muscle are serum creatine kinase (CK) and aldolase. Typically the determination of CK is used, but it is known that 5-20% of cases of dermatomyositis and polymyositis can present with a normal CK
activity. We performed a retrospective review of all adult patients from 2011 to 2014 who had parallel orders of serum CK and aldolase activity and found 9 patients who had serum CK within reference range but an elevated serum aldolase activity. Diagnoses were myositis (3), muscular dystrophy (1), dermatitis vesicularis (1), vasculitis (1), antisynthetase-syndrome with polyarthritis (1), polymyalgia rheumatica (1) and capillary leakage syndrome (1). Among the 3 myositis-patients the aldolase activity ranged from 0.19-0.21 μmol/sxl (upper limit of reference interval: 0.12 μmol/sxl). Neither the current guideline for myositis nor those for diagnosing myopathy in general consider the aldolase activity. According to the current literature however our data support the possible role of aldolase activity within the laboratory program. The issue needs to be addressed with an extended number of investigations.

P082

Analysis of the stimulatory potential of secreted and surface proteins from Streptococcus galloyticus subsp. galloyticus

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Introduction: Streptococcus galloyticus subsp. galloyticus (SGG) is an emerging pathogen in about 10% of infective endocarditis cases. In this study, surface and secretory proteins from SGG were analyzed on strain dependence and stimulatory potential.

Methods: Peptides from SGG were made by digestion with trypsin, proteinase K (shaved) or without a protease (shed). These peptides were separated by SDS-PAGE and stimulated the monocyte cell line THP-1. IL-6 concentration and cytokine gene expression (IL-1β, IL-6, IL-8, MCP-1, MIP-1β, TNF-α) were measured.

Results: SDS-PAGE showed typical patterns of bands from peptides for every SGG isolate. The data of monocyte stimulation demonstrate differences in the potential from peptides of different SGG isolates to cause IL-6 secretion with similar tendency in gene expression of cytokines. Peptides of the isolate 010672 did not stimulate monocytes. Marginal increases in IL-6 secretion were seen after stimulating with peptides from LMG17956. Peptides from BAA2069 stimulated THP-1 cells up to 6.7 times higher. Protease shaved peptides from UCN34 led to similar results. The highest stimulation was induced by shed proteins from UCN34 (up to 87 times higher than control).

Conclusion: Surface peptides and secreted proteins are distinct between different isolates of SGG. Proteins of SGG can be sufficient to cause an immune reaction. It seems that the native conformation of proteins is crucial for recognition by monocytes.

P083

TNF tolerance of human monocytes is regulated by A20-dependent mechanisms

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Objective: TNF tolerance, characterized by reduced sensitivity towards TNF stimulation following TNF pre-exposure, is considered as protective in sepsis and inflammation but may also represent a paradigm for immunoparalysis. The molecular basis of TNF tolerance is only poorly understood.

Methods: Using primary human monocytes and THP-1 cells, TNF tolerance is measured via IL-8 mRNA or IL-8 promoter-driven reporters as a read-out. Mechanisms were further analysed by siRNA, western blot, inhibitors, and overexpression experiments.

Results: We detected two forms of TNF refractoriness in human monocytes: absolute tolerance as a selective form, affecting a small group of powerful effector molecules and induction tolerance as a more general phenomenon. Following preincubation with TNF, a dose dependent blockade of 1xB-α proteolysis/phosphorylation and increased 1xB-α protein degradation were detected. Phosphorylation of p65 was dose-dependently inhibited on Ser536, and levels of p50 were increased under tolerance. Most important, we identified A20 as a key player for the induction of TNF tolerance and controlling TNF signaling cascades. In addition, interferon-dependent modulation of TNF signalling via A20 and cross tolerance towards PMA stimulation were observed.

Conclusions: Taken together, our results demonstrate that A20-dependent mechanisms induce TNF tolerance restricting TNF-induced signaling, potentially playing a role in the resolution of inflammation.
Automated Detection of Fecal Calprotectin with the new Phadia Calpro EliA – Comparison with the Established Bühmann Microtitre Assay

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Fecal calprotectin (migration inhibitory factor related protein; MRP 8/14) indicates leucocyte invasion into the gut lumen. It helps to distinguish the irritable bowel syndrome (IBS) from inflammatory bowel disease (IBD) and can be used for diagnostic, prognostic and therapy monitoring purposes. Until recently, mainly microtitre assays from several manufacturers were available. In our study, we compared our routine assay (Bühmann) with the new Phadia Calpro EliA designed for the automated Phadia 100/250/1000 series technology. 335 fecal specimens from healthy and clinical children (age range 0 – 17, mean 4.3 years) sent to our laboratory during the last 15 months were homogenized, weighed-in, extracted and tested in parallel due to the manufacturer’s recommendations. Overall we observed a satisfying congruence in results ($r > 0.80$), but with remarkable divergences in a number of single samples, partially depending on the level of measured values. Specimens below 100 mg/kg and thus near to the commonly accepted cutoff value (50 mg/kg) were more divergent ($r = 0.55$) than those with higher values. In the absence of a reference method or international standardization, there remains uncertainty about the “true” values. While the qualitative decision of no, middle or strong evidence for inflammation leads to similar answers with both assays, follow up samples with the need for more accuracy should always be examined with the same method. The possible need for individual cutoff values for each assay has to be taken into account for further studies. With this in mind, the Phadia Calpro EliA proved a reliable, quick and easy to perform automated method for the detection of calprotectin in human feces.

Heterotropic Modulation of Selectin Affinity by Allosteric Antibodies Affects Leukocyte Rolling

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During inflammation and immune surveillance leukocytes have to tether to and roll on the vascular endothelium. These first steps are mediated by the adhesion receptors of the selectin family, which are designed for efficient leukocyte tethering under shear conditions. To withstand premature bond disruption, selectin adhesiveness is enhanced by tensile forces that promote the conversion of a bent into an extended conformation of the extracellular domain. In contrast to integrins, nothing is known about conformation-specific antibodies for selectins. By force-free affinity assay, flow chamber and microkinetic studies, we could show that, in contrast to the anti-human L-selectin mAbs DREG-200, DREG-56 or LAM1-1, the mAbs DREG-55 and LAM1-5 dramatically reduce L-selectin-mediated lymphocyte rolling velocity under shear, consistent with the selective binding to a high-affinity conformation of L-selectin. These data introduce a new concept of heterotropic modulation of selectin affinity and demonstrate how such anti-selectin mAbs can provide further insights on the structure-function relationship. Furthermore, these results highlight the need for a new classification of anti-selectin mAbs according to their distinct stimulatory or inhibitory potential.


The Role of Metalloproteinase Adam17 in Atherosclerosis

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Objectives: The metalloproteinase Adam17 is a major sheddase of membrane-bound proteins, such as TNFα, TNF-receptors I and II (TNFRI, TNFRII). The current study aimed to investigate the influence of Adam17 deficiency on atherosclerosis.

Methods: Since mice with complete deficiency of Adam17 are not viable, we used Adam17 hypomorphic mice with ~5% residual Adam17 activity (Adam17<sup>−/−</sup>). Mice were bred onto the LDL-receptor-deficient background and atherosclerosis was quantified at the aortic root.
Genome-wide expression and pathway analyses were performed in livers, aortas, and bone marrow-derived macrophages (BMDM). Adam17 substrates TNFα, TNFRI and TNFRII were measured in plasma and supernatants of BMDM.

**Results:** Adam17 deficiency was associated with changes of atherosclerosis susceptibility (p=0.04) and altered aortic valve morphology. TNFα, TNFRI and TNFRII concentrations were significantly lower in plasma (p<0.05) and supernatants of BMDM (p<0.001) of Adam17ex/ex compared to ADAM17wt/wt mice. Pathway analyses predicted effects of Adam17 deficiency on inflammation, apoptosis and proliferation, which were validated in functional studies in primary cells and RAW264.7 cells using RNAi.

**Conclusion:** In this study, we demonstrate a role of Adam17 in mechanisms of atherosclerosis, likely mediated through different release of TNFRI and TNFRII. These findings are important due to the development of Adam17 inhibitory drugs and provide a plausible role of Adam17 in atherogenesis.

**P087**

**Cellular proteomics ("cytomics") for early detection and risk stratification in sepsis**

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Transcriptomic profiling of peripheral blood leukocytes has revealed major differences between patients with sepsis and SIRS. It is likely that these changes might reflect or cause changes in protein expression, signaling cascades and effector function, which can be directly measured on a single cell level by flow cytometry. Changes in the composition and/or antigen expression of the cytome during sepsis are directly related to pathogenesis and could be used for diagnosis, risk stratification and monitoring. In order to develop antibody panels for flow cytometry, we used in house generated as well as published transcriptomic data sets for the selection of markers. Starting with the top 450 transcripts we narrowed our selection according to several parameters (expression on cell surface, availability of antibody-fluorochrome conjugate, marker reproducibly found in different studies). We then constructed antibody panels by selecting backbone markers for identification of leucocyte subsets (n=9) and subsequently added the informative markers (n=31). To maximize resolution sensitivity we selected the reagents and predicted the panel performance by calculation of the spillover spreading error. Using this approach we developed 4 different 16-color panels for flow cytometric assessment of leucocyte changes in sepsis and SIRS. We are currently exploring the feasibility of this approach as diagnostic and prognostic in a cohort of patients.

**P088**

**Granularity index and delta hemoglobin: two novel cellular markers for inflammation diagnostics**

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**Background:** The humoral and cellular inflammatory response is very complex and offers many opportunities for diagnostic testing. Two novel cellular markers with potential for inflammation diagnostics are the granularity index and delta hemoglobin. We investigated whether these two parameters differ in cohorts with increasing extent of systemic inflammation.

**Methods:** We retrospectively analyzed the C-reactive protein concentration, granularity index and delta hemoglobin values of 289 patients. The extent of a systemic inflammation was assessed according to the C-reactive protein concentration and classified as “none”, “low grade”, “mild”, “moderate”, or “high grade” inflammation. Parameter values in these groups were compared with Kruskal-Wallis tests.

**Results and conclusions:** The values for delta hemoglobin (P<0.001) and granularity index (P=0.03) differed significantly between groups with increasing extent of systemic inflammation. Both parameters could provide useful additional information for inflammation diagnostics.

**P089**

**Impact of the macrophage stimulating protein on macrophage-associated inflammatory responses**

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**Introduction:** MSP (macrophage stimulating protein) modulates macrophage motility, shape changes and phagocytic activity. It is known to be involved in inflammation and carcinogenesis. The association of the common MSP-SNP rs3197999 with IBD (inflammatory bowel disease) was shown by several authors. We were also able to show the association of the SNP with a decreased risk for atherosclerosis.
Methods and Results: C2078T results in the substitution of an arginine by a cysteine at position 689 of the MSP protein. The effect of wild type and mutant type MSP on migration properties of THP-1 cells (AML cell line, macrophage-like phenotype) was investigated. Both wild type and mutant MSP stimulated migration and proliferation but the mutant type was significantly more effective at equal concentrations. The MSP expression depends on the genotype as well as on further parameters: In humans serum levels of mutant MSP are significantly lower as serum levels of wildtype protein. The serum levels are also modulated by chronic and acute inflammation. Similar effects were observed in mice.

Summary and Conclusion: The stimulatory function of MSP is modulated by various factors. Beside of the “gain of function”, which increases the stimulatory effect of MSP at similar concentrations, the expression of the protein is highly regulated. The underlying pathophysiological mechanisms need to be elucidated.

### Endocrinology

#### P090

Evaluation and comparison of a new generation of vitamin D assays


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Introduction: The recently released new 25-hydroxy-vitamin D (25(OH)D) assays are aligned to the National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM). The aim of this study was to compare the 25(OH)D determination results between one recalibrated and one previous version of a chemiluminescence immunoassay (CLIA), one enzyme linked immunosorbent assay (ELISA), and one liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

Methods: Blood samples from 198 patients were parallel measured by the recalibrated IDS-iSYS 25(OH)DS assay, the previous used IDS-iSYS 25(OH)D CLIA assay, the ORGENTEC 25(OH)D3/D2 ELISA assay, and the ClinMass® LC-MS/MS Complete Kit. Pearson’s correlation coefficients, Bland-Altman plots and Deming regression analyses were calculated.

Results: Compared to the LC-MS/MS method the Pearson’s correlation coefficients were 0.82 (IDS-iSYS 25(OH)DS assay), 0.81 (IDS-iSYS 25(OH)D assay), and 0.78 (ORGENTEC 25(OH)D3/D2 assay), the mean biases were 0.86 ng/ml (IDS-iSYS 25(OH)D assay), 6.17 ng/ml (IDS-iSYS 25(OH)D assay), and 6.63 ng/ml (ORGENTEC 25(OH)D assay).

Conclusions: The highly positive correlations and the low-to-moderate mean biases compared to the LC-MS/MS method demonstrated in this study are a clear indicator for a widespread introduction of a well standardized new 25(OH)D assay generation in clinical laboratories within the next years.

#### P091

Evaluation of the first fully automated Chemiluminescence Immunoassay for the Quantification of 1a, 25-Dihydroxy-Vitamin D compared to Liquid Chromatography/Tandem Mass Spectrometry


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Background: 1α, 25-Dihydroxy-Vitamin D is the active metabolite of vitamin D. LC-MS/MS was reported to have improved specificity compared to immunologic methods. A fully automated sensitive and highly specific immunoassay using a specific recombinant fusion protein for the capture of 1,25(OH)D was developed for the DiaSorin LIAESON XL analyzer.

Methods: Performance data like intra- and interassay precision, recovery, linearity, limit of detection and quantification of the DiaSorin 1,25(OH)D immunoassay were determined and compared to two different LC-MS/MS assays, the first with immuno extraction and derivatisation and the second with protein precipitation and a two dimensional chromatography (AB Sciex), and a commercial radioimmunoassay (IDS).

Results: 1.25(OH)D intraassay and total imprecision was determined between 1.4 - 5.2% and 3.8 - 7.1% with the immunoassay and 1.4 - 5.2% and 3.8 - 7.5% with the AB Sciex LC-MS/MS method, respectively. Limits of detection and quantification of the immunoassay were 0.7 ng/L and 5.0 ng/L and of the LC-MS/MS assay 1.8 ng/L and 5.4 ng/L, respectively. Methods comparison studies of the novel assay resulted in no
significant difference \( y = -0.3 + 1.0 x, r = 0.998 \) to the first LC-MS/MS method, but a proportional and systematic difference to the AB Sciex LC-MS/MS \( y = 7.2 + 0.68 x, r = 0.935 \), and a systematic difference to the RIA \( 12.3 + 0.97 x, r = 0.822 \).

**Conclusions:** The Liaison XL 1,25 (OH)\(_2\)D fully automated immunoassay appears to have improved comparability to LC-MS/MS with the LC-MS/MS \( y = 0.822 \), and a systematic difference to the RIA \( 7.2 + 0.68 x, r = 0.97 \), but a proportional and systematic difference to the AB Sciex \( -0.3 + 1.0 x, r = 0.998 \) to the first LC-MS/MS method, but a proportional and systematic difference to the AB Sciex LC-MS/MS \( y = 7.2 + 0.68 x, r = 0.935 \), and a systematic difference to the RIA \( 12.3 + 0.97 x, r = 0.822 \). All together our findings indicate that the novel test is highly robust and will in future add significant mean to measure 1,25 (OH)\(_2\) D in clinical samples.

**P092**

**The Hepatokine Betatrophin is increased in Nonalcoholic Fatty Liver Disease and may affect Insulin Secretion in Prediabetes**

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**Aims/hypothesis:** Animal data suggest that the liver-secreted protein betatrophin is elevated in insulin resistant states and improves glucose tolerance by expanding beta cell mass. We now investigated whether betatrophin is a hepatokine that may be increasingly produced in non-alcoholic fatty liver disease and may affect insulin secretion in humans.

**Methods:** In liver tissue samples the relationship of hepatic betatrophin mRNA expression with liver triglyceride (TG) content (N=92) and circulating betatrophin (N=29) was studied. The associations of the cholesterol-regulating single nucleotide polymorphism (SNP) rs2278426 in the betatrophin-encoding gene DOCK6, with insulin secretion was studied in a cross-sectional study (N=2136) and during 9 month of a lifestyle intervention (N=344).

**Results:** Betatrophin mRNA expression correlated positively with liver TG content (r=0.31, p=0.0025) and with betatrophin serum levels (r=0.41, p=0.03). The minor T allele of the SNP rs2278426 associated with higher insulin secretion, adjusted for sex, age and insulin sensitivity, in subjects with prediabetes (p=0.044), but not in subjects with normal glucose regulation (p=0.78). The T allele also predicted a larger increase in adjusted insulin secretion during the lifestyle intervention in subjects with prediabetes (p=0.01) and associated with higher arginine-stimulated insulin secretion (p=0.04) in 97 subjects during a hyperglycaemic clamp.

**Conclusions:** We provide novel information that the liver-secreted protein betatrophin is increasingly expressed in fatty liver and that it may not only be involved in cholesterol metabolism, but also in insulin secretion in subjects who are at increased risk for type 2 diabetes.

**P093**

**Comparison of salivary stress biomarkers in children with psychiatric disorders**


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Response patterns of hypothalamic-pituitary-adrenal (HPA)-axis and autonomous nervous system (ANS) to the acute laboratory stress test and under basal condition have not been clearly characterized in children with internalizing or externalizing disorders yet. Saliva samples of 169 children (11.09 ± 1.86 yrs) with/without psychiatric disorders were measured for cortisol, cortisone, and alpha-amylase during a stress test (Trier Social Stress Test for Children, TSST-C) and under basal condition. Cortisol and cortisone were simultaneously measured by LC-MS/MS. Only at TSST-C, patients showed reduced reactivity of cortisol and cortisone (p<0.0001) compared to controls, but not in alpha-amylase. Internalizing patients had discerning difference in cortisol [% increase (median, range): Int-Pt. (59.1%, -22.6 to 1631) vs. controls (186%, -31.8 to 407) (p=0.007)]. Externalizing patients showed pronounced difference in cortisone [% increase: Ext-Pt. (29.1%, -23.2 to 400) vs. controls (84%, -17.8 to 679) (p<0.018)]. Paralleled measurement of three salivary biomarkers demonstrated patient-group specific correlation patterns between the HPA-axis and the ANS [Internalizing patients: almost no correlation between the HPA-axis and the ANS vs. Externalizing patients: higher correlation between HPA-axis and ANS]. Measurement of the activity of HPA-axis in saliva under acute stress test is a proper experimental condition to evaluate the physiological difference between children with psychiatric disorders and healthy controls. Salivary cortisol is useful biomarker to assess the response of internalizing patient and salivary cortisone is for externalizing patients.
P094
Role of p45-NF-E2 in regulating syncytiotrophoblast formation in human placenta

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p45-NF-E2 transcription factor, known to regulate megakaryocyte maturation, has been found to be required for normal syncytiotrophoblast formation in mice. Within the current study we evaluate the role of p45-NF-E2 in regulating syncytiotrophoblast formation in human placenta. Expression of NF-E2 was studied in the human choriocarcinoma cell line Bewo after stimulation with 8-Br-cAMP to induce syncytiotrophoblast formation. NF-E2 expression and alterations in acetylation were studied in human placenta tissues from healthy controls and patients with pregnancy complications. Syncytia formation was studied after NF-E2 knock down (kd) in Bewo cells. Further, the effect on Gcm-1 acetylation was studied. 8-Br-cAMP treatment elevated the expression of syncytiotrophoblast marker genes. In parallel, it lowered the expression of NF-E2 and altered Gcm-1 acetylation, indicating a mechanistic role of NF-E2. Indeed, NF-E2 kd was sufficient to induce syncytia formation. The diseased human placenta samples showed enhanced syncytiotrophoblast formation, altered acetylation and reduced expression of NF-E2 as compared to controls. The current observation in human trophoblasts matches those primarily made in mice. Reduced p45 NF-E2 expression in placenta may be an early cause and a suitable marker of placental dysfunction. Further studies are being conducted to gain more mechanistic insights and to evaluate biomarkers associated with altered acetylation and p45 NF-E2 expression.

P095
Gender verification in amniotic fluid via LC-MS/MS

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Introduction/Objectives: Mass-spectrometry is an established clinical tool for the detection of 21-OH-deficiency in amniotic fluid of early pregnancy. Although reference values for this pathology exist for GC-MS, the sensitivity and accuracy of LC-MS/MS for gender verification in healthy subjects has not been studied so far.

Methods: Amniotic fluid of 78 male and 93 female healthy newborns was analyzed by LC-MS/MS at 16 weeks of gestation. Statistical data analysis was performed using cross-validated logistic regression modelling (R Software for Statistical Computing).

Results: LC-MS/MS revealed high levels of sensitivity and accuracy in the determination of testosterone. Interestingly, testosterone levels yielded highly accurate predictions for male gender (0.9932). Additional analysis of further steroidal hormones did neither increase accuracy (0.9805) nor sensitivity (females: 0.9950; males: 0.9637) of this prediction. The cut-off value for amniotic testosterone levels was 0.074 ng/ml for male newborns.

Conclusion: The determination of testosterone in amniotic fluid by LC-MS/MS in early pregnancy of healthy subjects is a reliable method for determination of gender.

P096
Effect of life style mediated weight loss on the serum bile acid profile in patients with metabolic syndrome

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Rising obesity rates and related disorders such as insulin resistance, hyperlipidemia and high blood pressure are epidemic and belong to the most serious health challenges worldwide. Co-occurrence of these disturbances is defined as metabolic syndrome, a major risk factor
for cardiovascular disease and type 2 diabetes mellitus. As serum bile acids (BA) are discussed as powerful regulators of metabolism and as biomarkers for metabolic diseases, our study was designed to investigate the effect of lifestyle-induced weight loss on serum BA profile and obese biomarkers. 74 non-smoking, non-diabetic men (45-55 yr) with metabolic syndrome have been randomized to a lifestyle-induced weight loss program (reduced caloric intake and enhanced physical activity) or a control group. Serum BA profile and metabolic parameters were determined before and after the 6 months intervention period. In our study lifestyle-mediated weight loss (13.1 ± 5.0%) lead to a significant reduction of HbA1c, blood glucose, LDL-C, TG and IL-6 whereas adiponectin and HDL-C were increased. In parallel we observed a significant decline of serum BA, which derived from reduced amounts of cholic acid (CA), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA). The decline of BA did significantly correlate with HOMA-Index (r=0.278), fasting triglyceride (r=0.326) and weight loss (r=0.394), fasting triglyceride (r=0.326) and weight loss (r=0.394), and significantly reduced insulin sensitivity (HOMA-IR). Notably, no BA was enhanced during lifestyle mediated weight loss.

**P097**

Inhibition of Src homology 2 domain-containing phosphatase 1 (SHP-1) increases insulin sensitivity in high-fat diet-induced insulin resistant C57Black6 mice

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**Background:** Insulin resistance plays a crucial role in the development of type 2 diabetes. Insulin receptor (IR) signaling is antagonized and tightly controlled by protein tyrosine phosphatases (PTPs). The precise role of PTPs in insulin resistance, however, has not been explored.

**Results:** Male C57BL/6 mice were fed a high-fat diet (HFD, 60% kcal from fat) to induce insulin resistance, or a low-fat diet (LFD, 10% kcal from fat) for 10 weeks. Afterwards, HFD mice were treated with PTP-inhibitors for additional 6 weeks. Mice under HFD exhibited a significant increase in body weight as well as decreased respiratory quotient and adiponectin levels, and were characterized by impaired insulin- and glucose tolerance. Organ-based gene expression analyses in insulin-resistant mice demonstrated upregulation of SHP-1, PTP1B, LAR, and DEP-1 in insulin-sensitive organs. SHP-1 was further explored in vitro. Insulin stimulation in murine liver cells induced site-selective hyperphosphorylation at IR tyrosine-sites Y1158, and Y1361 after inhibition of SHP-1. Furthermore, SHP-1 impairment time-dependently enhanced insulin-induced Akt- and Erk-phosphorylation, and resulted in elevated glucose uptake in skeletal muscle cells. Administration of a SHP-1 inhibitor (Sodium Stibogluconate) and a brought pan-PTP inhibitor (BMOV) in HFD mice led to improvement of both insulin- and glucose tolerance. Finally, BMOV- and SHP-1 treatment also resulted in reduced body weight.

**Conclusions:** The results indicate a central role of PTPs and, in particular, of SHP-1 as endogenous antagonists of the IR. Taken together targeting PTPs led to beneficial effects in insulin resistance, and may thus improve metabolic diseases.

**P098**

Thyroid dysfunction as risk factor for a negative cardiac outcome: Results from the Heinz Nixdorf Recall cohort


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Thyroid hormones are crucial for normal development and physiological organ function. T3 and T4 pass via the blood stream to target organs such as brain, heart and muscle. Several population-based cohort studies indicate a negative impact of thyroid disorders (hypothyroidism and hyperthyreoiditis) to cardiovascular diseases. In this study, we analyzed data from the Heinz Nixdorf Recall (Risk Factors, Evaluation of Coronary Calcium and Lifestyle) study (HNR), a population-based, prospective cohort study. The HNR cohort was set up in a study population with an age range start at 45-75 years. After baseline assessment, a 5 year follow up was performed. Investigations of the HNR cohort indicated the Coronary Artery Calcium (CAC) score as a strong qualitative marker for atherosclerosis. In the present study we address thyroid dysfunction as a risk factor for negative cardiac outcome. Thus data from the HNR cohort are analyzed with regard to 1) longitudinal distribution of normal and pathological thyroid function in the HNR cohort?; 2) link between thyroid dysfunction and development of risk CAC-score?; 3) influence of subclinical as opposed to overt thyroid dysfunction on cardiac events? In this project, the strength of the comprehensive study design is used to get new insights into the role of thyroid hormones and their potential negative effects on the cardiac health.
P099

Adreno-cortical dysfunction in critically ill patients – Development of a sensitive 2D-UHPLC-MS/MS-Method for the simultaneous quantification of seven corticosteroids

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Background: Relative adreno-cortical dysfunction was recognized as a potential complication in critically ill patients. To investigate the corticosteroid pathway under this condition we developed a high-throughput isotope-dilution UHPLC-MS/MS method for the quantification of cortisol, cortison, corticosterone, 11-desoxycortisol, 17-OH-progesterone, 11-desoxycorticosterone and aldosterone.

Methods: First the samples were spiked with deuterated internal standards. Subsequently deproteination as the only manual step in sample preparation was carried out and the samples were analyzed by a 2D-UHPLC-MS/MS system (HLB, trapping column; RP18, analytical column) in 7 minutes. Special focus was put on the separation of isomeric compounds with the same mass transitions.

Results: The method provided high selectivity since the isomeric compounds were separated to baseline. Evaluation demonstrated acceptable values for accuracy (94 – 98.4%) and reproducibility (CV 3.1% - 8.5 %) as well as a high sensitivity for all analytes. In a preliminary study the impact of ACTH stimulation on the corticosteroid pattern was investigated in critically ill patients.

Conclusions: This novel method is convenient for studies with large cohorts as the sample preparation is quick and simple and the run time is short. Monitoring of the corticosteroid pathway – instead solely assessing serum cortisol - might enable important insights into the pathophysiology of adreno-cortical dysfunction in critically ill patients.

P100

Salivary diagnostics for the assessment of the HPA-axis in children with psychiatric disorders

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Salivary cortisol (F) has been used as a surrogate marker for serum free F and routinely measured by immunoassay (IA). Salivary cortisone (E) has emerged as a new surrogate marker of serum free F due to metabolic activity of the enzyme 11β-HSD2 in parotid tissue. Liquid chromatography tandem mass spectrometry (MS) enabled the simultaneous measurement of F and E. In our study, 2780 saliva samples of 169 children were measured by IA (F) and MS (F and E). Method comparison between IA and MS with Passing-Bablock regression and Spearman’s correlation showed that cortisol concentration (nmol/L) by IA (y) was about twice higher than the corresponding values by MS (x) with high correlation (y = 2.38 x + 0.03, rho = 0.957, p < 0.0001). Bland-Altman plot indicated that two analytical techniques well concord in the concentration higher than 5 nmol/L. Cross-reactivity with cortisone was not significant in cortisol IA. Cortisol response in functional test (Trier Social Stress Test for Children) yielded similar clinical output by both IA and MS [Cortisol % increase (median, range): IA (196%, -32 to 3806) vs MS (186%, 31.8 to 2407): Correlation between IA and MS: rho = 0.946, p < 0.00011]. Activity of 11β-HSD2 (E/(E+F) x 100%) reached a steady-state as molar ratio of cortisone to cortisol increases. Our data suggest that both IA and MS are largely comparable in its utility of discriminating small cortisol changes during a functional test in pediatric patients with psychiatric disorders. Analytical data comparison showed cortisol readouts by IA are about twice higher than those by MS, which could be attributed to the different standardization of each assay.

P101

Implausible constellations of TSH, FT3 and FT4, analytical causes and clinical consequences

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Objective: Since most FT3- and FT4-assays are based on immunological principles, system-specific autoantibodies can be a major cause of analytical interference resulting in over- or underestimation of free thyroid hormone concentrations. We tried to improve an existing algorithm to identify those analytically biased samples. Therefore, according to predefined criteria we collected conspicuous samples over a period of 8 month, determined total T3 and T4 and remeasured FT3 as well as FT4 by employment of an assay from a different vendor.
Methods: 124 out of 26700 patient samples were selected from routine diagnostics of the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig. Measurements of TSH, FT3, FT4 as well as total T3 and T4 were performed with the Roche Elecsys system. For verification and comparison of FT3 and FT4 results we employed the Liaison (Diasorin) assay. Analytical system-specific interfering antibodies are determined at Roche.

Results: 29 out of 124 selected patient samples (relative frequency of 0.1%) were identified with contradicting results in FT3 and FT4 determinations in relation to total T3 and T4 concentrations. Furthermore a distinct divergence between Roche and Diasorin FT3 and FT4 results was observed in these samples. Therefore, the respective samples are currently investigated with regard to interfering antibodies at Roche.

Conclusion: Analytical system-specific, antibody-based interferences may lead to over- or underestimation of FT3 and FT4 concentrations. These interferences have to be considered for the interpretation of laboratory measurements of thyroid function as well as treatment decisions.

Lipidomics/Other Topics/Biobanking/Laboratory Management/Neurodegeneration, Ageing, Dementia/Infectious Disease

P102

The lipid profile of brown adipose tissue is sex-specific in mice

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Brown adipose tissue (BAT) is a vital organ of non-shivering thermogenesis in small mammals and neonates and a potential metabolic drug target in adult humans. By using high-resolution LC-tandem-mass spectrometry, we performed an extensive lipid profiling of BAT and two different white adipose tissue (WAT) depots, subcutaneous and gonadal, from female and male mice. We quantified more than 300 lipid species from 17 (sub)classes, including FFA, TAG, DAG, and several phospha- and sphingolipid classes. In addition, acyl (alkenyl/alkyl) chains could be assigned to all phospho- and sphingolipid species. The overall levels of phospholipids and FFA were higher in BAT, while DAG and TAG were higher in WAT. The lipids that showed the greatest specificity for BAT were dominated by the fatty acid residue docosahexaenoic acid which influences membrane fluidity. Multivariate analysis indicated a clear separation of BAT samples from female and male mice. Quantitatively, female BAT contained less TAG and more phospholipids than male BAT. Qualitatively, a set of fatty acids including arachidonic and stearic acid occurred more frequently in female BAT phospholipids, while another set including linoleic and palmitic acid was higher in males. The different WAT depots, in contrast, were comparatively similar. The observed differences in the fatty acid composition of phospholipids could greatly affect mitochondrial membranes and other cellular organelles and thereby regulate the function of BAT in a sex-specific manner.

P103

Teaching in Laboratory Medicine: Complex problems provide the potential to increase the intrinsic motivation to learn

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Background: Since 2004 learning on the basis of paper cases is integral part of medical education in Germany. These paper cases namely problems are crucial for the intrinsic motivation to learn, the study time and achievement. We asked how the experienced difficulty and the intrinsic motivation are associated, which variable influences the perception of difficulty and if the intrinsic motivation to learn differs between the students working with complex or non-complex problems.

Methods: 173 students were included in the study, performed in the module “Diagnostic Medicine” at the University Hospital Hamburg-Eppendorf. In addition to laboratory diagnostics as a main part, the problems dealt with imaging, endoscopic and histological examinations. 88 students worked on complex and 85 on non-complex problems.

Results: In both groups experienced difficulty was negatively associated with the intrinsic motivation to learn. The better the tutor was rated, the less the problems were considered as difficult. When complex and non-complex problems were perceived as equally difficult, the intrinsic motivation to learn and the individual study time were higher in students working with complex problems.

Conclusions: In combination with a good tutor performance complex problems may reach levels of intrinsic motivation which are unattainable with non-complex problems.
P104

TOSOH HLC-723G: comparison of haemoglobin variant detection under routine conditions using different measuring modes on a G8 analyzer

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HbA1c is a well established parameter for monitoring long-term glycemic control. The Tosoh Glycohemoglobin-analyser HLC-723G8 (G8), using ion-exchange principle for separation of HbA1c has two different working modes. One mode (VAR mode) will detect most of the common hemoglobin (Hb) variants while the second mode (STD mode) is quicker but due to a shorter separation time less adapted to detect variants, possibly leading to an increased risk for false measurements. We intended to clarify to what extent Hb-variants are detected via STD-mode. Therefore, the two G8 modes were compared under routine conditions, and effects of specific Hb-variants on HbA1c analysis in STD mode were investigated. 1578 left over-samples were measured on the Tosoh G8 analyzer in both measuring modes under routine conditions. Further, approximately 18,000 samples run over the G8 systems in the VAR mode were analyzed for system messages (“flags”) indicating the presence of a Hb-variant in the sample. The respective left-over samples were measured in STD mode after adapting flags in the system software. 79 % (n = 23) of Hb variants detected in VAR mode could also be found in the STD mode. Thus, a possible routine strategy could be to use flags to perform routine measurement in the STD mode. By this strategy, according to our experience 79% of Hb variants present in a European routine setting will be detected on a G8 in STD mode.

P105

Variability of the human serum albumin gene in the Swiss blood donor population

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Background: Human serum albumin (HSA) exerts versatile functions in the maintenance of the plasma oncotic pressure as well as in solubilization and transport of various ligands such as bilirubin and drugs. Its disturbed synthesis, physical loss or compromised function may affect detoxification processes and impair ligand homeostasis. About 10% of patients with chronic hepatitis C or alcohol abuse develop cirrhosis, and an altered HSA oxidative state in cirrhotic compared to non-cirrhotic patients was recently reported. A potential role of genetic HSA variants in the development of cirrhosis has not been studied yet. So far, 65 rare mutations have been reported to result in bisalbuminemia, but their clinical significance as well as prevalence is unknown. We thus aimed to characterize the genetic variability of the albumin gene (ALB) in the general Swiss population to obtain base-line data for studying variation of ALB in the context of cirrhosis.

Methods: All exons and flanking intronic regions of ALB were sequenced in 300 volunteers recruited by the Blood donation center in Bern, Switzerland.

Results: An initial analysis including 293 individuals revealed 35 single nucleotide polymorphisms, of which 15 were novel. Moreover, nine exonic mutations were observed, including four synonymous and five missense mutations. The minor allele frequencies of the missense variants were between 0.1 and 0.4% with a carrier frequency of 2.4%.

Conclusion: Since these missense variants may have a role in the development of cirrhosis, in a next step, we aim to assess their phenotypic effect on HSA and potential association with cirrhosis.

P106

Clinical, coagulation and hematologic samples handled by Laboratory Automation – Let’s process it all

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State of the art in laboratory automation improves fast as more and more analysis systems and support units (aliquot and storage module) are combined. Reduction in turnaround time is the main reason to go for a more automated solution. Clinical, coagulation and hematologic samples (N = 19075) were processed by laboratory automation. A vision detection module (VDM) gathers information on sample identification
number (SID) and cap color. Thus, any inconsistency of material type (e.g., serum, citrate) and analysis program is recognized (< 1%). Cap color of lithium-heparin is similar to serum tubes (Sarstedt). VDM is not able to differentiate same sized tubes. Furthermore, short tubes (Becton Dickinson 2.7 ml citrate and 2.0 ml K-EDTA) pose a problem. The entire SID is often not recognized, since parts of it is concealed by a transport cup. Within the first two weeks 18.4% of all samples were unreadable. We started to teach, where and how to stick a SID. We observed a significant reduction in unreadable samples. Laboratory automation will only live up to its promise if tube types of different manufacturers can be distinguished, SID is printed in good quality and aligned properly to each tube.

P107

Implementation of an automated -80 °C biobank

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Objective: Long term reliability of automated biobanks is essential. Even small numbers of errors will affect the use and availability of the biobank. We therefore evaluated the -80°C Kiwi (LiCONiC) with the connected -20°C picking module using 96 plates with 1.1 ml cryo vials (FluidX) for long term routine use.

Material and Methods: Evaluation included technical reliability of mechanical processes during loading and unloading of cryo vials. Failures of power and compressors were imitated and liquid nitrogen emergency refrigeration including messaging of alarms at any day time were tested. Ten times 10,000 cryo vials containing plasma were loaded and unloaded after freezing of one to seven days. Additionally, 50,000 vials were unloaded with the help of data file import and export. Sample unloading time was measured. The picking module was tested by scanning and picking of 100,000 cryo vials.

Results: Problems with power supply and instrument reliability were improved during the implementation period by several modifications by the manufacturer including exchange of three out of 240 cassettes. Optimization of liquid nitrogen emergency refrigeration took three months. Specific racks had to be chosen to fit labeled cryo vials. Error rates for loading and unloading were reduced to 1-3%. Picking errors were reduced from 10% to less than 1% by adjustments.

Conclusion: After 12 months of evaluation and adjustments the Kiwi biobank was successfully introduced to routine use and proves to be reliable for at least 18 months.

P108

Biobanking Concept in the German Centre for Cardiovascular Research (DZHK)

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The German Centre for Cardiovascular Research (DZHK) has started to establish a harmonised decentral biobanking. Biobanking is the basis for biomarker research. It will gain even more importance in the future. Biobanking includes several processing steps: patient identification, sampling, transport, aliquoting, long term storage, retrieving of suitable samples, thawing, mixing, analyzing and re-storage, if appropriate. This impacts the biobanking structure. Quality of the samples is mainly affected by processing time and temperature. Aliquoting of samples after thawing for analysis should be avoided, because manual processing requires time and is an additional source of errors; both aecting the quality of biomarker research. The DZHK has seven partner sites including overall 20 university hospitals. One focus of the DZHK is on clinical research. Besides the definition of storage conditions state of the art biobanking needs reliable structures for collection of standardised clinical data. In addition, use & access rules have been defined and legal regulations including data privacy protection and ethical aspects have been taken into account. From all patients recruited within the DZHK a pseudonymised basic-sample-set will be collected independent of scientific questions. Primary and aliquot tubes suitable for robotics and small aliquot sizes with 1D and 2D barcodes are chosen. The tubes will be pre-labeled at the Institute of Clinical Chemistry and Laboratory Medicine of the University Medicine Greifswald and will be sent to all study centres. All samples will be stored in decentral biobanks at -80°C.
P109

Prism effect of the specimen receiving – Generation of fundamentals for the smooth progress of the analytical process, fast and valid medical reporting and accounting

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A fast and efficient sample receiving (SR) process is requisite for a fast analytical process, an important part of the quality management, a correct medical report and happy clients. We developed an algorithm for sample and requisition handling aimed at structuring and organizing the workflow. The procedures in SR are comparable to characteristics of a prism. Like a prism, which separates the white light into its constituent colours, the SR has to register and route the flow of the arriving samples. The arriving specimen and test requisitions are checked according to usual standards of quality (e.g. physical integrity) and sorted during the initial unpacking. Two different test requisitions consequential sorting categories are possible: Electronic order entry or test order requisition by paper. This differentiation is important for processing of the samples (e.g. sorting, barcoding, registration, conformation of the sample material) and in consequence for medical reporting and billing. In this context the more complicated steps for the registration process of samples of paper based ordering are analyzed, discussed and optimized. Every query with samples or requisition should be given to the customer service to call the sender. Logistical aspects like aliquoting for external testing are integrated in the algorithm. Different requisition and billing categories in various countries and states were taken into account. Integration of high urgent samples in the process is shown.

P110

The future of the laboratory information system – What are the requirements for a powerful system for a laboratory data management?

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The laboratory information system (LIS) were introduced into clinical chemistry in the 1970’s. Although permanent improvements are made there is a growing number of users calling for a better, more powerful and contemporary LIS. In the near future we will have an increasing amount of data (e.g. individualized medicine). Therefore some questions arise: Can the contemporary LIS manage the new tasks? Is it sufficient to add parts to the contemporary LIS or should the LIS be completely restructured? In a fish bone diagram (Ishikawa diagram) the main tasks (preanalytical and postanalytical management, quality management, pre-, post- and analytical phase) for a LIS are shown. For each main task subtasks are illustrated, analyzed and discussed. Basically mechanisms are presented and reviewed. Based on the tasks of a future LIS, we think this is only solvable by a new concept, the laboratory data management (LDM). A LDM should be a leading system that has to coordinate specific programs for different specialized tasks. It has to process and provide laboratory data for specialized programs, other programs have to process and provide data for the LDM (‘symbiosis’ of programs). The LDM is a modular system with an individual adaption (e.g. high specialized laboratory or laboratory with a high amount of samples). It is advisable for a high flexibility and change of programs that the data (e.g. measurement data, medical reporting) are saved in a form that can be easily read by another software.

P111

Evaluation of a fast single sample pneumatic tube system

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Objectives: A new Danish pneumatic tube system (PTS; TEMPUS600®) which does not require packing and unpacking of samples, transports samples with 10 m/s. It allows sending of the samples directly into the bulk loader of a laboratory automation system. We investigate influences of this transport type on the quality of samples.

Methods: Duplicate blood samples of 20 volunteers were analyzed after transporting one set of samples by courier and the other by PTS. During transport, a mini data logger (MSR145®) continuously measured temperature and accelerations. After transport clinical chemistry, hematology and coagulation parameters were measured, compared and the corresponding g-forces were calculated.
**Results:** Though samples in PTS were subject to maximum accelerations of 18 g compared to 6 g of courier transport, the cumulated forces (vector sum) did not reveal relevant differences between the conventional and single sample PTS. Medians of results differed less than 10% for all investigated analytes. Relative differences of medians for LDH and free Hb were 8.1% and 6.3% respectively, both showing lower values in PTS samples. There was no difference between medians for potassium.

**Conclusion:** The impact of the acceleration forces during transport on analytical results is comparable to the conventional PTS and within the magnitude of the imprecision of the utilized assays. The overall workflow is improved by decreasing hands on time on the ward and in the laboratory.

**P112**

**Hereditary Spastic Paraplegia in mice: in vivo models for neurodegeneration**

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**Background:** Hereditary spastic paraplegias (HSPs), a heterogeneous group of progressive gait disorders, are due to degeneration of upper motoneuron axons. HSP type SPG8 is an autosomal dominant form caused by heterozygous mutations in *KIAA0196*. The encoded strumpellin protein resides to endosomes where it may play a role in intra-cellular membrane traffic.

**Methods and Results:** By targeting the homologous gene in mice, we generated a knockout (KO) line as well as a knockin (KI) line which carries a pathogenic missense mutation. The homozygous KI is viable, whereas the homozygous KO shows early embryonic lethality. Preliminary phenotyping of heterozygous animals suggests a gait deficit for the KI but not the KO line. Transferrin-based assays as performed on primary neurons from different genotypes have failed to reveal an endosomal uptake deficit. Together with mRNA and protein expression data, these observations challenge the previously favored hypothesis of a loss-of-function mechanism in SPG8.

**Conclusion and Outlook:** Our KI line seems to represent a valid disease model for defining the pathomechanism in SPG8. Our KO line, which is also available in the conditional mode, is a potentially valuable tool for determining strumpellin's cellular function. To address pertinent questions in the future, we are currently extending biochemical membrane analyses to include endolysosomal and recycling pathways.

**P113**

**Neurochemical dementia diagnostics: urgent need for standardization**

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Neurochemical dementia diagnostics comprises of CSF surrogate biomarkers associated with CNS parenchymal degeneration. The German S3 guideline recommends the analysis of β-amyloid 1-42, total tau protein and hyper phosphorylated tau. There is significant evidence for the additional benefit of calculating the β-amyloid ratio $aβ_{1-42} \times 10/aβ_{1-40}$. Until recently most of the German CSF laboratories used test kits of only one supplier (Innogenetics/Fujirebio [I/F]). Nevertheless broad divergence in results was observed in national and international quality surveys. Meanwhile several new test kits from different suppliers are available (i.e. IBL Japan, IBL Germany, Euroimmun). Moreover, I/F has revised calibration of their tau, ptau and $aβ_{42}$ kits and released an own $aβ_{40}$ assay. In our study we cross-checked several combinations of the new assays with the routine I/F values. Not surprisingly, gross differences of results were observed in most comparisons, both in calibration and in variance. Correlations ranged from 0.62 ($aβ$ ratio, I/F new vs. IBL Germany) up to 0.97 (ptau, I/F old vs. I/F new), and calibration revealed differences in results of up to 50%. Obviously, proprietary reference values have to be established for the majority of new assays, and results will be even less comparable from lab to lab than nowadays. We feel that there is an urgent need for standardization in neurochemical dementia diagnostics. Otherwise, its clinical use will be hampered by particularization.

**P114**

**Evaluation of four different anti-HBc blood donation screening assays**

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**Objective:** One of the regulations issued by the Paul Ehrlich Institute require retesting of repeatedly anti-HBc reactive blood or stem cell donations by two different anti-HBc assays which need to be independent from the anti-HBc assay used for initial screening. We investigated, if the Axsym core results were independent from the screening test results obtained with the Architect Anti-HBc II assay.
Methods: Blood samples, reactive in the Architect Anti-HBc II screening assay (Abbott), were retested with the AxSYM core (Abbott), Liaison Anti-HBc (Diasorin) and Vidas (Biomérieux) assays.

Results: Concordant results were obtained with all anti-HBc assays when 14 strong positive samples were investigated. Only 8 of 11 samples with weak positive results in the screening assay were also positive in the three additional tests. One sample was tested negative with the Vidas but positive with the other anti-HBc assays indicating a false negative result with the Vidas assay. Two samples were tested positive with both assays from Abbott, but negative with the Liaison and Vidas. One of these samples originated from a 29 year old blood donor who had been vaccinated against Hepatitis B. The other sample was from a 64 year old patient with hepatitis C and the non-specific anti-HBc results are known for 2 years. The AxSYM core and the Architect Anti-HBc II assay share common antigens produced in E. coli.

Conclusions: The sensitivity of the Vidas assay is questionable. Furthermore, it remains unclear whether the two Abbott assays represent analytically independent test systems.

P115

What is the difference among High-Level Aminoglycoside resistant and virulent Enterococcus faecalis isolates of human, animal and environmental sources?

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Background: Enterococcus faecalis and *E. faecium* are the leading cause of nosocomial infections, and therefore a persisting clinical problem globally. This study was undertaken to compare the virulence and the high-level resistance towards aminoglycosides (HLAR) in *E. faecalis* of different sources in Nigeria.

Methods: We studied 106 *E. faecalis* isolates (year 2009: human (n=65), stool isolates of healthy pigs (23), chicken (21), cattle (10) and water body in Lagos (n=7). PCR was used to investigate the presence of high HLAR genes (*aac(6′)-Ie-aph(2″)-1a, aph(2″)-Ib, aph(2″)-1c, aph(2″)-ld, aph(3″)-IIia, ant(4′)-Ia*) and seven putative virulence genes (esp, cyl, gelE, hyl, ace, efaA, asa1). The clonality was analysed by multilocus sequence typing (MLST).

Results: No acquired vancomycin resistance gene was detected. HLR to gentamicin was observed in 46.7% of clinical isolates and 13.7% of animal isolates (chicken) and was accompanied by HLR to kanamycin, encoding of the *aac(6′)-Ie-aph(2″)-1a* gene. Isolates with HLR to streptomycin harboured the *aph(3″)-IIIa* gene. MLST analysis revealed that 33.3% of HLAR isolates were ST6 belonging to clonal lineage CC2 and 25% were ST116. Other STs determined included ST40, ST28 and ST16. The virulence genes *esp* and *hyl* occurred only in clinical isolates (6.4% and 2.1%) while *cyl* was found in both clinical (8.5%) and animal (2%) isolates. The only HLAR water isolate was typed as ST202.

Conclusion: The spread of *aac(6′)-Ie-aph(2″)-1a* and *aph(3″)-IIIa* gene is responsible for HLR among *E. faecalis* in the South-western of Nigeria. Animal isolates belonged to ST116 whereas human isolates were mostly typed as ST6.

P116

Induction of platelet aggregation by *S. gallolyticus subsp. gallolyticus*, an emerging pathogen in infective endocarditis

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Introduction: *S. gallolyticus subsp. gallolyticus* (SGG) can be identified as causative pathogen in approximately 20% of all streptococcal infective endocarditis (IE) cases. Throughout the establishment of endocardial vegetation, induction of platelet aggregation by bacterial structures could have strong impact on IE development. Therefore, this study focusses on the characterization of the induction of platelet aggregation by SGG.

Material and methods: Platelet rich plasma (PRP) of 4 healthy probands was used for characterization of platelet aggregation by 9 different SGG isolates. SGG was diluted up to approximately 4×10^5 cfu/mL in DBPS. PRP (200×10^3 plt/μL) was inoculated with SGG isolates and aggregation was recorded using light-transmission aggregometry.

Results: The study shows individual platelet host response and phenotypic diversity of SGG isolates. It is particularly interesting that only 4 out of 9 SGG isolates were able to induce platelet aggregation of proband 4. The other probands showed that SGG isolates are able to induce platelet aggregation and only individual isolates fail to induce aggregation.

Conclusion: The induction of platelet aggregation could be an intriguing virulence factor for SGG in the course of establishment of vegetation on endocardial structures. The study showed individual host components as well as different bacterial aggregation potentials.
Proteomics / Mass Spectrometry

P117

Mass spectrometry based quantification of bradykinin in human blood

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The inflammatory mediator bradykinin (BK) is a vasoactive peptide hormone causing vasodilatation and increase of vascular permeability. It is also known for its involvement in diseases, such as diabetes, hypertension, sepsis and hereditary angioedema (HAE). BK is generated from the cleavage of high molecular weight kininogen (HK) by the serine protease plasma kallikrein (PK) and acts on G-protein coupled BK receptors B1R and B2R. A reversed phase high performance liquid chromatography (HPLC) coupled to a tandem mass spectrometer (LC-MS/MS) was used for quantifying BK by selected reaction monitoring (SRM) like mode. BK’s short half life, due to rapid degradation by kininases, as well as induction of the contact activation system, where BK is generated ex vivo, represent the major challenges for its accurate quantification. In order to circumvent these problems we developed a protocol including addition of protease inhibitors and application of polypropylene vials, from blood collection until LC-MS/MS analysis. Furthermore we tested several precipitation methods as well as solid phase extraction (SPE) as appropriate sample preparation steps. We will present data showing the limit of detection (LOD) and limit of quantification (LOQ) for determining the concentration of BK and its main degradation product in human blood plasma.

P118

Protease Profiling for Diagnosis of Systematic Aspergillus Infections in Neutropenic Patients with Acute Leukemia

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Background: The lethal infection caused by Aspergillus species in immunocompromised patients is concomitant with secretion of a wide spectrum of pathogenically relevant proteases. These could serve as potential diagnostic biomarkers for the detection of invasive Aspergillosis (IA) in human serum. Here we describe a MS-based protease profiling approach to detect secreted proteases using designated synthetic reporter peptides as substrates.

Methods: A concise peptide library was synthesized and tested in serum and in Aspergillus cell culture supernatant. Suitable peptides were added to serum specimens from patients with Aspergillus infections and to healthy controls and incubated under standardized conditions. The respective proteolytic fragments were detected using LC/MS.

Results: Reporter peptides spiking has revealed numerous peptides that are stable in serum but in contrast are cleaved in the cell culture supernatant. Furthermore, MS data showed that the cleavage rates of the reporter peptides were significantly higher in specimens of patients with IA when compared to healthy controls.

Conclusion: Here, we identified a set of reporter peptides that might improve the timely diagnosis of IA in the future.

P119

Cellular proteome analysis of lipopolysaccharide-stimulated hepatic stellate cells

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Background and aims: Bacterial lipopolysaccharide (LPS) induces the acute inflammatory response upon infection. In the liver, Kupffer cells (KC) and hepatic stellate cells (HSC) express the LPS receptor TLR4 and are central providers of the "cytokine storm" that initiates the acute phase response (APR). Our aim was to delineate in greater detail molecular changes in HSC during initiation and sustenance of this severe and acute inflammatory reaction.

Methods: We isolated primary HSC from BALB/c mice, kept them in culture and treated them with LPS for 24 hours on day 2, when they are considered to represent the quiescent stage in vivo, and on day 5, when HSC are considered to be activated, i.e. represent the transdifferentiation
stage towards myofibroblasts in vivo. Differentially expressed proteins were identified and quantified by liquid chromatography/mass spectrometry (LC/MS), and subjected to pathway analysis to identify significantly regulated processes.

**Results:** 725 differentially expressed unique protein fragments were identified, corresponding to 498 protein IDs. Pathway enrichment analysis revealed significant enrichment of the following processes: Valine, leucine and isoleucine degradation ($P=1.5\times10^{-6}$), citrate cycle (TCA cycle) ($P=5.6\times10^{-5}$), focal adhesion ($P=2.0\times10^{-5}$), fatty acid metabolism ($P=1.7\times10^{-4}$), glycolysis / gluconeogenesis ($P=3.4\times10^{-4}$) and regulation of actin cytoskeleton ($P=5.1\times10^{-3}$). CTGF (fibrogenesis) and MMP3 (tissue remodelling) were upregulated 2.5-fold and 5-fold, respectively.

**Conclusion:** The LPS-induced inflammatory response of HSC is underpinned by substantial changes in metabolism as well as cellular scaffold and adhesion molecules.

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**P120**

The immunomodulatory function of CAAP48

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**Objectives:** Discrimination of Systemic Inflammatory Response Syndrome (SIRS) due to infection or non-infectious causes remains a challenge in the clinic, because to date there is still no reliable biomarker which facilitates this diagnosis. In a previous study we identified a proteolytic fragment of alpha-1-antitrypsin (CAAP48) as potential sepsis biomarker. CAAP48 allows discrimination of patients with severe sepsis from severe SIRS with high sensitivity and specificity (AUC: 0.96). Currently we analyze the biological function of CAAP48 on human neutrophils.

**Methods:** Human polymorphonuclear neutrophils (PMN) were isolated from peripheral blood of healthy donors using Polymorph-Prep. PMN were incubated with different concentrations of synthetic CAAP48 and several control peptides and the expression of activation markers were analyzed by fluorescence-activated cell sorting (FACS). The PMN oxidative burst was measured using a commercially available test kit. PMN viability was determined based on Annexin V and propidium iodide staining.

**Results:** PMN were highly activated by CAAP48. After incubation of PMN with CAAP48 the activation markers CD66b, CD63 and CD69 were up-regulated, which confirmed massive degranulation. There was also an effect of CAAP48 on viability of PMN in concentrations up to 100 μM. CAAP48 induced oxidative burst of PMN.

**Conclusion:** Based on our previous results we propose that CAAP48 is a promising discriminatory sepsis biomarker, with immunomodulatory functions, particularly on human neutrophils, supporting its role in the pathobiology of the sepsis host response. In ongoing research the immunomodulatory effects on other immune cells like monocytes or natural killer cells will be evaluated.

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**P121**

LC/MS based monitoring of endogenous decay markers for quality assessment of serum specimens

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**Introduction:** Preanalytical variations have major impact on most biological assays. There is no comprehensive technique available to measure the preanalytical quality of a given sample regarding its peptide and protein content. Here, we show a method for determining the preanalytical quality of serum by monitoring the ex vivo time dependent decay of endogenous peptides with LC/MS.

**Materials and Methods:** Serum specimens from patients with colorectal malignancies (n=50) were analyzed for a set of endogenous peptides. These proteolytic fragments of serum proteins were monitored with LC/MS at different preanalytical timepoints at room temperature ranging from 1h to 48h after blood withdrawal. The peak areas of these peptides were extracted from LC/MS data. An algorithm was constructed to estimate the time at room temperature for an unknown sample.

**Results:** With a set of 61 endogenous decay markers we were able to characterize the time dependent changes of serum peptide profiles. By combination and comparison against each other it was possible to compensate the adverse effects of great biological variance. The
mathematical analyses of the peptide intensities and their change over time led to a classification scheme for estimation of specimen age according to the respective preanalytical time span.

**Conclusion:** The endogenous peptides are continuously processed in blood specimens in a time dependent manner. This ‘proteomic degradation clock’ can be used to estimate the preanalytical quality of serum prior to other proteomic profiling approaches. This approach might be integrated as a quality control tool for collection and storage of specimens from biobanking repositories.

**P122**

**Multiplex profiling of tumor associated proteolytic activity in serum of colorectal cancer patients**

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The monitoring of tumor associated proteolytic activity in blood specimens has recently been proposed as new diagnostic tool in cancer research. In this paper, we describe the screening of a peptide library for identification of reporter peptides (RPs) that are selectively cleaved in serum specimens from colorectal cancer patients and investigate the benefits of RP multiplexing. A library of 144 RPs was constructed that contained amino acid sequences of abundant plasma proteins. Proteolytic cleavage of RPs was monitored with mass spectrometry. Five RPs that were selectively cleaved in serum specimens from tumor patients were selected for further validation in serum specimens of colorectal tumor patients (n=30) and nonmalignant controls (n=60). RPspiking and subsequent quantification of proteolytic fragments with liquid chromatography mass spectrometry showed good reproducibility with coefficients of variations always below 26%. The linear discriminant analysis and principal component analysis revealed that a combination of RP markers for diagnostic classification is superior to single markers. Classification accuracy reached 88% (79/90) when all five markers were combined. Functional protease profiling with reporter peptides might improve the laboratory based diagnosis, monitoring and diagnostic of malignant disease and has to be evaluated thoroughly in future studies.

**P123**

**Peptide microarrays as diagnostic tool for cancer associated protease profiling**

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Proteases play an important role in progression and metastasis of cancer [1]. Some are secreted from the tumor and can be found in the bloodstream [2-6]. Functional protease profiling aims at discovering tumor associated protease activity in clinical specimens which could be used for diagnostic purposes. Therefore, synthetic reporter peptides are incubated with blood specimens ex vivo and exclusive peptide cleavage by tumor associated proteases is monitored [2-5]. The on-chip synthesis of the peptides is directly performed on coated glass-slides using a proprietary laser printing technique [7]. In order to identify relevant reporter peptides we focus on the development of a new array-based peptide assay for screening. The employed microarray platform is standardized using antibody staining after proteolytic digestion of its epitope (anti-FLAG & anti-HA, fluorescently labeled) with Trypsin as model protease. Actually we are using a biotin-streptavidin-system (printed biotin s.o.: fluorescently labeled streptavidin) which allows us to measure a signal decrease after peptide cleavage compared to the buffer control. So far we could obtain good cleavage efficiencies with Trypsin even for low concentrations using our standardized peptide content (=6 U/ml; incubation 2h, 37°C). Preliminary data with further model proteases like Carboxypeptidase Y using our biotin-streptavidin-system showed also promising results. Future experiments will focus on the investigation of the sensitivity and reliability of the established assay platform as well as on an on demand solution using a fluorophore based method. Finally cancer related proteases and clinical samples will be analyzed.

**References:**


P124

Signals of three peptides, six proteins and concentrations of six phosphatidylcholines are biomarker candidates for the detection of an infectious etiology of febrile neutropenia

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**Objectives:** Neutropenia is a common side effect of myeloablative chemotherapy for haematological malignancies. A substantial fraction of neutropenic patients develops fever (FN), associated with risk of progression to severe sepsis/septic shock. We sought to identify proteomic and metabolic biomarker candidates to aid the diagnosis of infection and to monitor the course of the disease in FN.

**Methods:** 48 febrile episodes of neutropenic patients were investigated. Blood plasma aliquots were collected at fever onset and two and four days after. 20 patients with fever of unknown origin (FUO) were compared to 28 patients with proven infection or a subgroup of 17 patients with bacteremia. A combined approach of proteomic and metabolomic analyses was applied and logistic regression models were fitted for each signal. Moreover, procalcitonin (PCT), C reactive protein (CRP) and interleukin 6 (IL 6) were investigated.

**Results:** We found three peptide signals (m/z 1,017.4 – 1,057.3) and six phosphatidylcholine metabolites that occurred differently in the patient groups at fever onset and six protein signals (m/z 6,881 – 17,215) two and four days after fever onset. CRP and IL 6 were different two and four days after fever onset. PCT showed a differentiating potential four days after fever onset. In addition, changes of four lysophosphatidyl cholines and the amino acids threonine and tryptophan were significantly associated with resolution/no resolution of FN.

**Conclusion:** 15 promising biomarker candidates for the early detection of an infectious origin of FN and for monitoring the course of the disease were found. Validation in independent patient cohorts and functional analyses are needed before practical therapy concepts can be adopted. Acknowledgement: supported by the Paul-Martini Research Group (Clinical Septomics), funded by the Ministry of Thuringia (Pro-Excellence; PE 108-2), the Thuringian Foundation for Technology, Innovation and Research (STIFT), the German Sepsis Society (GSS) and the Center of Sepsis Control & Care (CSCC); funded by the German Ministry of Education and Research (BMBF).

Hematology & Hemostasis

P125

The role of Developmental endothelial locus-1 (Del-1) in hematopoietic stem cell niche

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Bone marrow niche, composed by mesenchymal stromal cells (MSC), osteoblasts and endothelial cells, play a critical role in hematopoietic stem cells (HSC) function during normal and stress hematopoiesis. Adhesive interactions, like VLA-4/VCAM-1 and LFA-1/ICAM-1 between the
HSC and bone marrow niche, regulate HSC retention in the bone marrow. Del-1 is an endogenous inhibitor of inflammatory cell recruitment by antagonizing LFA-1-dependent leukocyte adhesion to endothelial ICAM-1 and is expressed and secreted by endothelial cells. Here we found that Del-1 is expressed in the bone marrow, especially in endosteal part by MSC and endothelial cells, but not HSC. Reduced numbers of Lin- Scal1-c-kit+ (LSK+) multipotent progenitor cells (MPP) and HSC were observed in Del-1-/- mice compared to littermates. In the stress condition, Del-1-/- mice demonstrated better chemoresistance with higher HSC recovery after 7 days of 5-fluorouracil (5-FU) administration. Additionally, Del-1-/- mice showed better LSK+ mobilization after G-CSF stimulation although there were decreased numbers of HSC in steady state. Upon G-CSF administration, the level of Del-1 in endosteal is decreased, which is reminiscent of the reduced expression of CXCL12, Kit ligand and angioptoeitin1, which are important factors for HSC retention. Finally, static adhesion assay showed that alphavbeta3 integrin contributes to the interaction between LSK+ and Del-1. Our results suggested Del-1, as a negative regulator for maintenance, chemoresistance and G-CSF-induced mobilization of HSC, and blocking Del-1 could be beneficial for HSC protection during chemotherapy and for efficient HSC mobilization in the context of HSC transplantation.

**P126**

Folate and vitamin B12 reference intervals in a Norwegian population

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**Introduction:** Folate and vitamin B12 are important coenzymes of the homocysteine-degrading remethylation and transsulfuration pathways. Deficiencies of folate and vitamin B12 lead to an elevated serum concentration of homocystein. Reference intervals for serum-folate and vitamin B12 vary significantly among populations for which dietary intakes may be different. The aim of this study was to establish reference intervals for serum-folate and vitamin B12 in a Norwegian population.

**Methods:** Blood samples were taken from 324 volunteers, no blood-donors, aged 18-65. Subjects were excluded from participation if they were pregnant or taking vitamin supplements or drugs. Serum folate- and vitamin B12 concentrations were measured on the Abbott Architect i2000. Reference values were calculated using nonparametric method.

**Results:** Mean serum concentrations of folate and vitamin B12 were 13,4 nmol/l and 329,6 pmol/l respectively. Reference intervals (2.5-97.5 percentile) were calculated to 5,3-30,5 nmol/l (CI: 4,1-6,8; 26,3-32,6) for folate and 138,6-593,0 pmol/l (CI: 129,5-161,8; 534,3-620,6) for vitamin B12. A statistically significant difference between gender was observed for vitamin B12.

**Discussion:** Serum-folate levels < 8 nmol/l usually indicate folate insufficiency. According to the conclusions of a WHO technical consultation on folate (<10 nmol/l) and vitamin B12 (<150 pmol/l) deficiencies, 29% of the Norwegian subpopulation have folate- and 3,5% vitamin B12-deficiency.

**P127**

Regulatory Function of Polypolishosphate on Coagulation Factor V

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Platelet polyphosphate influences activation of the blood coagulation factor XII, an effect that we confirmed by chromogenic assay for factor XII short-chain polyphosphate (PP45). Long-chain polyphosphate (PP700), however, had weak effects. To examine the influence of polyphosphate on factors of the common final pathway in greater detail, we developed a chromogenic prothrombinase assay. In this assay, factor V and factor X react with diluted Russells viper venom reagent (dRVV) and phospholipids. The resulting thrombin converts a chromogenic thrombin substrate (Pefachrom) that is read by an ELISA reader at 440 nm. In this assay, we noted an inhibitory effect with n = 45 and 70 polyphosphate. In contrast, long-chain polyphosphate (PP700) had little influence on thrombin formation and thus on the prothrombin complex. The inhibitory effect of the polyphosphate can be detected only in the copresence of factors X and V. Polyphosphate has no effect on the incubation of factor X with dRVV reagent alone. We conclude that polyphosphate has a specific inhibitory effect on coagulation factor V. This regulatory mechanism prevents platelets from overactivation of coagulation, with little polyphosphate release. Thus, this mechanism ensures that individual degranulating platelets are insufficient to form a clot.
P128
Phthalate detection during allogenic stem cell donation
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Introduction: Phthalates are chemicals that are added to polyvinylchloride-based containers to provide them with soft and flexible properties. The use of plasticised blood bags is therefore accompanied by the possible contamination of blood products, especially with di(2-ethylhexyl)-phthalate (DEHP)- and the respective hydrolytic cleavage product mono-ethylhexyl-phthalate (MEHP). In our study, we observed the contamination of stem cell products by MEHP and DEHP.

Material and Methods: In allogenic stem cell donation (peripheral stem cell apheresis (PBSC) (n = 10), bone marrow (BM) (n = 7)), MEHP and DEHP concentrations were measured in peripheral blood (PB) samples before, during and after the donation procedure and in the respective stem cell product undergoing different storage conditions. MEHP and DEHP concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Apheresis procedures as well as the BM puncture showed no effect on the MEHP/DEHP concentrations in the PB of the donors. During storage of the stem cell products, leaching of DEHP as well as MEHP was observed dependent on storage duration: For example, the DEHP concentration in PBSC products was found to be 11.5 ± 4.9 μg/mL immediately after preparation and increased to 121.9 ± 17.3 μg/mL after 48h of storage at 4°C. Phthalate behaviour was comparable in PBSC and BM products.

Conclusion: A phthalate contamination of the peripheral blood of allogenic stem cell donors was not observed. In contrast, phthalate leaching into stem cell products was clearly proven, reaching phthalate concentrations comparable to MEHP/DEHP levels of plasma and red cell products well known from the literature.

P129
Influence of hemodilution on coagulation tests
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Introduction: In special clinical situations (e.g. traumatic bleedings) it has to be expected that blood coagulation is impaired by therapeutic side effects. So a hemodilution in order to stabilize the circulation could lead to decreased coagulation factor activities and platelet count. Therefore in this study we start to look for the effects of different hemodilutic solutions on standard coagulation tests.

Material and Methods: In the citrated whole blood of healthy volunteers (n = 6) coagulation was induced and examined by laboratory standard tests: Plasmatic clotting was observed by PT, aPTT and thrombin time (Siemens Healthcare Diagnostic, Germany) and thrombocytic clotting by PFA-100 (Siemens Healthcare Diagnostic, Germany) as platelet function test. For hemodilution physiologic NaCl solution and hydroxyethyl starch (Braun, Germany) were used.

Results: PT, aPTT and thrombin time were prolonged dependent on the degree of hemodilution: For example, aPTT was elevated from 30 ± 3 sec to 79 ± 6 sec (n = 6) at a dilution factor of 50%. Overall a linear correlation between coagulation times and degree of hemodilution could be observed. Differences between the dilution solutions could not be shown. The platelet function test was completely diminished by hemodilution.

Conclusions: Hemodilution induced clear alterations of the coagulation test systems: It was able to conclude from the alteration of the plasmatic coagulation times on the amount of hemodilution. In contrast, hemodilution diminished the platelet function test in its entirety, and therefore the degree of hemodilution could not be detected by this procedure.

P130
Acute Coronary Syndrome and primary hemostasis - risk factors in patients with manifest atherosclerosis
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Background: The acute coronary syndrome ACS is one of the leading causes of death worldwide. While risk factors for atherosclerosis are well established, it is still unclear which aspects of primary hemostasis augment the risk for an ACS in patients with manifest atherosclerosis.
Methods: We measure parameters in patients from the Leipzig (LIFE) Heart Study with confirmed coronary artery disease CAD and with or without myocardial infarction. Picturing the coagulation proteins and prothrombotic characteristics of platelets, the measurements include plasma levels of fibrinogen, Willebrands-Factor and shedded platelet glycoprotein VI as well as Ristocetin-cofactor activity, platelet count and mean platelet volume. The findings will be referred to data from genome wide association studies GWAS.

Results: The acute thrombotic event in CAD is initiated by atherosclerotic lesions or plaque rupture, but it is based on the processes of platelet adhesion and aggregation (primary hemostasis). Individual differences in platelets’ phenotype, levels of coagulation proteins and underlying genetic variability are suspected to be responsible for the occurrence of a cardiovascular event. We want to identify those links between high-risk phenotypes and their genetic determination and aim to develop a hemostatic risk profile for ACS in patients who are prone to CAD.

P131

Novel transgenic FII mouse models to functionally ablate and monitor ectopic thrombin expression dynamics by noninvasive in vivo live imaging

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Thrombin (FII) is involved in numerous physiological processes beyond blood coagulation ranging from angiogenesis, embryogenesis, to tumorigenesis and inflammation, and de-regulated FII expression (even as little as 1.6-fold) can have detrimental clinical consequences. Further FII is increasingly understood to exert various roles at the extravascular site. Although FII is extrahepatically hyper-expressed during some inflammatory processes, little is known about the role and consequences of ectopic FII production in these conditions and whether FII is driver or passenger in these processes. Here, we set out to generate novel mouse models with the aim to interrogate and functionally ablate ectopic FII expression dynamics in intact animals by means of pre-clinical non-invasive in vivo 3D-imaging, Diphtheria toxin-mediated cell depletion and inducible shRNAs directed against FII. By using these mouse models we recapitulated extrahepatic FII expression dynamics during embryogenesis, indicating that the transgenic lines are functionally fully active and are ready-to-use to monitor ectopic FII dynamics in various disease modalities. With these tools we expect to obtain important insights with the potential to systematically exploit therapeutic FII inhibition for hemostatic and non-hemostatic disease processes. Ultimately, these studies may also illuminate novel extravascular roles of FII and help to understand the potential risks and benefits of its therapeutic inhibition.

Metabolomics

P132

Metabolic Phenotyping: A New Tool Enabling Drug Response Prediction and Personalized Medicine

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Quantitative metabolic phenotyping provides individual snapshot metabolic signatures as surrogate marker of individual genetic disposition, somatic changes, acquired adaptations and exposition to pathogens, environment and alimentation. The supplied fingerprints even enable the identification of pathophysiological processes underlying a specific disease, possibly providing early stage diagnosis and prognosis yet lacking of diagnostic parameters. By applying Biocrates Kit solutions it was possible to generate standardized and quantitative data for more than 200 relevant metabolites. The analytes were quantified by triple quad mass spectrometry generating highly accurate and precise data. The highly efficient sample preparation permits analyses in several different biological matrices and low sample volumes. Another aspect is technology standardization and harmonisation. By the provided software all data can be collected and processed, allowing reliable inter-laboratory and inter-instrument comparisons, enabling retrospective analyses even with inter-instrument or inter-laboratory measurements. In contrast to single disease markers, snapshot metabolic signatures provide individual and deep system biological insights into the patients’ status, being more exhaustive. For the investigated pathology a whole panel of metabolites can be identified, helping researchers to understand the pathogenesis and clinicians to predict and monitor diseases and therapies. To underline this potential, impressing examples from cancer research and neurology will be given.
P133

The Clinical Metabolomics Facility (CMF) – accelerating translational research

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Metabolic profiling has opened new prospects to discover biomarkers in clinical samples. Clinical applications of metabolomics may provide a better understanding of pathophysiological mechanisms in disease as well as give new insights into therapeutic treatment efficacy. Currently, limited access to the state of the art instrumentation and the related scientific expertise are the major hurdles for its application in clinical research setting. To provide high quality metabolomics services for clinical research, the Clinical Metabolomics Facility (CMF) was founded at the Bern University Hospital (Inselspital Bern) with the aim to strengthen interdisciplinary research by accelerating the discovery and validation of clinically relevant biomarkers and signatures. The CMF offers a variety of analytical techniques, including liquid or gas chromatography coupled with mass spectrometry and nuclear magnetic resonance spectroscopy. Our metabolomics workflow has two major steps: in a first step, a non-targeted metabolic profiling based on a holistic, hypothesis-generating approach can be used to identify potential candidate signatures in a wide range of materials (e.g., tissues, blood, cell lysates). In a second step, discovered biomarkers are further validated by targeted metabolite analysis. Our new CMF brings metabolomics, bioinformatics and medical experts together and accelerates translational research by linking basic research, clinical diagnostics and biobanking services.

P134

Are plasma amino acid concentrations predictors of mortality in patients with liver cirrhosis?

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Objectives: In patients affected by liver cirrhosis mortality is predicted by MELD-Score that is used for organ allocation. Complications such as ascites, hepatic encephalopathy and cachexia are often observed in end-stage liver disease. Amino acids play an important role in metabolic balance. It is known that concentrations of several amino acids change during progression and complications of cirrhosis. Aim of this study was the evaluation of the prognostic value of amino acid concentrations and the identification of amino acid constellations that predict mortality.

Methods: Amino acid concentrations of 231 citrated plasma samples from 173 patients were measured by LC-MS/MS. Patients with renal replacement therapy were excluded from analyses. For all samples, corresponding MELD scores consisting of the parameters bilirubin, creatinine and INR were available.

Results: Plasma concentrations of branched chain amino (BCAA) acids especially valine decreased significantly with increasing MELD score (r=-0.279; p<0.001). In contrast, levels of aromatic amino acids (AAA) such as phenylalanine increased (r=0.314, p<0.001). The ratio of valine/phenylalanine seems to be a strong predictor for mortality in patients with advanced liver diseases (r=-0.405, p<0.001).

Conclusion: The BCAA plasma level decrease might be explained by changes in skeletal muscle metabolism whereas AAA increase may promote hepatic encephalopathy. Interestingly valine/phenylalanine ratio could be an innovative biomarker in prediction of mortality in end-stage liver disease. Further studies for identification of underlying pathophysiological mechanisms and potential treatment strategies i.e. BCAA supplementation are necessary.

P135

Role of PI3K? in inflammatory phagocytes

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Sepsis is a systemic inflammatory reaction of the immune system to bacterial infections. This depicts one of the most critical clinical states with a high rate of mortality and is frequently associated with long-term handicaps for survivors. Lipopolysaccharide (LPS, endotoxin), a surface component of gram negative bacteria, activates the immune system via the toll like receptor 4 (TLR4) and induces an inflammatory immune response. An in vivo injection of LPS may cause a systemic inflammation with sepsis-like symptoms. The pro-inflammatory cytokine

Unauthenticated
TNF-α is one of the main pro-inflammatory mediators during sepsis and throughout the initiation of septic encephalopathy. Augmented production by activated monocytes/macrophages and other leucocytes during the acute phase response causes an increase in TNF-α serum concentration, e.g. in response to bacterial substances as LPS. Phosphatidylinositol-3-kinases (PI3Ks) are key mediators of inflammatory responses and related signaling cascades. In particular the class I PI3Ks PI3Kγ and PI3Kδ, preferentially expressed in cells of the immune system, are in focus of ongoing research. Recent own data demonstrated an expression of PI3Kγ in microglia, the resident immune cell population in the central nervous system, and an upregulation of PI3Kγ expression in response to LPS stimulation. Current findings indicate PI3Kγ as mediator of LPS-induced cytokine and ROS production as well as phagocytic activity of bone marrow derived macrophages (BMDM).

P136

Metabolic Phenotyping of Bile Acids - Standardized quantitative bile acids analysis in human plasma/serum and mouse plasma using (U)HPLC-MS/MS

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Bile acid analysis provides a powerful tool for applications in precision medicine, toxicology, and clinical biomarker research. We have developed and validated a standardized (U)HPLC-ESI-MS/MS assay for the analysis of ca. 20 bile acids from only 10 μL human plasma/serum or mouse plasma samples. The panel consists of cholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid, muricholic acids and their glycine as well as taurine conjugates. 10 μL sample and 10 μL IS mixture are pipetted onto the paper filter spot suspended in a 96-well filter plate. Bile acids are extracted with 100 μL methanol, which is filtered through into the 96- deep well receiving plate under light centrifugation. 60 μL water is added to the extract before injecting into the (U)HPLC-ESI-MSMS for analysis. The analysis runtime for UHPLC and HPLC is 5 and 11 min, respectively. Bile acids detection is performed using MRM in negative ESI mode. 7-points calibration curves are used for quantitation. The assay has been rigorously validated according to the EMA guideline. Using this very simple and robust bile acids kit, the analysis of human and mouse plasma samples reveals that the bile acid profile of mice is quite different from that of human. While taurine conjugates of bile acids are prevalent and glycine conjugates are almost absent in mouse plasma, the situation is reversed in human. Moreover, the male/female differences found in mouse plasma is much more profound than that found in human samples.

P137

Distinct urinary metabolic phenotypes associated with TSH levels and FT4 serum concentrations

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Background: Thyroid hormones (THs) affect virtually all tissues and are essential for maintaining energy metabolism, cellular growth and development. Their release depends on a complex feedback regulation including thyrotropin (TSH) out of the pituitary, offering a unique individual set point compared with a broad interindividual variance. The aims of the present study were firstly to screen the urine metabolome for associations with serum TSH and free thyroxine (FT4) concentrations and secondly, in an attempt to join their metabolic associations and taking into account a tight individual set point, to analyze the relations with the ratio log(TSH)/FT4.

Methods: The urine metabolome of 3327 participants of the population-based Study of Health in Pomerania was characterized by 1H-NMR spectroscopy. Subsequently, multivariate linear as well as multinomial logistic regression models were used to detect possible associations.

Results: We observed differing association patterns for serum TSH levels or FT4 concentrations. Associations with circulating FT4 included various amino acids as well as citrate, formate and ethanolamine, whereas TSH was associated with members of tyrosine metabolism. Overlap existed towards glycine, histidine and glycolate. The log(TSH)/FT4 ratio was able to mirror many of the associations and revealed new associated metabolite ratios towards glycine and succinate.

Conclusion: Our findings confirmed metabolic consequences of TH actions, thereby emphasizing the need for distinct interpretation of associations related to serum TSH or FT4 concentrations. In particular, the log(TSH)/FT4 ratio was able to join their metabolic impact, probably offering a new path for thyroid function characterization.
P138
Determination of 6-α-D-glucopyranosyl-maltotriose in Pompe disease by LC-MS/MS and correlation with the genotype

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Background: Pompe disease, also classified as glycogen storage disease type II (GSD II), counts to the rare lysosomal storage disorders with an incidence of 1 in 40000 births. As a biomarker for Pompe disease the tetrasccharide 6-α-D-glucopyranosyl-maltotriose (Glc4) was identified.

Method: A LC-MS/MS method was established on a Thermo TSQ Quantum system in order to measure Glc4. Calibration was achieved using standardized urine spiked with raffinose as internal standard and Glc4. For sample preparation 75 μL urine, control and standard were diluted with water and spiked with internal standard. After centrifugation 20 μL of supernatant was injected. The chromatographic separation was achieved by a hypercarb column using gradient elution.

Results: To separate Glc4 chromatographically from other tetrasccharides, one run took 14 minutes. The method was linear from 0,25 – 500 μmol/L, the intra-assay CV was 2,3 % and the inter-assay CV at 50 μmol/L (respectively 15 μmol/L) was 6,5 % (5,2 %).

Conclusion: The established LC-MS/MS method allows the specific and sensitive analysis of Glc4 in urine. Furthermore, previous results show predominantly a good agreement between the Glc4-concentration and the severity of mutation in the GAA-gen.

P139
Plasma Sphingosine-1-Phosphate Levels and Coronary Artery Disease: Results from the Leipzig Heart-Study

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Objectives: Coronary artery (CAD) disease is the number one cause of death in developed countries. Sphingosine-1-Phosphate (S1P) is related to inflammatory processes as well as to myocardial ischemia.

Methods: We conducted a study nested within the Leipzig Heart Study including 184 cases with coronary stenosis grade III and 230 coronary healthy controls. S1P levels were determined in plasma after protein precipitation by hydrophilic interaction liquid chromatography tandem mass spectrometry. Differences in S1P levels were calculated by the Mann-Whitney-U-Test.

Results: After adjustment for age, BMI, gender, smoking and diabetes, median levels of plasmatic S1P in cases (184.6 ng/mL) were significantly decreased compared to the control group (186.6 ng/mL, p=0.025). The normalization of S1P concentrations to HDL-cholesterol (CH) levels resulted in higher levels in the case group (0.38 ng/μg CH vs. 0.40 ng/μg CH, p=0.016).

Conclusion: S1P levels in EDTA-plasma differ significantly between CAD patients and coronary healthy subjects. It has to be further investigated whether prediagnostic S1P levels could serve as marker to predict risk for the development of CAD.

DVTA POSTER
Aus Wissenschaft und Forschung

P140
Technique-affinity and e-learning behaviour in continuing vocational training

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With MySpace, Wikipedia, Facebook and StudiVZ the new millennium brought us one with the term Web 2.0 combined virtual world, which reveals new social qualities through their new architecture of communication [1]. Even the professional everyday life is peppered Web 2.0 [2]. Terms such as e-learning, tele-learning and online learning that are considered new web-based opportunities for professional qualification are established. New forms of learning back into the focus of adult education. Assistant medical technician whose professional environment is characterized technologically, eg if there concern the functioning and operation of innovative devices in radiology or laboratory should have no fears with this new technological world. In the study, the affinity for technology and e-learning behavior based on pre-determined research hypotheses in the target group of the MTA occupations were explored empirically. The aim was to formulate on the basis of the results of research, requirements for a range of qualifications in vocational training for MTA professions to simultaneously counteract the training needs previously identified. The training needs arising, inter alia, be as a result of...
a new task profile and the associated new requirements

- a future skills shortages
- increasing demand for medical technology and skilled personnel to operate [3,4].

The evaluation of the expert interviews was used to develop the items for the standardized questionnaire, which was prepared as an online survey connected to the target group. The results should lead to answer the question whether adult education based on e-learning is suitable for the target group. Initial analysis of the research results give a classification of respondents in five technology groups, which were named according to their forms as “Technikaffine” (technology affine), “Technikinteressierte” (interested in technology), “Technikakzeptierende” (technology is accepted), “Technikunentschlossene” (wavering) and “Technikverweigerer” (technology objectors). The evaluation of all the results cannot determine a relationship between the sizes affinity for technology and e-learning open-mindedness. However, there is a trend that tech-affine MTA professions tend to a more positive attitude towards online learning. It is interesting that less technology experienced want to participate in an exciting online training for them. They want more online training, which is used in the continuing training of the MTA occupations little. The open questions of the online survey underpin, that participation in e-learning courses is subject to specific criteria.

References


P141

Analysis and Appearance of Physiological Amino Acids in Nature - From Serum to Vineyard Soil -

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This diploma thesis of the discipline ‘Biomedical Analysis’ aimed to establish a method for analysis of physiological amino acids from extracts of vineyard soil. This is the first time an analysis of free amino acids took place in the soil extracts of the intensely researched vineyard ‘Schloss Vollrads’ in the Rheingau of Germany by the Hochschule Geisenheim University. The commitment of the different validation parameters happened according to the European Pharmacopoeia and to the Official Journal of the European Communities for analysis of amino acids in feedingstuff. For the detection of the amino acids in a picomol range, an amino acid analyzer with a post-column ninhydrin derivatization was applied just like a post-column derivatization with orthophthaldialdehyde. The successful certification of 30 physiological amino acids from lyophilized serum was undertaken for this purpose in 2013 at ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) in a clinical worldwide comparison of methods at monthly intervals over one year by using the post-column ninhydrin derivatization. As a significant result a strong increase of physiological amino acid nitrogen appeared in high mineralic manured vineyard soil surfaces in springtime 2013. In conclusion, high application of mineralic fertilization causes an impact of the appearance and the composition of physiological amino acids in vineyard soil.

P143

Effect of one year B and D vitamins supplementation on telomere length in elderly

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Background: Telomere length declines with age and telomere dysfunction has been proposed as a biomarker for age-related diseases. B and D vitamins are essential cofactors for numerous cellular processes and their deficiencies are risk factors for the development of age-related diseases. The aim of this study was to evaluate the effects of B and D vitamin supplementation on telomere biology in elderly people.

Methods: In a double-blind study 60 subjects (>54 years) were randomly assigned to receive a daily combination of vitamin D3 (1200IU), B12 (0.5mg), B6 (50mg), folic acid (0.5mg), and calcium carbonate (456mg) (GroupA) or vitamin D3 and calcium carbonate (GroupB) for 1 year. Fasting blood was collected at baseline and after 1 year.

Results: Baseline gender- and age-adjusted telomere length correlated with methyltetrahydrofolate (r=0.35), 5,10-methylenetetrahydrofolate (r=0.36) and total folate (r=0.33). At the end of the study gender- and age-adjusted telomere length showed the following correlations: GroupA:
methylmalonic acid \((r=-0.46)\) and choline \((r=0.39)\); GroupB: 5,10-methylenetetrahydrofolate \((r=-0.57)\), dimethylglycine \((r=-0.39)\), and LINE-1 methylation \((r=-0.43)\).

**Conclusions:** The present results provide evidence for an active involvement of D and B vitamins in telomere biology. One year of B and/or D vitamin supplementation changes the pattern of correlations observed at baseline. The inverse relationship between telomere length and DNA methylation could be an explanation that links telomere length with B vitamins.

**P144**

Testing of the in vitro-effects of UV-C-irradiation, thyme oil and Bprotect C on the growth of Hypomyces rosellus

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Hypomyces rosellus belongs to the phylum of sac fungi and is a vermin in the cultivation of edible mushrooms. His presence on the soil is quite disliked by the agriculturalists, because losses in revenue come along with it. The aim of our study was to test physical and biochemical ways to arrest the growth of the pathogen. All experiments where performed by both project partners and each carried out a triplicate-testing on two kinds of agar plates (Potato-dextrose-agar, Corn-meal-agar) simultaneously. We tested increasing irradiation times of UV-C-light as a possible physical inhibitor of the mold and thyme oil as well as a plant strengthening agent called Bprotect C to examine possible biochemical antagonists. For thyme oil and Bprotect C the effect was investigated with the help of two kinds of methods called disk diffusion assay and agar dilution test. The usage of pure thyme oil and Bprotect C indicated good inhibitory effects in vitro, whereas the results of the diluted fluids showed a little variance according to the used method. Furthermore UV-C-light was proven to have a good germicidal effect on H. rosellus. For follow-up in vitro-testings, the examination of mold, taken directly from germ-infested soils or champignons, would be a further step towards the implementation of UV-C-light, thyme oil and Bprotect C in the fight against H. rosellus. Subsequently the examination of the practicality of our tested inhibition methods in the field should be studied further.

**P145**

Rapid identification of microorganisms in urine using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS)

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The straight identification of microorganisms by using mass spectrometry is a new approach within microbiological laboratories. This whole new perspective could lead to quicker diagnoses and earlier, better calculated antibiotic therapies. The focus of the project, which was performed in the Klinikum Klagenfurt for three months, was the analysis of germs out of urine samples using the MALDI-TOF MS (Bruker Daltonik). The main goal was to test this method and create a convenient protocol. Only the samples which showed a germination number of \(= 10^5 \) /ml after being run through the UF-1000i (Sysmex) \((n=497)\) were used for further tests \((n=202)\). After working with the formic acid ethanol extraction method, the samples were tested with the mass spectrometry technique. Regular microbiological methods, such as the common growth method or possible further biochemical analyses, provided the necessary references. Results showed that in 60% of all samples it was possible to perform at least one germ identification at a genus level within two to four hours of receiving the specimen. It also appeared that samples with higher germination number increased the chance of identification \((10^5 / \text{ml} \approx 38.5\% / 10^6 / \text{ml} \approx 63.2\% / 10^7 / \text{ml} \approx 88.5\%)\). In the case of polymicrobial samples the maximum of only one germ could be identified. This study led to six misidentifications. This method would absolutely be suitable within routine study, extending its repertoire beyond using the current techniques.

**P146**

Study on the Professionalization in Biomedical Laboratory Science in Germany

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Biomedical laboratory science (BMS) is an interdisciplinary subject between natural science, medicine and engineering. The aim of this investigation as a sociological analysis is to analyze the tasks of the BMS practitioner in process of the biomedical analytics. Furthermore, it
shall be investigated to which extend these tasks can be carried out by the Biomedical Scientists and how the professional decision-making and responsibility for the execution of the tasks is assessed. Characteristics of biomedical analytics as a profession were compared to a model/theory of professionalization developed by Oevermann and other to assess the extent to which biomedical laboratory science has acquired attributes of a profession.

**P147**

Empirical study for testing the cleaning efficiency of Hollu chemical agents compared with Enjo fibre-products used only with clean water

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Cleaning is the process of removing contamination and conduces to cleanliness and preventing of infection. Cleaning surfaces should lead to a reduction or removal of bacterial count and to a prohibition of bacterial proliferation. Chemical cleaning agents contain several substances that can harm the environment and the user. This is why cleaning only with water has advantages over cleaning with chemical agents. The aim of this study is to find out whether cleaning with cleaning cloths and clean water only is as efficient as cleaning with chemical agents. Therefore Enjo fibre-products are compared with Hollu chemical agents in two experimental arrangements. On the one hand, the cleaning efficiency is tested in a kindergarten, where the cleaning products have to remove an existent mixed bacterial flora, on the other hand the cleaning efficiency is tested under controlled laboratory conditions, where the cleaning products have to clean surfaces which have before been contaminated with Escherichia coli. The outcomes of the study are compared and discussed with other existing studies. In the kindergarten the Hollu cleaning agents reached a mean microbe-reduction of 63%, whereas the Enjo-fibres reached a mean microbe-reduction of 74%. Under controlled laboratory conditions both cleaning methods reached a mean microbe-reduction of nearly 100%.

Summing up, on the basis of the outcomes of this study one can say that cleaning with Enjo fibre-products is at least as efficient as cleaning with chemical cleaning agents.

**Entwicklungsprojekte aus der Laborpraxis**

**P148**

The optional certificate for further education for acceptation continuing education relating to medical technologists

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The learning of adults is a lifelong process of socialization, cultural imprint, the way of becoming an individual and of the process of forming the identity. With a view to the information and knowledge society which is connected with further education and qualification, it is important to support the potential of personal development and possibilities to learn, especially in the adulthood [1]. Education is the most significant resource while competing for a job, especially in the industry sector or other sectors which require a wide educational background [2].

The always changing structures in working life execute a certain pressure on the employee, who has the duty to refresh their profiles of qualification. It is the same in the job profile of medical technologists [3]. The DIW-MTA believes in the term of Life-Long-Learning. Together with the professional association DVTA, the DIW-MTA initiated the optional certificate for further education for medical technologists in the year 2009 in order to support a documentation of the own activity in the field of advanced training as well as including methods which proof the quality of what is taught. The participants appreciate the offered opportunities with high interest, which can be seen on the following information:

More than 1,250 registered participants, more than 6,000 validated courses, more than 500 awarded certificates within four years. Among the technical realization as an online-tool and other numerous innovative implementations (e.g. the use of bar codes, the integration of new ways of learning like e-learning or job shadowing), a palette of elements for quality assurance have been developed. The optional certificate supports the postulate of Life-Long-Learning and the acceptance of professional and soft skills. The autonomy of every individual person and the own engagement are also underpinned. The concept is in principle transferable to other careers.

**References**


Ausbildung in Theorie und Praxis

P155

Excellence and System Manager in Health Care in the tradition of a transformative education

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Scientific training allows affiliation to current training needs, and is good for an advanced subject-specific training and for job specialisation. Offers in further education which apply to health care spread out. That is a hint for the needs of qualification related to the target group [1]. Training organizations are confronted with the challenge of demand-supply planning, to qualify, professionalize and prepare adequate study material, taking account of the development of health care and the labor market. The penetration of various management system concepts in health care facilities is a reality [2]. But so far lacks an overarching and unifying all management systems training which will meet this structural change. The second problem is, that with the transition to a knowledge and information society the concept of education as a reference category for the establishment and pedagogical preparation of training opportunities will be restructured. How can the new critical handling of information be realized? The DIW-MTA has taken up the issues in the planning model of the new study course. Participants complete a part-time modular study. The course entitled to use the designation “Excellence and System Manager in Health Care (DIW MTA)”. Education in the sense of a transformation takes place when people have experiences that are not sufficient to overcome them their previous ways so that new figures from the world and self-relationship arise [3], to deal with professional problem situations. The implementation of transformative education processes happen e.g. through the integration of an internship with the networking of students, internship site, lecturer and the DIW-MTA as a learning organization and exchange of experience with a reflexive feedback on their action expertise. Also reactions of the learning processes at the micro-didactic level and the selection of appropriate learning environments play a crucial role for a transformative education. The interdisciplinary approach tries to recognize synergies through the application of integrated management systems, which aim to set common elements across different systems interact. The study course “Excellence and System Management” combines, among other things management systems: quality-, organizational- and hygiene management, regulatory affairs, environmental-, energy-, and safety management, IT security management, so that learners are empowered in middle and senior management of health just these management systems are not detached from each other, but to build an integrative.

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