Abstracts*)

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Personalisierte Medizin-Onkologie

V01 – Talk Kloke

Development of individualized Immunotherapies – from bench to bedside

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Mutations are regarded as ideal targets for cancer immunotherapy. As neo-epitopes with strict lack of expression in any healthy tissue, they are expected to be safe. The systematic use of mutations for vaccine approaches, however, is hampered by the uniqueness of the repertoire of mutations ("the mutanome"). We have recently proposed a personalized immunotherapy approach targeting the spectrum of individual mutations. Pre-clinically we could show in three independent murine tumor models that a considerable fraction of non-synonymous cancer mutations is immunogenic and that unexpectedly the immunogenic mutanome is pre-dominantly recognized by $CD4^+$ T cells ("the $CD4^+$ immunome"). Vaccination with such $CD4^+$ mutations confers strong anti-tumor activity. Encouraged by these findings we set up a process comprising mutation detection by exome sequencing, selection of vaccine targets by solely bioinformatical prioritization of mutated epitopes predicted to be abundantly expressed and good MHC class II binders and rapid production of synthetic mRNA vaccines encoding multiple of these mutated epitopes. We show that vaccination with such poly-neo-epitopic mRNA vaccines induces potent tumor control and complete rejection of established aggressively growing tumors in mice. End of 2013 this approach has been translated from bench to bedside when a first in human clinical study started demonstrating the clinical feasibility of the approach. This tailored immunotherapy approach may be regarded as a universally applicable blueprint for comprehensive exploitation of the huge neo-epitope target repertoire of cancers enabling the treatment of patients by targeting every patient's tumor with individual "just in time" produced vaccines.

Referenzintervalle und Leitlinien-Entwicklung

V02 – Talk Rauh

Establishment of continuous, age-related reference intervals for children, using an indirect statistical approach on data from multiple centers

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Background: Laboratory test results in children and adolescents have to be interpreted in the context of age- and gender-dependent dynamics. The current concept of reference intervals (RI) as presently defined for separate age groups can only approximate the age-related dynamics encountered in pediatrics. Indirect methods address these issues by deriving RIs from clinical laboratory databases.
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 635,367-635,619 individual samples contributed by 4 diagnostic laboratories. Patient samples were binned into 197 overlapping age intervals, and a density function of the proportion of healthy samples was estimated for each age group.
Results: The resulting RIs were merged and continuous percentile charts were calculated and plotted. The majority of the quantities analyzed showed substantial age-specific dynamics, especially in the first months and years of life and after the onset of puberty.
Conclusions: Disease markers depending strongly on covariates such as age and sex require large numbers of reference individuals to establish continuous reference intervals and peripheral percentiles with sufficient precision. This is feasible through the use of indirect methods and collaborative data sharing.

Labormedizin in der Gesundheitsvorsorge

V03 – Talk Friedrich

Vitamin D from an epidemiological perspective

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Recent clinical and experimental studies suggest that vitamin D in addition to its crucial function in bone metabolism also could play an important role in the pathogenesis of cardiovascular disease, cancer and autoimmune diseases. These findings together with the world-wide high prevalence of vitamin D deficiency represent a major economic burden on the health system. However, the positive effects shown by some meta-analyzes or randomized controlled trials of vitamin D supplementation on fractures, tendency to fall or mortality are currently a subject of controversial discussion due to the fact that recent studies partially repudiated these findings. The talk attempts to summarize and evaluate new results from clinical and epidemiological studies with respect to vitamin D.

V04 – Talk Winter

3 years of Cystic Fibrosis screening for Newborns in Mecklenburg-Western Pomerania

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Objectives: Despite being part of screening programs in many EU states, the Cystic fibrosis newborn screening (CF-NBS) is not part of the German screening panel. To gain further practical experience in terms of feasibility within the German newborn screening system, the CF-NBS was implemented state wide in Mecklenburg-Vorpommern (MV) starting in October 2012, free of charge in due course of an EU founded Interreg IVa project.
Methods: First, the immunoreactive trypsinogen (IRT) was analyzed. Results > 55 ng/ml (98.5Perc) resulted in the pancreatitis associated protein (PAP) analysis. CF-NBS screening was positive, if IRT and PAP (>1.0 ng/ml / 1.8 ng/ml, IRT dependent) were pathological. A fail safe strategy (IRT > 140 ng/ml (99.9Perc)) was additionally implemented. According to the AWMF-guideline, sweat tests were strongly recommended to perform in certified CF centers.
Results: Between January 2013 till March 2015, 27,121 newborns were screened for CF (94% of all newborns in MV). 544 had an increased IRT and were tested for PAP. For 127 (0.5%) newborns, a sweat test was recommended. 15 failed to turn up/refused, 112 (88%) sweat tests were performed and five children with CF were detected within an age range of one to three months. 107 of 112 sweat tests were performed in certified CF centers.
Conclusion: The high participation rate proves wide acceptance for clinics and parents. Further improvement can be achieved by fewer false positives (e.g. cutoff adjustments) and examination of suspected newborn in accordance to the AWMF-guideline. The MV trial gives valuably experience in terms of relevant cutoff values, laboratory workflows and guidelines approved confirmation steps and will help in the nationwide implementation in the near future.

V05 – Talk Rigter

Prioritize genetic testing for high risk of serious disease over whole genome sequencing

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Objectives: Whole genome sequencing promises to improve the predictive ability of genetic testing. At present, analysis of the sequence is still highly demanding in terms of resources (personnel, equipment, databases of gene variants with phenotype). The presentation will discuss how to prioritize tests with clinical utility.
Methods: Recent policy literature from Europe and USA is summarized and discussed.

Results: Resources are currently too limited to fund all the beneficial genetic testing services available in the next decade. According to EUROGENTEST/PPPC-ESHG, prioritization should be based on considerations of medical benefit, health need and costs. The American College of Medical Genetics and Genomics recommends that laboratories performing clinical sequencing seek and report mutations of specified classes or types in genes, all of which involve serious disease with a high recurrence risk (BRCA, Lynch, HCM). Although the setting proposed (always reporting as incidental finding) has been highly disputed, the list may give insight in the type of disorders to prioritize. Later, informed choice was included in the model, and the approach in minors adapted. The ESHG adds that the use of genome-wide analysis requires a justification in terms of necessity (the need to solve a clinical problem) and proportionality (the balance of benefits and drawbacks for the patient). The primary objective of genetic screening using WGS should be the targeted analysis and identification of gene variants conferring a high risk of preventable or treatable conditions.
Conclusion: Sequencing technologies open many new possibilities. Targeted analysis of genes with potential for prevention in high risk of serious disease should be prioritized.

Biobanking und Big Data

V06 – Talk Mooser

High Participation Rate in a Systematic, Hospital-Based Biobank with Broad Consent

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Background: Little is known about hospital patient's willingness to engage into genomic research.
Methods: Within the framework of the Lausanne University Hospital Institutional Biobank (BIL), inpatients are systematically invited to grant researchers access to their clinical data and to donate blood for future genomic analyses. Participants are offered the options to be re-contacted in case of incidental findings and to receive an electronic newsletter. Multivariable logistic regression analysis was used to identify personal factors associated with willingness to participate in BIL and with interest in these options. Analyses were restricted to the initial 11099 invited patients for whom full dataset was available.
Results: Overall participation rate was 82.4% (9141/11099) and was higher in the < 64-year old group (odds ratio [OR] 1.70; 95% CI 1.53 to 1.90). In the = 64-year old group, participation was lower among women (OR 0.77; 95% CI 0.68 to 0.89), among non-Swiss citizens (OR 0.66; 95% CI 0.55 to 0.79) and those with emergency admissions (OR 0.59; 95% CI 0.51 to 0.69). A total of 8576 (93.8%) and 3020 (33.0%) participants were willing to be re-contacted for incidental findings and to receive the newsletter, respectively.
Conclusions: A large proportion of patients are willing to actively participate in this hospital-based genomic research program. Hospitals adopting broad consent represent an efficient setting to recruit participants into precision medicine initiatives.

V07 – Talk Haferlach

Next Generation Sequencing and DNA Storage: Who, how, and what?

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Objectives: The need for molecular diagnostics in haematology is increasing. This is caused by new findings in the recent years with respect to molecular changes. In addition, many new targeting drugs are developed. Better and targeted treatment leads to longer survival, in some cases cure. Therefore the investigation of *minimal residual disease* (MRD) is also increasing.
Methods: Next generation sequencing is soon a routine method in hematology. It allows not only to investigate single genes with high throughput and acceptable turnaround time but also leads to patient specific or disease specific use of gene panels for sequencing, even whole *exome sequencing* or *whole genome sequencing* are applied in selected cases. In addition, viable cells, cell pellets, or DNA or RNA have to be stored for future investigations or for backtracking.
Results: The number of genes and the respective mutations as well as the samples exploded in the last years, lot of software tools and new instruments have been developed and implemented already in routine diagnostics. However, it is crucial to discriminate between real mutations, variants in the genome with unknown significance, and polymorphisms or SNPs without any meaning for the diagnosis of a *malignant hematological disease*.With respect to biobanking automatic systems, completely controlled by barcode readers.
Conclusions: Next generation sequencing allows to investigate hundreds of genes in parallel. Software tools need to help us for interpretation of data before the results can be delivered to patients and doctors. Storing of material is mandatory for future studies as well as for backtracking. It's a big challenge to organize all that.

Kardiovaskuläre Biomarker und Konsequenzen

V08 – Talk Kronenberg

Lipoprotein(a): renaissance of an atherogenic lipoprotein

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Plasma lipoprotein(a) [Lp(a)] is a quantitative genetic trait with a very broad and skewed distribution which is largely controlled by genetic variants at the *LPA* locus. Based on genetic evidence provided by studies conducted over the last two decades, Lp(a) is currently considered to be the strongest genetic risk factor for coronary heart disease (CHD). The copy number variation of kringle IV in the *LPA* gene has been strongly associated with both Lp(a) levels in plasma and risk of CHD, thereby fulfilling the main criterion for causality in a Mendelian randomization approach. Alleles with a low kringle IV copy number which together have a population frequency of 25–35% are associated with a doubling of the relative risk for outcomes, which is exceptional in the field of complex genetic phenotypes. Drugs that have been shown to lower Lp(a) have pleiotropic effects on other CHD risk factors and more and more interesting options to lower Lp(a) became recently available. It has been established in proof of principle studies that lowering of very high Lp(a) by apheresis in high-risk patients with already maximally reduced LDL cholesterol levels can dramatically reduce major coronary events. We can therefore expect interesting studies in the upcoming years which will hopefully deepen our knowledge on this highly atherogenic lipoprotein.

Inflammation

V09 – Talk Herwald

Host-parasite interactions under systemic conditions

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Despite modern antibiotics, bacterial infections account for a considerable clinical burden that is often combined with high morbidity and mortality rates. Though the molecular mechanisms behind invasive and severe bacterial infections are far from being understood, it is generally believed that dysregulated immune reactions play an important role in these states of disease. In severe infectious diseases, the host response is because of the rapid progression almost entirely dependent on the innate immune system. Considering the amplitude of some innate defense mechanisms, their selectivity for pathogenic microorganisms is not always guaranteed and thus, there is a risk that some immune reactions may also have a deleterious impact on the host. For instance, modulation of pro- and anti-inflammatory cascades, hemostatic disorders, and impairment of vascular integrity can contribute to a great deal to the pathogenicity of the disease process. A better understanding of the molecular mechanisms behind the induction of such complications, will open new possibilities for the discovery of more advanced strategies to develop novel anti-microbial treatments and tools for diagnosis.

Volkskrankheit Allergie? Sinn- und Unsinn von Labordiagnostik

V10 – Talk Treudler

Component resolved diagnostics in food allergy

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Component-resolved diagnostics (CRD) utilize purified native or recombinant allergens to detect IgE sensitivity to individual allergen molecules and have become of growing importance in clinical investigation of IgE-mediated allergies. In food allergy, CRD allows to some extent to discriminate between clinically significant and irrelevant sIgE results and to establish sensitisation patterns with particular prognostic outcomes. Overall, CRD may decrease the need for provocation testing and may also improve the specificity of allergen specific immunotherapy. The important plant food allergens belong to a few protein families, the most well-known of which are the Bet v 1 homologues, lipid transfer proteins (LTP), profilins and storage proteins. Great progress has already been made in applying the CRD to well-defined patients' collectives

verified by DBPCFC. For example, sensitisation to the storage protein Ara h 2 (peanut) is associated with severe immediate-type reactions and sensitisation to the storage protein rTri a 19 (?-5-gliadin; wheat) is observed in wheat-dependent exercise-induced anaphylaxis (WDEIA). By contrast, sensitisations to Bet v 1 homologues Mal d 1 (apple) or Cor a 1 (hazel) correlate with clinically mild reactions. There are also efforts, to apply CRD in targeted specific immunotherapy in patients with certain food allergies (i.e. against Cyp c 1 from fish or Pru p 3 from peach).

V11 – Talk Renz

Molecular Allergy Diagnostics

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The in-vitro allergy diagnostics is rapidly advancing. This is primarily due to the development of component based diagnostic tools. The availability of allergen components now allows a more precise and patient-tailored diagnostics which has implications for therapeutic strategies including the decision about specific immunotherapy. Furthermore, the differential diagnostics of food intolerances and food allergies is also advancing due to novel test. Another area of advancement is the cellular diagnostic, primarily based on the basophile activation tests. These recent developments will be discussed in this article.

BNLD-Session: Modernes Hygienemanagement

V12 – Talk Gässler

Rapid and completely Laboratory Diagnostic is essential, for example MRSA-Diagnostic

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Generally MRSA-Infections means increasing activity in treatment and care with the patient and growing costs for therapy, ca. 15.000 € in direct costs. Each other spreading induce additional costs and damaged the reputation of the hospital. Additional costs are indirect costs, e. g. procedures for insulation the patients and others more.
Methods: MRSA-Screening takes place in our central accident and emergency department. The time for the admission of patient is insignificant longer. If the result is positive, the patient has to be insulated. In our laboratory we use the system "Xpert MRSA-PCR" from Cepheid, a single-locus PCR. After lysis of samples and transfer in a test catridge everything happens automatically, viz. after 60 minutes the result is ready. Parallel the traditional bacteriology diagnostic is proceeded as normal.
Results: Until now we have tested 115 patients with PCR-Screening; 88 results of these are negative. All these results were confirmed with our traditional methods (negative predictive value 100 %). The other 27 results are positive. Five results were not confirmed with the culture methods (positive predictive value 81,5%).
Conclusion: We could show that we didn't have to insulate 15 patients or we could finish the insulation two days earlier. For these MRSA-positive patients we had costs of 19.950 €. If the indirect costs, e. g. for more personal activity in care, additional costs for material and less capacity for free beds, are considered, the value must be multiplicated with an factor "X".

Validierung oder Verifizierung-Qualitätsanforderungen an Testentwicklungen

V13 – Talk Gurr

Method validation and verification-data cemetry or decision support?

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DIN EN ISO 15189 and the „Richtlinien der Bundesärztekammer für die Qualitätssicherung in medizinischen Laboratorien (RiliBÄK)" are demanding for the validation of analytical methods. Validation (or verification, if a validated method shall be installed in a medical laboratory)

means that it has to be be checked whether the method fulfills the diagnostic requirements. Unfortunately both DIN EN ISO 15189 and the RiliBÄK neither specifies the experiments being indispensible parts of validation procedures nor specifies what the diagnostic requirements of an analytical procedure are. In consequence, frequently the validation procedures performed by the users did not lead to valid results and assessments of the data were lacking. Validation procedures are described elsewhere (1) and standards of assessment to judge e.g. uncertainty of measurement are published recently (2). The lecture will give ideas of a) which experiments are necessary for method validation and verification procedures (e.g. which kind of imprecision), b) how to perform the experiments (e.g. how much tests per series), c) how to assess the results, and d) discuss conclusions to be drawn from the assessments. (1) Haeckel R, edt. Evaluation methods in laboratory medicine. VHC Verlagsgesellschaft 1993 (2) Haeckel R et al. Clin Chem Lab Med 2015; 53:1161-71

V14 – Talk Streichert

New model for defining performance criteria

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Objectives: The first EFLM Strategic Conference on "defining analytical performance goals" in 2014 focused on three models for defining analytical performance goals in laboratory medicine. Model 1 based on the effect of analytical performance on clinical outcomes addresses clinical needs but is limited by complex studies and restricted to selected analytes. Model 2 uses biological variation and could be applied to most measurands nevertheless current data on biological variation are insufficient. Model 3 defined by the state-of-the art criteria is based on empirical data but might not relate to what is needed. A working group of the German Society of Clinical Chemistry and Laboratory Medicine (DGKL) proposes a combination of model 2 and 3 to overcome some disadvantages inherent to both models.
Methods: The biological variation is derived from the reference interval and used for the calculation of the permissible analytical uncertainty. The permissible imprecision is not defined as a constant proportion of biological variation, but by a non-linear relationship between permissible analytical and biological variation. Furthermore, the permissible imprecision is referred to the target quantity value.
Results: Deriving biological variations from the reference ranges leads to results correlating well with the values taken from the literature (Database, Ricos et al.) and the derived permissible analytical uncertainty clearly corresponded to the RMSD of the RiliBÄK 2008 (column 3, Table 2a).
Conclusion: The empirical (biological) variation derived from the reference range could serve as a surrogate for the biological variation and thus for the determination of permissible analytical uncertainty.

V15 – Talk Zauke

Verification and validation on the part of the IVD manufacturer

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Objectives: IVD industry is operating in a highly regulated environment in Europe as well as for example in the United States. The performance characteristics of IVD products must be extensively verified and validated to meet these demands.
Methods: Numerous guidelines from non-governmental and noncommercial organizations exist. It is generally recognized that guidance documents from CLSI (Clinical and Laboratory Standards Institute) are most accepted by government agencies, such as the FDA in the US to prove assay performance in Verification and Validation studies. Internal Standard Operating Procedures in the IVD industry are aligned to adhere to continuously developing requirements from such guidelines.
Results: To support standardized experimental protocols across different technologies for Clinical Chemistry, Immunology, Coagulation etc., industry is using up-to-date statistical methods and in house developed Lab IT tools thereby exploiting connectivity to the automated lab analyzers. Areas of application comprise diagnostic accuracy, precision, analytical specificity including interfering substances, diagnostic specificity and assay sensitivity assessment (detection as well as quantitation limits, diagnostic sensitivity).
Conclusions:. In IVD industry processes are in place to support Verification and Validation activites in R&D and Clinical Study Management Teams.

Immunhämatologie und cell signalling

V16 – Talk Kracht

Inflammatory signal transduction networks

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Soluble mediators secreted by cells of the innate immune system are fundamental drivers of inflammation. A central factor in this scenario is interleukin(IL)-1 which plays a key role in sterile but also pathogen-driven acute and chronic inflammatory diseases. In most cell types, IL-1 induces a fast, strong and transient expression of 50-250 genes many of which have established functions in signal transduction and in initiation or resolution of inflammation. Our lab has a long-standing interest in the comprehensive understanding of the signal flow from the IL-1 receptor to the level of chromatin, mRNA synthesis and translation. Based on existing knowledge, we have compiled a canonical map of the IL-1 pathway that includes 86 molecules involved in 186 reactions. Using logical operators we constructed a mathematical model which can simulate activation or inhibition of IL-1 signal transduction. These maps visualize all known components and their connections and can be continuously extended. To find further signaling elements we have performed time kinetic mass spectrometry screens for major post-translational modifications. Upon IL-1 treatment, 1,710 proteins showed a twofold regulation of at least one phosphorylation site. 749 or 197 proteins were inducibly modified at at least one lysine residue by ubiquitination or acetylation, respectively. Further in depth filtering and bioinformatics analysis revealed that most of these proteins have not been implicated in IL-1 biology. In conclusion, our results suggest that the number of intracellular control points by far exceeds that of extracellular mechanisms. Thus, intracellular signaling nodes offer multiple new ways of therapeutically targeting the IL-1 response in inflammation.

Frühe Indikatoren des metabolischen Syndroms

V17 – Talk Jonas

Modulation of liver fat content and its impact on the metabolic syndrome

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Objectives: Type 2 diabetes (T2D) is the result of chronic insulin resistance and loss of functional pancreatic beta-cell mass. Interventions such as caloric restriction and estrogen supplementation are known to improve insulin resistance in several species. The aim was to test the hypothesis that fasting and estrogen mediate protective effects on T2D by modulating liver fat content.
Methods: New Zealand Obese (NZO) mice, a model of polygenic obesity and T2D were put on (1) dietary interventions or (2) treated with estrogen. Liver fat content, hepatic diacylglycerol (DAG) concentration and blood glucose levels were analyzed.
Results: NZO male that had ad libitum (AL) access to a high-fat diet showed a diabetes prevalence of 43%, whereas mice that were either 10% caloric restricted (CR) or fasted every other day (IF) were completely protected against hyperglycemia. In addition, accumulation of DAGs was markedly diminished in livers of CR and IF mice. This effect was associated with a decreased PKCe activation and might explain the improved insulin sensitivity. By computed tomography we furthermore demonstrated that early liver fat content (>10% in week 10) is a valuable predictor for later hyperglycemia. Treatment of diabetes-prone NZO mice with estrogen prevented hepatic fat accumulation, reduced expression of genes involved in lipid metabolism, and prevented beta-cell loss.
Conclusion: Our data indicate that protection against diabetes upon dietary intervention and estrogen is linked to modulation of hepatic fat storage.
Funding: BMBF, DZD, grant 01GI0922

Ermittlung von Referenzgrenzen aus vorhandenen Datenpools Konzept der AG Richtwerte der DGKL

V18 – Talk Haeckel

Estimation of reference limits from existing data pools. Concept of the working group Guide Limits of the German Society of Clinical Chemistry and Laboratory Medicine (DGKL)

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Results of laboratory investigations require guide limits for their interpretation (Richtwerte in German). A classification of guide limits is proposed: reference intervals, decision limits, action limits and therapeutic limits. Benefits and disadvantages of the various classes will be discussed. Focus is directed to reference intervals. 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. 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 DGKL working group provides an Excel program for deriving reference intervals on the home page of the DGKL and has also developed an Excel program for the calculation of the quantity quotient and the estimation of permissible uncertainties.

V19 – Talk Wolters, Arzideh, Hoffmann

Reference Limit Estimator

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Reference limits (RL) are essential for the interpretation of laboratory 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). The determination and periodical review of RL by calculation of intra-laboratorial RL is reasonable. The mathematical ambitious indirect retrospective method developed by Arzideh et al. is using large data pools of routine laboratory results. The reference interval is calculated after Box-Cox transformation of data and a truncation algorithm. We present the latest version of the graphical frontend "Reference Limit Estimator" (previously named Guide Limit Calculator) based on Microsoft Excel® and the free statistical software R. The participants of the course will be acquainted with the basic features and discuss typical problems and limitations of the "Reference Limit Estimator" using large data pools. Further developments are underway. The "Reference Limit Estimator" will be continuously extended by the members of the working group 'guide limits' of the DGKL. This includes a more flexible frontend, which accepts different input formats (csv, txt, xls, xlsx etc.) and performs an automated plausibility check, before data and configuration parameters are fed into the program. Another upcoming feature is the estimation of continuous RL shifts over age.

V20 – Talk Zierk

Indirect determination of continuous pediatric reference intervals – Experiences from a multi-center Analysis

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Background: Indirect procedures allow the creation of continuous pediatric reference intervals from clinical laboratory data with an exact representation of age- and sex-dependent dynamics. However, the procedure's performance depends on the size of the examined dataset,

reducing accuracy or excluding certain analytes from analysis when limited data is available. Aggregate data from multiple centers increases the number of patients and samples available for analysis, improving the accuracy of indirect procedures and enabling the examination of less common analytes.
Methods: We analyzed samples from 135 000 different inpatients and outpatients (635 000 samples per analyte) from 4 German pediatric tertiary care centers with an established indirect statistical approach. Center-specific analysis as well as analysis of the combined dataset was performed to examine analytical differences between centers.
Results: Center-specific analysis showed no substantial differences of reference limits and their age-dependent dynamics between centers, allowing the combined analysis of data from all 4 centers. We established continuous reference intervals from birth to adulthood for 9 hematology analytes (hemoglobin, hematocrit, red cell indices, red cell count, red cell distribution width, white cell count, and platelet count) and alkaline phosphatase, which show superior accuracy in comparison to single center reference intervals.
Conclusions: Aggregation of data from multiple centers can enhance indirect methods for reference interval creation. The increased sample size improves the accuracy of age- and sex-related dynamics and allows the application of indirect methods when data sets from individual centers are underpowered.

Freie Vorträge / Free Talks Personalisierte Medizin-Onkologie

FV01 – Talk Amstutz

Polymorphisms in MIR27A associated with early-onset toxicity in fluoropyrimidine-based chemotherapy

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Background: The microRNA miR-27a was recently shown to directly regulate dihydropyrimidine dehydrogenase (DPD), the key enzyme in fluoropyrimidine catabolism. A common polymorphism (rs895819) in the miR-27a genomic region (*MIR27A*) was associated with reduced DPD activity in healthy volunteers. Here, we assessed the association of *MIR27A* variants with early-onset FP toxicity in cancer patients.
Methods: *MIR27A* was sequenced in 514 cancer patients receiving fluoropyrimidine-based chemotherapy. Associations of *MIR27A* polymorphisms with early-onset toxicity were assessed in the context of known risk variants in the DPD gene (*DPYD*) and other covariates associated with toxicity.
Results: An association of rs895819 with early-onset fluoropyrimidine toxicity was observed, which was dependent on *DPYD* risk variant carrier status (interaction p=0.0025). In patients carrying *DPYD* risk variants, rs895819G was associated with a strongly increased toxicity risk (OR: 7.6; 95% CI: 1.7-34.7; p=0.0085) with 71% (12 of 17) of carriers of both rs895819G and a *DPYD* risk variant experiencing severe toxicity. Conversely, an opposite effect was observed in patients without *DPYD* risk variants (OR: 0.62; 95%CI: 0.43-0.9; p=0.012).
Conclusions: Our results indicate a clinically relevant role of miR-27a for fluoropyrimidine toxicity risk stratification in carriers of *DPYD* risk variants, in whom direct suppression of DPD by miR-27a may predominate due to the reduced DPD activity. In patients with normal DPD activity, this effect may be outweighed by miR-27a regulation of additional targets, explaining the negative association with fluoropyrimidine toxicity.

FV02 – Talk Sollfrank

New insights in the mutation spectrum and the genotype-phenotype relationship of patients with paraganglioma (PGL) / pheochromocytoma (Pheo) syndrome

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Objectives: Aim of the study was to design and evaluate an NGS panel to offer extensive, time and cost efficient genetic testing to PGL/Pheo patients (up to 40% suffer from an autosomal dominant syndrome) and expand knowledge on genotype-phenotype correlations.
Methods: 74 PGL/Pheo pre-characterized index patients (mut), carrying 54 different mutations in 10 different genes, 115 PGL/Pheo patients (PP) with no mutation detected by Sanger sequencing, and 112 wild type controls (wt) were subjected to a hybridization-based targeted resequencing approach (16 genes including splice-sites and UTR's) carried out on an Illumina MiSeq instrument. Bioinformatic analyses were performed combining the output of NextGENe® with in-house PERL scripts. New results were confirmed by Sanger sequencing.

Results: NGS resulted in an average of 97% matched reads with a mean coverage of 651 reads in ROI/sample. Approx. 2 interesting variants/patient were defined by the bioinformatic procedure and further evaluated. All mutations in the mut collective were detected. In the PP collective 6 probably disease-causing mutations were identified. Though assumed to be already completely characterized, 8 further known mutations/most likely pathogenic variants were found in the mut group, suggesting the existence of disease- causing mutations on the one and penetrance- affecting variants on the other hand. Genotyping of the family members and LOH analysis in tumor samples is still pending.
Conclusion: Further research is necessary to establish the significance of 2 mutations in a PGL/Pheo patient. NGS is not only reliable, time and cost efficient, and facilitates extensive genotyping, it may also help to identify patients with a particularly high disease risk.

Neue Erkenntnisse zur Pathogenese und Früherkennung seltener Erkrankungen

FV03 – Talk Ziegler

Novel mutations in Pakistani population in the major Fanconi anemia gene "FANCA"

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Introduction: Fanconi anemia (FA) is a rare hereditary disease which results in decreased building and higher degradation rate of leucocytes and erythrocytes. This leads to different congenital malformations, a higher rate of tumor neoplasms and bone marrow failures. Because of its autosomal-recessive inheritance, FA is more frequently observed in societies with a high rate of consanguineous marriages. Our aim is to investigate if FA patients from Pakistan have a similar mutation pattern in the subgroup FANCA as observed in other well studied populations (Japanese, Brazilian etc.).
Methods: A diepoxybutane test was performed to confirm the FA diagnosis. Primer pairs which cover the entire FANCA-gene (43 exons) were designed and optimized. The DNA was amplified by PCR, purified, sequenced and sequences were analysed with the help of DNA Dynamo software.
Results: Two patients showed novel duplications of 16bp in exon 3 which have not been described before. Two patients had novel insertions in exon 29, one patient a novel mutation in exon 15 and two patients showed known mutations in exon 9. In total 7 out of 26 FA patients showed mutations in the FANCA-gene, 5 of them have not been previously reported.
Conclusion: The results show that the Pakistani population harbour novel FANCA mutations, possible due to their special cultural setup. However, the mutation detection rate in FANCA-gene is 26% in our studies so far (compared to reported 60% in other populations) and demands a more detailed analysis of the other FA-genes.

FV04 – Talk Jäger

Telomerase Immortalization of Human Skin Fibroblasts from Tangier and Progeria Patients as a Tool for Characterization of Disease Phenotype

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Human telomerase reverse transcriptase (hTERT) is a cellular reverse transcriptase that can compensate for the erosion of telomeres by synthesizing new telomeric DNA. In contrast to cells transformed with oncogenes this cells have normal cell cycle controls, are contact inhibited and possess a normal karyotype. hTERT immortalization allows study of cells from rare genetic diseases at comparable conditions. We present two examples showing the advantages of hTERT immortalization for studying disease phenotype: the familial HDL deficiency syndrome Tangier disease (TD) and the premature aging syndrome Hutchinson Gilford progeria (HGP). TD is a human genetic disorder with mutations in the ATP-binding cassette protein A1 associated with defective lipid efflux and increased atherosclerotic susceptibility. We show that ectopic expression of hTERT extended the life span of TD skin fibroblasts. The lipid efflux in TD cells was significantly improved after immortalization (up to 40%). Our findings indicate the existence of an ABCA1-independent lipid removal pathway that may help to prevent early atherosclerosis in TD and may help to understand the differential atherosclerotic susceptibility in TD. HGP is a model for replicative aging, in which premature aging and telomere attrition occur secondary to a mutation in *laminA*. Patients have a very short life span due to early onset of aging and premature atherosclerosis. By comparison of the expression of telomeric genes in HGP fibroblasts before and after immortalisation candidate genes for a telomere position effect have been identified. Some of these genes influence cell cycle regulation or cholesterol efflux and may help to understand the severe atherosclerosis in this disease.

Referenzintervalle und Leitlinien-Entwicklung

FV05 – Talk Hoffmann

Indirect Estimation of Reference Intervals – Method Comparison and Practical Application Examples

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Background: According to international guidelines, reference intervals are defined as the central 95% of values observed in apparently healthy persons [1]. The direct standard method determines the respective 2.5 and 97.5 percentiles in at least 120 reference individuals.
Problem: Since the standard method is sometimes too intricate to perform, simpler indirect procedures are needed, which estimate reference intervals from routine laboratory data [2].
Methods: We present an indirect quantile-quantile plot (QQ plot) method [3], which can be performed on an Excel sheet without any additional statistics software. The results are compared with a somewhat more elaborate kernel density method developed by the DGKL working group "AG Richtwerte" [2].
Results: Both methods yielded comparable results, which agreed with established reference intervals. It is shown that the values, which follow a Gaussian distribution (after logarithmic transformation if necessary) form a straight line in the QQ plot. Various distribution types with corresponding graphs will be discussed. The QQ plot method is useful for many practical applications, including preliminary and confirmatory estimates of reference intervals or z-transformation ("normalization") for multivariate analyses, continuous color coding of pathological values, etc.
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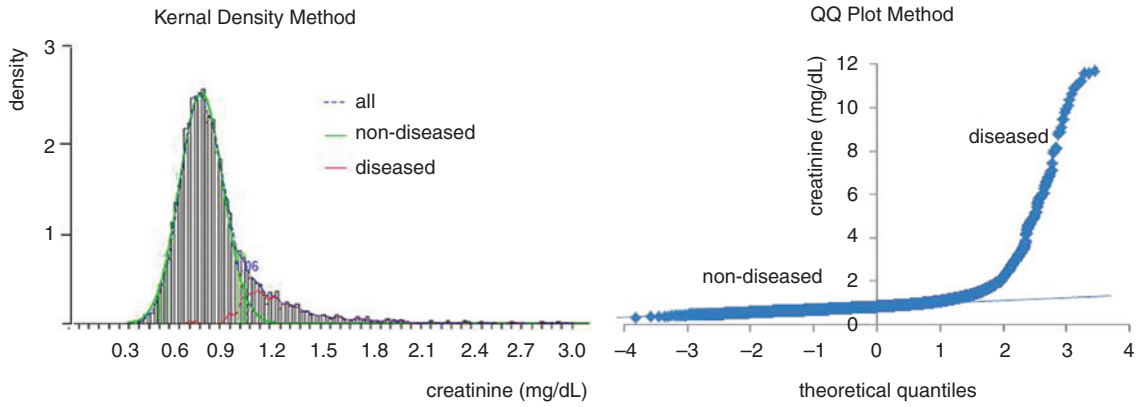

FV06 – Talk Hermanns

Reference Intervals for Aldosterone, Renin, and the Aldosterone-to-Renin Ratio in the Population-Results from the Gutenberg Health Study

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Objectives: Renin-angiotensin-aldosterone system (RAAS) plays a key role in the regulation of human blood pressure. A higher aldosterone level and aldosterone-to-renin-ratio (ARR) serve as markers of increased risk for hypertension.

Methods: Plasma aldosterone concentration (PAC) and plasma renin concentration (PRC) were measured by chemiluminescent immunoassay (CLIA) (LIAISON®, DiaSorin). Both, PAC and PRC were available from 10,392 participants of the population-based Gutenberg Health Study (GHS). A reference population was selected by excluding all participants with suspected hyperaldosteronism, hypokalemia, hypertension, renal insufficiency, and intake of antihypertensives. The reference interval was defined as the central 95% range between the 2.5th and 97.5th percentiles. Biomarkers and the ratio were log-transformed.
Results: The mean age of the sample population was 55 (IQR 46/65) years and 50.5% were female participants. PRC decreased with age in males and females whereas PAC decrease was significant in females only. Increase in ARR with age was comparable in both sexes ($ß_{log\ (ARR)}$ per decade: 0.11 [95CI:0.09/0.13]; $p<0.0001$). Thus, sex-specific reference limits and categories indicating the grade of deviation from the reference were calculated and nomograms for PAC, PRC, and the ARR were created by quantile regression. Exemplary calculations result an ARR [(ng/L)/(µU/mL)] of 5.0 (1.6-26.1) and 7.9 (2.4-47.4) in 40-year old, and 6.3 (1.8-40.9) and 9.4 (2.4-62.2) in 60-year old males and females, resp.
Conclusion: A sex-specific distribution and reference ranges for PAC, PRC, and the ARR in central Europeans is provided. The data support the use of age- and sex-specific reference limits in clinical practice.

Labormedizin in der Gesundheitsvorsorge

FV07 – Talk Koal

Potential of metabolome analysis for early diagnosis of Alzheimers disease

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disease of the brain that gradually leads to severe cognitive impairment. Currently, the diagnosis of AD within the clinical routine is based on a time consuming combination of psychological testing, imaging and the analysis of three well-established biomarkers in the cerebrospinal fluid (CSF). However, there is an important need to improve diagnostic performance by new and more selective and specific biomarkers in CSF. Furthermore, due to the invasive nature of CSF collection, blood biomarkers need to be found, to allow early screening and multiple analyses of patients.
Methods: The targeted quantitative metabolome analysis for one hundred CSF samples (n=50 AD, n=50 controls), and plasma samples from controls (n=35) and mild cognitive impairment (MCI) (n=33) and AD (n=43) patients were carried out. The targeted quantification of more than 180 metabolites, including 40 acyl carnitines, 21 amino acids, 19 proteinogenic amino acids, 15 sphingolipids and 90 glycerophospholipids were performed using the AbsoluteIDQ® p180 Kit (BIOCRATES Life Sciences).
Results: Our data show that sphingomyelin SM (d18:1/18:0) were significantly altered in the CSF of AD patients compared to controls. It proved to be a specific (76%) and sensitive (66%) biomarker with a defined cut-off of 546 nM. Additionally, we found that different PC and lysoPC are altered in plasma of AD and MCI patients compared to healthy controls. Our data suggest that the ratio of PC aa C34:4 to lysoPC a C18:2, representing the pathophysiological changes of phosphatidylcholines, might be highly useful as a novel plasma biomarker to diagnose dementia with an accuracy of 82-85%.

Biobanking und Big Data

FV08 – Talk Baber

One-year biostability of clinical relevant blood biomarkers under multiple biobank associated conditions in a long-term storage study

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Background: Biobanking is a promising tool for personalized medicine and biomarker research. The primary tasks of biobanking are to reflect the biological or biochemical state of the donor at the time point of sampling and to control the quality of stored biospecimen.
Problem: Different biobanking conditions and long-term storage might have a strong impact on the validity of the later biomarker analysis.
Methods: We are performing a long-term study to investigate potential effects of biobank associated variables like time of storage, temperature, consumables (straw, cryo tube, normal screw cap vial), filling level (full, half full) and freeze-thaw-cycles on different biospecimen. We

used pooled rest samples of EDTA-plasma, citrate plasma and serum from patients of the University Hospital of Leipzig after routine analysis at the Institute of Laboratory Medicine and produced multiple aliquots for the next 10 years. Samples were stored in ultra-low upright freezers (- 80 °C) and Askion C-line hermetic storage devices in the gas phase of liquid nitrogen (<-150 °C) and a baseline laboratory analysis of biomarkers was performed before freezing. We analyzed a set of 58 markers with potential clinical relevance (electrolytes, enzymes, hormones, metabolites, coagulation factors and others) using different analytical methods.
Results: Here we report about the one-year results (3, 6 and 12 months; 4 freeze-thaw-cycles) of storage at – 80 °C and in the gas phase of liquid nitrogen (<-150 °C) and their potential implications for biobanking.

FV09 – Talk Lehmann

Control of biobank sample quality using a novel, robust biomarker identified by non-targeted metabolomics

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Background: Variabilities in biobank sample quality can heavily bias conclusions drawn from clinical trails. Pre-analytical inaccuracies may result in poor blood sample quality, but up to the present no general accepted tools exist to directly control the quality of biobank serum or plasma samples using a biomarker.
Objectives: 1) Identification and validation of a biomarker reflecting pre-centrifugation delay, one of the most critical steps in processing of blood; 2) define confidence intervals in serum and plasma; 3) evaluate the sample quality of biobanks from Germany, France and China.
Methods: Non-targeted metabolomics by UPLC-tripleTOF-MS was applied to detect a suitable biomarker. Subsequently targeted UPLC-MS analysis was used for all further steps.
Results: We identified a robust biomarker suitable to assess the quality of serum and plasma samples stored in biobanks or planned to be stored. The definition of confidence intervals enabled us to classify biobank samples into excellent, good (< 2h exposure of whole blood to room temperature, RT), fair (< 4h exposure of whole blood to RT) and low quality (> 4 h exposure to RT). In contrast to the provided biobank SOPs and precentrifugation protocols the quality of a considerable number of biobank samples was only fair or low. This novel quality control (QC) step offers a unique possibility for valid pre- and post-storage QC of serum and plasma suitable to be implemented in future biobank reporting recommendations.

Kardiovaskuläre Biomarker und Konsequenzen

FV10 – Talk Mirtschink

HIF-driven SF3B1 induces KHK-C to enforce fructose metabolism and heart disease

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Alternative pre-mRNA splicing plays a crucial role in determining the metabolic phenotype of mammalian cells but the mechanisms by which alternative splicing is linked to metabolism and regulated by specific signaling pathways remain unclear. Here we report that in human and mouse models of heart disease, activation of hypoxia-inducible factor (HIF)1a is mechanistically linked to increased fructose metabolism via direct transcriptional induction of the gene encoding splicing factor SF3B1 and consequent SF3B1-mediated alternative splicing of ketohexokinase (KHK) pre-mRNA. This results in a shift in isoform expression from KHK-A to KHK-C. KHK-C displays a higher affinity for fructose and accordingly, stimulates increased fructose-to-lipid conversion and cell growth. Heart-specific depletion of Sf3b1 or genetic ablation of *Khk,* but not *Khk-A* alone, in mice, suppressed pathologic stress-induced fructose metabolism, growth and contractile dysfunction. Thus, cardiac hypertrophy is enforced by the activation of a HIFa-SF3B1-KHK-C axis dedicated to couple microenvironmental hypoxia to the activation of fructose metabolism and growth.

FV11 – Talk Holdt

Non-coding RNAs in Atherosclerosis

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Background: The chromosome 9p21 (Chr9p21) locus of coronary artery disease (CAD) is the strongest genetic factor of atherosclerosis known today. Chr9p21 encodes the long non-coding RNA(ncRNA) *antisense non-coding RNA in the INK4 locus* (*ANRIL*), which is associated with the Chr9p21 genotype and correlated with atherosclerosis severity.
Objectives: The aim of the present study was to unravel the molecular mechanisms of *ANRIL* in atherogenesis.
Methods: We established stably over-expressing cell lines and investigated *ANRIL*-mediated *trans* regulation of target genes and keycell functions of atherosclerosis. Using RNA-immunoprecipitation followed by mass-spectrometric analysis, we identified *ANRIL* binding proteins that were followed up in functional and mutagenesis experiments.
Results: We show that *ANRIL* isoforms regulate target gene expression through recruiting epigenetic effector proteins or through regulating RNA translation, thereby controlling cell proliferation, adhesion and apoptosis. Epigenetic *trans*-regulation was dependent on Alu motifs, which marked the promoters of *ANRIL* target genes and were mirrored in *ANRIL* RNA transcripts. Effects on RNA translation were mediated through a subset of *ANRIL* isoforms that could be reversed using siRNAs specifically targeting these RNA transcripts. Molecular mechanisms were confirmed using genome-wide expression analyses in 2280 individuals with and without CAD and functionally validated in primary cells from patients carrying the Chr9p21 risk and protective allele. Our study provides molecular mechanisms for non-coding RNA in atherogenesis and suggests that the balanced expression of different isoforms is critical for atherosclerosis risk.

Neue orale Antikoagulantien-sicher ohne Labor

FV12 – Talk Kuhn

UPLC-MS/MS method for simultaneous measurement of the direct oral anticoagulants dabigatran and rivaroxaban in human plasma and its comparison with coagulation-based assays

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Background: Commercially available coagulation assays were usually used for coagulation diagnostics of direct oral anticoagulants (DOACs). However, these assays give no direct information about the drug concentration in patient blood or which kind of drug the patient has taken.
Issues: The fast, precise, and direct measurement of DOACs such as dabigatran and rivaroxaban in patients' plasma gives information about the drug level in patients' blood and is therefore helpful in critical bleeding events, such as emergency situations.
Methods: Internal standards were added to the samples and after protein precipitation, the samples were separated on a reverse phase column. After ionization of the analytes ions were detected using electrospray ionization-tandem mass spectrometry. Run time was 2.5 minutes per injection.
Results: The calibration curves of dabigatran and rivaroxaban were linear over a working range between 0.8 and 800 µg/L ($r > 0.99$). Limit of detection in the plasma matrix was 0.2 µg/L for dabigatran and 0.3 µg/L for rivaroxaban. The interassay CVs were < 6% for dabigatran and < 9% for rivaroxaban. Inaccuracy was < 5% for both drugs. The mean recovery was 104.5% for dabigatran and 87.0% for rivaroxaban. A method comparison between our UPLC-MS/MS method, the commercially available automated Direct Thrombin Inhibitor assay for dabigatran measurement from CoaChrom Diagnostica, as well as the automated anti-Xa assay for rivaroxaban measurement from Chromogenix showed a high degree of correlation. In conclusion, we developed and validated a sensitive and specific LC-MS/MS assay for the quick and specific measurement of dabigatran and rivaroxaban in human plasma.

FV13 – Talk Nazir

Defining the differential effects of novel anticoagulants: effects of fXa versus fIIa inhibition on coagulation and inflammation

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Background and Aims: Thrombin is the key protease in regard to thrombus formation, while in regard to protease dependent signaling other proteases (e.g. fXa, aPC) may be equally important. Hence, we postulate that inhibition of either fXa or fIIa has comparable effects in regard to coagulation, but convey different effects in regard to inflammation and receptor dependent signalling.

Methods: WT mice were either treated with low and high doses of dabigatran or rivaroxaban for 1 week and then were analyzed by tail bleeding assay, arterial injury induced thrombosis formation, or LAD ligation induced myocardial infarction (ischemic/reperfusion).
Results: Dosage regiments of rivaroxaban and dabigatran with comparable effects in regard to bleeding and thrombosis were established using *in vivo* models (tail bleeding assay, arterial thrombosis formation). At dosages with comparable antithrombotic efficacy of rivaroxaban and dabigatran, areas of infarcted myocardium were similar in both groups. However, fXa inhibition dampened proinflammatory cytokines (IL-6, TNF-alpha) and myocardial macrophages infiltration. Similar, NF-Kβ activation was significantly reduced in the fXa inhibitor treated group as compared to fIIa inhibitor treated group. Importantly, plasma levels of endogenously activated protein C (aPC) were higher in the fXa inhibitor treated group as compared to the group receiving the fIIa inhibitor.
Conclusion: These results demonstrate that inhibition of fIIa and fXa have differential effects on the inflammatory response despite similar anticoagulant profiles. Inhibition of fIIa, but not of fXa, dampens aPC-dependent anti-inflammatory effects, thus paradoxically promoting tissue inflammation.

Massenspektrometrie in der endokrinologischen Labordiagnostik: Wann notwendig, wann verzichtbar?

FV14 – Talk Bae

Immunoassay or LC-MS/MS for the measurement of salivary cortisol in children?

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Background: Dysregulation of the adrenal cortex has been assessed with measurement of salivary cortisol. So far salivary cortisol is routinely measured with immunoassay (IA). However, liquid chromatography-tandem mass spectrometry (MS) is known to offer better specificity. We compared the concentrations of salivary cortisol measured by MS and IA at basal and stress induced conditions and evaluated reasons for the difference in method-dependent cortisol results.
Methods: Saliva samples (n=2893) were collected from 169 children (age range: 8-14 yrs.; 81 healthy children; 55 with internalizing and 33 with externalizing disorders) under circadian conditions and during the Trier Social Stress Test for Children (TSST-C). Biochemical analyses were performed with MS for cortisol and cortisone, IA (IBL, RE62011) for cortisol, and enzyme kinetic assay for alpha-amylase.
Results: MS and IA showed mostly comparable results for circadian activity and TSST-C response with similar statistical power. However, IA measured cortisol concentrations about 2.39-folds higher than MS. We found that this difference in measured values between MS and IA was mainly due to different standardization of IA compared to MS. At low concentration range below 5 nmol/L, cross-reactivity with cortisone depending on cortisone/cortisol ratio ($p<0.001$) and the interference from alpha-amylase ($p<0.05$) were found to contribute to the lower concordance between MS and IA.
Conclusion: Immunoassay and LC-MS/MS were largely comparable in the interpretation of salivary cortisol dynamics in stress research. But the IA method revealed a restricted accuracy in the measuring range below 5 nmol/L.

FV15 – Talk Peitzsch

3-Methoxytyramine, the O-methylated metabolite of dopamine, is an important marker to include into the biochemical diagnosis of Pheochromocytoma and Paraganglioma

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Background: Pheochromocytomas and paragangliomas (PPGLs), tumors arising in adrenal or extra-adrenal chromaffin cells, are biochemically diagnosed by measurements of plasma free normetanephrine (NMN) and metanephrine (MN), the respective O-methylated metabolites of norepinephrine and epinephrine. Recently, the O-methylated dopamine metabolite 3-methoxytyramine (MTY) demonstrated use in identification of rare dopamine-producing PPGLs as well as a marker for malignant disease.

Objective: Does routinely measured MTY in addition to commonly used NMN and MN improve the performance of the biochemical diagnosis of PPGL?
Methods: MTY, NMN and MN in plasma samples from 1495 patients (745 female, 750 male; median age 54, ranging from 10-93 years) with excluded PPGLs and 164 patients (95 female, 69 male; median age 50, ranging from 11-82 years) with confirmed PPGLs were analyzed by liquid chromatography tandem mass spectrometry [1,2].
Results: Median concentrations of MTY, NMN and MN were respectively determined 2.8fold, 9.4fold and 2.6fold higher ($P \leq 0.0001$) in patients with confirmed PPGLs than in patients without disease. ROC curve derived diagnostic power, evaluated by comparisons of areas under curves (AUC), showed different AUC ($P \leq 0.05$) using combinations of 3-methoxytyramine, normetanephrine and metanephrine (0.975), and normetanephrine and metanephrine (0.965).
Conclusions: Addition of plasma MTY to commonly used metanephrines for diagnosis of PPGL improves the diagnostic test performance. This is particularly important for identification of dopamine producing PPGLs.
References:
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Volkskrankheit Allergie? Sinn- und Unsinn von Labordiagnostik

FV16 – Talk Junge

Sensitization against PR10, Profilin, non-specific lipid transfer proteins (nsLTP) and storage proteins (SP): Which recombinant allergens are required for detection?

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Background: PR10 and Profilin may cause oral allergy syndrome (OAS), nsLTP and SP are responsible forsystemic reaction (SR).
Method: 117 samples (43 female,2-68y; 74 male,1-74y) with at least one positive result for specific IgE (sIgE) 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) or SP (Ara h1, Ara h2, Ara h3, Cor a9, Cor a14). sIgE cutoff for evaluation: 0.35kU/l, 0.10kU/l, resp.
Results: At the cutoff of 0.35 kU/l the recomb. allergens required to clearly detect sensitization are Bet v1 (mean 25.7kU/l; range 0.84-100kU/l) and Cor a1 (16.7;0.47-100kU/l) for PR10, Bet v2 (2.61;0.34-56.2kU/l) and Pru p4 (2.67;0.38-39.7kU/l) for Profilin, Pru p3 (2.39;0.41-22.8kU/l) and Ara h9 (1.62;0.36-15.9kU/l) for nsLTP, and Ara h1 (7.53;0.41-100kU/l), Ara h2 (9.58;0.36-100kU/l) Ara h3 (2.79;0.36-100kU/l), Cor a9 (3.02;0.39-100kU/l) and Cor a14 (4.94;0.41-100kU/l) for SP. At the cutoff of 0.10 kU/l: a slightly different recombinant allergen pattern of Bet v1 (24.4;0.14-100kU/l), Cor a1 (15.7;0.23-100kU/l) and Ara h8 (0.83;0.11-58.9kU/l) for PR10, Bet v2 (6.14;0.11-56.2kU/l) and Phl p12 (0.76;0.12-27.3kU/l) for Profilin, Pru p3 (0.90;0.11-22.8kU/l) and Ara h9 (0.64; 0.11-15.9kU/l) for nsLTP, and Ara h1 (3.74;0,14-100kU/l), Ara h2 (3.71;0.11-100kU/l), Ara h3 (1.54;0.13-100kU/l), Cor a9 (1.38;0.11-100kU/l) and Cor a14 (0.96;0.12-100kU/l) for SP as required for straightforward sensitization detection. Using cutoff of 0.10 kU/l we detected more cases of sensitization against PR10(+3%), Profilin(+73%), nsLTP (+64%) and SP(+38%), resp.
Conclusion: Only selected recombinant allergens are required to detect sensitization depending on the cutoff.

Immunhämatologie und cell signalling

FV17 – Talk Ziogas

Endothelial-Specific X-box Binding Protein 1 Deficiency Limits Tumor Necrosis Factor-Induced Leukocyte Recruitment and Vasculitis

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Objective: Endothelial cell activation by tumor necrosis factor (TNF) and associated leukocyte infiltration are hallmarks of vasculitis. Here we investigated the potential role of the cellular stress-associated endothelial X-box binding protein 1 (XBP1) transcription factor in TNF-induced endothelial inflammation and vasculitis.
Methods: Mice bearing endothelial specific XBP1-deficiency were engaged in the vascular inflammation model of TNF-induced Local Shwartzman Reaction (LSR), which displays small vessel vasculitis in the skin. To address the contribution of XBP1 to the TNF-mediated inflammatory response in endothelial cells, we studied the expressional activation of XBP1 by TNF, as well as the effect of XBP1 knockdown in endothelial cells on TNF-induced signaling, pro-inflammatory gene expression and leukocyte-endothelial adhesion.
Results: The active spliced form of XBP1 (XBP1s) was triggered in endothelial cells by TNF. Additionally, endothelial XBP1 contributed to the sustained TNF-triggered NF-κB-dependent transcriptional activation of pro-inflammatory molecules, associated with leukocyte-endothelial adhesion. In LSR, endothelial specific XBP1-deficient mice displayed significantly less vascular damage, accompanied by reduced perivascular neutrophil infiltration as compared to XBP1-proficient mice.
Conclusions: Endothelial XBP1 is activated by TNF and regulates leukocyte-endothelial adhesion *in vitro* as well as neutrophil infiltration and vascular damage in murine vasculitis.

FV18 – Talk Huber

C/EBPß-LAP*/LAP expression during monocytic differentiation is mediated by RSK/eIF4B-dependent signalling boosted by increased protein stability

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Objective: To assess the expression of transcription factor C/EBPβ during monocytic differentiation and its (post)transcriptional regulation (transcription, translation, protein stability) by signalling pathways.
Methods: We used PMA-/VitD3-treated THP-1 or MM-6 cells as a model for monocytic differentiation. Mechanisms were further analysed by western blot, pRT-PCR, inhibitor and siRNA experiments as well as protease activity assays.
Results: In differentiated THP-1 and MM-6 cells, a dramatic increase in C/EBPβ-LAP*/LAP protein occurred, whereas mRNA levels were only modestly increased. LAP*/LAP levels were also increased in differentiating primary murine bone marrow-derived cells. Following PMA stimulation, an enhanced expression/activation of RSK (but not mTOR) and PKR was observed. Selected proteins of the translation initiation complex (eIF4B, rpS6) were induced and activated by RSK and PKR in an eIF2α-independent manner. The half-life of LAP*/LAP was significantly increased under these conditions, whereas the proteasomal chymotrypsin-like activity and the calpain activity were reduced. These effects appeared to be independent of RSK activity. The expression of calpastatin, however, was concomitantly increased.
Conclusions: The switch from mTOR- to RSK-mediated signalling to orchestrate eIF4B-dependent LAP*/LAP translation, accompanied by increased protein stability, may be a prototypical example for the regulation of protein expression during monocytic differentiation.

Validierung oder Verifizierung-Qualitätsanforderungen an Testentwicklungen

FV19 – Talk Moog

Validation flowzytometrischer Leukozytenrestzellzahlbestimmungen bei der Qualitätskontrolle von Erythrozytenkonzentraten

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Background: To compare two different flow cytometers for the quality control of white blood cell (WBC) reduced blood components.
Methods: The BD Leucocount Kit was used at Becton Dickinson FACS Calibur (BD Heidelberg) and the BC Leucosure Kit at Beckman Coulter C FC500 (BC Krefeld). Every following described investigation was performed in both manufacturers. Linearity: At first a double

filtered leuco-reduced red blood cell concentrate was constructed and spiked with a defined buffy coat concentration. 5 defined concentrations stock solutions were measured 3 times in series and linear regression analysis was performed. Precision: Red blood cell- and platelet samples were prepared and measured 5 times in series and the coefficients of variation were calculated and compared with manufacturers declarations.
Accuracy: In count 90 residual WBCl enumerations were compared in red blood cell -, platelet- and plasma samples.
Results: The BD Leucocount kit provides linearity from 1 to 350 WBCs/µl and the BC Leucosure kit a dynamical range from 0 to 400 WBCs/µl, respectively. We tested a sensitive range of 0- 5 WBCs/µl were the manufacturers provide a coefficients of variation of 19-43% (BD) or 16-33% (BC) for red blood cell concentrates and received for FACS Calibur 0.9807x − 0.0563 and for FC500 1.0114x + 0.0207, respectively. On precision analysis of both manufacturers achieved coefficients of variation in a similar manner under their declarations. During the parallel measurements comparable results were analyzed.
Conclusions: The counting of residual WBCs in leuco-reduced blood products can be performed in a comparable way with BD FACS Calibur and BC FC500.

FV20 – Talk Lehn

GCP Conformity in Clinical Studies: Guidelines for Laboratories

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Background: "GCP (Good Clinical Practice) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects". According to European GCP-ICH guideline 2001/20, laboratories have to expose the evidence of quality controls and reference ranges for services related to clinical studies. More detailed specifications can be found in "Reflection paper for Laboratories that perform the analysis or evaluation of clinical trial samples"(European Medicine Agency 2012), which is usually referenced when public authorities review GCP-conformity in spite of its missing regulation by law.
Problem: Recommendations with respect to GCP-conformity vastly exceed conventionally requested qualifications and put heavy demands on labs with a clear focus on routine clinical chemistry.
Method: Many requests of the reflection paper are already complied within laboratories that are accredited according to DIN ISO 15189. Additional specific requirements, for example the laboratories obligation to give advice or services that used to be solely within the investigator's responsibility, have to be newly defined and implemented.
Results: We developed work-flows to already acquire additional basic data of the specific profile of laboratory parameters when clinical studies are centrally registered. Based on this data, consulting, cost calculations, organizational processes (human resources planning, validations, stocking of reagents) and other laboratory services, just as the whole study sample management can be planned and implemented according to GCP guidelines and specifications.

FV22 – Talk Chatzigeorgiou

Robo4 is a gatekeeper of the pancreatic endothelium ameliorating islet inflammation during MLDS–Induced Diabetes in Mice

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Background: In type 1 diabetes mellitus (T1DM), leukocyte infiltration of the pancreatic islets and the resulting immune-mediated destruction of beta-cells precedes hyperglycemia and the clinical symptoms of the disease. However, the role of pancreatic endothelium as a regulating barrier during autoimmune tissue destruction remains scarce. Signalling through Robo4 (an endothelial-specific receptor of the Robo family) is known to promote vascular stability under inflammatory conditions by reducing endothelial permeability.
Aim: To study whether Robo4-deficiency induces endothelial hyper-permeability in pancreas leading to increased inflammatory response during T1DM development.
Methods: The Multiple Low Dose Streptozotocin (MLDS) model of autoimmune diabetes was performed in Robo4-deficient and sufficient mice. Blood glucose and insulin levels were monitored every 3 days upon starting MLDS induction. Insulitis development and pancreatic islet leukocyte infiltration were evaluated by histological studies, while vascular permeability was evaluated by an in vivo permeability assay.

Results and Conclusion: Robo4 was expressed in the pancreatic endothelium. Robo4-deficient mice displayed increased permeability of the pancreatic endothelium and increased leukocyte infiltration of the pancreatic islets leading to exacerbated hyperglycaemia, reduced insulin levels and faster diabetes development upon MLDS as compared to the Robo4-sufficient mice. Together, Robo4 is a gatekeeper of the pancreatic endothelium ameliorating islet inflammation during MLDS–induced Diabetes.

Früherkennung/seltene Erkrankungen/Volkserkrankungen

P001

Urinary peptidomics in a rodent model of diabetic nephropathy highlights epidermal growth factor as a biomarker for renal deterioration in patients with type 2 diabetes

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Albuminuria is the gold standard diagnostic and prognostic urinary biomarker for nephropathy in patients with diabetes, however many patients with declining renal function remain normoalbuminuric. To identify alternative biomarkers we performed urinary peptidomic analysis in a rodent model in which hyperglycaemia and hypertension synergise to promote renal pathological changes consistent with human Diabetic Nephropathy (DN). We identified 297 increased and 15 decreased peptides in the urine of DN rats compared with controls, including peptides derived from proteins associated with DN and novel candidate biomarkers. We confirmed by ELISA that one of the parent proteins, urinary epidermal growth factor (uEGF), was more than x2-fold reduced in DN rats in comparison with controls. To assess the clinical utility of urinary EGF we examined renal outcomes in 642 participants from the Edinburgh Type 2 Diabetes study (ET2DS) who were normoalbuminuric and had preserved renal function at baseline. A lower uEGF:creatinine ratio was associated with new-onset eGFR <60 ml/min/1.73m^2, rapid (>5% per annum) decline in renal function or the composite of both outcomes (OR 0.50; 95%CI 0.34-0.75; p=0.001). These associations were independent of established renal risk factors. The Integrated Discrimination Improvement (IDI) to predict the composite outcome was increased when uEGF:creatinine was added to the panel of established risk factors (IDI=0.018, 95% CI: 0.007-0.029, p=0.002). The utility of low uEGF concentration as a biomarker of progressive decline in renal function in normoalbuminuric patients should be assessed in additional populations.

P002

PSA velocity and early detection of prostate cancer (PCa)

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Background: There are many controversy about the use of PSA testing for the early detection of PCa. The PSA test has its limitations. However, it can help to save lives by the diagnosis of cancer in a curative stage.
Aims: The question whether the determination of PSA velocity (PSAV) contributes to the early detection and prognosis of prostate cancer, should be examined by a long-term study.
Materials and Methods: The study was started in 2010. Since that time, 700 voluntary men were enroled. Their PSA levels were quantified yearly or every 2 years. The familial disposition and the PSA baseline were taken into account. Urologic relevant treatments or medications were recorded. Pathological PSA values and PSA rise were controled by repeated examinations. Histological examination was carried out when other causes of PSA increase could be ruled out. The calculation of PSAV was carried out according to the two-point method.
Results: The average age at study entry was 49 years, the average BMI 27,8 kg/m^2. During the 5 years, 61 participants had at least one pathological PSA level. In 21 men a histological examination was carried out. In 30 participants, the PSA level elevation was not confirmed. 10 prostate cancers have been detected histologically. 7 men had a primarly pathological PSA level. 1 carcinoma was discovered by accident and 2 carcinomas were diagnosed by a PSA rise. The prostate cancers were clinically relevant. They were curatively treated (radical prostatectomy). The average age at diagnosis was 49 years. The cancer rate is currently 1.4% in the whole study group.

P003

Hemoglobinopathies in Germany aren't rare diseases! – Models of prevention programs, requirements and standards in laboratory diagnosis of hemoglobinopathies

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Sickle cell disease, one of the most important hemoglobinopathies, was the most prevalent target disease in the Berlin Newborn Screening in 2011/2012. Migration from high prevalence regions towards Germany is continuously increasing. WHO resolution in 2006 demanded the member states to establish prevention programs for this increasing worldwide health problem. Germany as an immigration country has difficulties so far developing guidelines and measurements for the prevention of hemoglobinopathies. International screening programs which have been established in various countries are mostly based on laboratory screening methods. Newborn screening e.g. aims to identify patients with sickle cell disease and provide optimal treatment. Prenatal carrier screening for hemoglobinopathies aims to provide informed choice to couples at risk to have an affected child. A quality assured diagnostic work-up for hemoglobinopathies in laboratories compasses a reliable detection of hemoglobin variants, a reliable quantification of hemoglobin HbA2 and the correct interpretation of results of hematologic, biochemistry and molecular analysis.

P004

PPARgamma stimulation attenuates monocrotaline induced pulmonary arterial hypertension by affecting cardiac and vascular remodelling in rats

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Background: Pulmonary arterial hypertension (PAH) is characterized by vasoconstriction and increased vascular remodelling of small pulmonary arteries. This results in enhanced pulmonary vascular resistance, elevated mean pulmonary artery systolic pressure (PASP), and deterioration of right ventricular (RV) function and heart failure. Recent findings indicate decreased expression of the nuclear hormone receptor peroxisome proliferator-activated receptor gamma (PPARγ) in lungs of patients with PAH. Therefore, we hypothesized that a PPARγ activating therapy with pioglitazone improves pulmonary hemodynamics and RV remodelling.
Methods: PAH was induced in adult male Sprague Dawley rats by injection of monocrotaline (MCT). Pioglitazone was administered in a chow diet starting 3 weeks after MCT injection. At day 35 after PAH-induction PASP was determined, together with histological and gene expression analyses for determination of tissue proliferation and inflammatory processes.
Results: Pioglitazone treated rats showed improved survival rates and were characterized by a significant reduction of PASP. The decrease in PASP was associated with a significant reduction of both the muscularization of the small pulmonary arteries and medial wall thickness. Further, MCT-induced RV hypertrophy was reduced by pioglitazone, along with downregulation of BNP gene expression and decreased collagen content. Finally, macrophage pulmonary infiltration was significantly less in rats receiving pioglitazone compared to the MCT-group.
Conclusion: Thus, activation of PPARγ might be a novel target to improve survival, attenuate mean PASP, reduce RV hypertrophy, vascular pulmonary remodelling and pulmonary inflammation in PAH.

P005

A Loss-of-Function Mutation in Acvr1c Protects Mice from Atherosclerosis and Obesity

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Background: Two novel quantitative trait loci (QTLs) of atherosclerotic lesion size and white adipose tissue (WAT) mass, which co-localized on mouse chromosome 2, were previously identified in a F2 intercross of atherosclerosis-susceptible C57BL/6 (B6) and atherosclerosis-resistant BALB/cByJ (BALB) mice lacking the LDL receptor.
Objectives: The aim of the present study was to identify a causal genetic variant for both phenotypes and to clarify its functional role in atherogenesis and adipogenesis.

Methods: Both QTLs were validated in an independent F2 intercross. Genetic variants between B6 and BALB mice were identified using next-generation sequencing. Expression data of WAT, aortic and hepatic tissues isolated from F2 mice of both intercrosses were used for expression QTL (eQTL) mapping. Pathway analyses of candidate genes were performed.
Results: A nonsense mutation in *Acvr1c* was identified in BALB mice that co-segregated with QTLs of atherosclerosis and WAT mass. Tissue profiling showed high mRNA expression of *Acvr1c* in WAT of F0 mice corresponding to high protein levels. The mutation caused a dramatic reduction of *Acvr1c* expression in BALB F0 mice as well as in F2 mice carrying two BALB alleles at this locus. Functionally, the nonsense mutation in *Acvr1c* led to a truncated protein with reduced kinase activity and subsequently to a reduced Smad2-phosphorylation. This, in turn, modulated downstream target genes affecting pro-inflammatory and adipogenic pathways. Functional studies of these pathways are currently performed. In summary, truncated ACVR1C in BALB mice was shown to decrease Smad2-mediated *trans* regulation of genes thus protecting these mice from atherosclerosis and obesity.

P006

Enhanced TNFR2 Signaling in Hypomorphic Adam17-Deficient Mice

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Background: Expression QTL mapping indicated that reduced mRNA expression and activity of ADAM17 were associated with increased atherosclerosis at the aortic root (AR) suggesting that ADAM17 confered atheroprotection. The metalloprotease ADAM17 is a major sheddase of membrane-bound proteins, such as TNF-α and TNF-receptors 1 and 2.
Objectives: The current study investigated the effect of *Adam17* on atherosclerosis in a hypomorphic *Adam17*-deficient mouse model and elucidated potential mechanisms of atherogenesis.
Methods: Since *Adam17* knockout mice are not viable, we used hypomorphic *Adam17* mice (*Adam17*$^{ex/ex}$) that have barely detectable levels of *Adam17* in all tissues. Mice were bred onto the LDL receptor-deficient (*Ldlr*$^{-/-}$) background and atherosclerosis was quantified at the AR. ADAM17 substrates were measured in plasma and the supernatants of bone marrow-derived macrophages (BMDM) and protein levels were analysed in cell lysates and cell surface proteins. Functional experiments of atherogenesis were performed in primary and RAW264.7 cells.
Results: *Adam17*-deficiency increased atherosclerosis at the AR. sTNF-α, sTNFR1 and sTNFR2 were significantly reduced released in plasma and supernatants of BMDM of *Adam17*$^{ex/ex}$.*Ldlr*$^{-/-}$ mice. Consistently, we detected higher TNFR2 protein levels in whole cell lysate and on the cell surface of BMDM of *Adam17*$^{ex/ex}$.*Ldlr*$^{-/-}$ mice leading to increased proliferation and decreased apoptosis. Both, increased proliferation and decreased apoptosis were confirmed in RAW264.7 cells after *Adam17* siRNA mediated knock-down.Together, results of the current study provide evidence for an atheroprotective role of ADAM17 through activation of the TNFR2 signaling pathway.

P007

Differential Eicosanoid Response on Gene Expression and Mediator Level in Patients with or without Coronary Artery Disease

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Background: Eicosanoids generated from arachidonic acid (AA) serve as important mediators of inflammation and are thought to play a role in coronary artery disease (CAD).
Objectives: Based on the hypothesis that differential regulation of AA metabolism may affect coronary risk, we aimed to investigate the individual qualitative and quantitative eicosanoid response on gene expression and mediator level in patients with or without CAD.
Methods: Heparinized whole blood from patients without (n=40) or with (n=20/32) angiographically confirmed CAD (<50%/≥50% stenosis) was incubated with or without LPS (c=100 ng/mL) for 4 and 24 hours. RNA was isolated and target genes of the AA pathway (*cyclooxygenase* (*COX*) *1* and *2*, *5-lipoxygenase activating protein* (*FLAP*)) were analyzed by quantitative RT-PCRs. Corresponding metabolites (AA, hydroxyeicosatetraenoic acids (HETEs)) were analyzed in supernatants using liquid chromatography tandem mass spectrometry (LC-MS/MS).
Results: Patients with CAD ≥50% showed an increased *COX-1* and *FLAP mRNA* expression after 24h ($P<0.01$) and a reduced *COX-2 mRNA* expression after 4h LPS activation ($P<0.05$) compared to patients without CAD. Patients with CAD revealed a reduced release of AA, 12-HETE and 5-HETE after 4 and 24 h LPS activation ($P<0.05$). ROC curve analysis combining differentially regulated target genes (*COX-1, COX-2),* mediators (AA), age and gender revealed an area under the curve of 83.6. In summary differential expression of target genes and eicosanoid response on mediator level in patients with and without CAD suggest that individual regulation of AA metabolism may represent a marker of CAD risk.

P008

Impact of HbA1c and Prolactin as Biomarker of Prediabetes in Middle-aged Women. PSYRECA-Study

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According to new ADA criteria for prediabetes, patients with HbA1c (5.7-6.4%) (39-46 mmol/mol) are at increased risk for prediabetes. In the SHIP study, serum prolactin has been inversely associated with the risk of development a type 2 diabetes.
We investigated a potential association of serum prolactin concentration with prediabetes according to HbA1c criteria in a small sample of middle-aged women.
Methods: Data from 336 nondiabetic women (40-69 years old at baseline) of the PSYRECA-study were used to reclassify them according to HbA1c at baseline and two years later. Baseline serum concentration of prolactin was measured in groups of stable nondiabetics (ND), stable prediabetics (PD) and unstable prediabetics (NDPD/PDND).
Results: 75% of old-fashion ND remained nondiabetics (stable-ND). Based on HbA1c criterion, 22% became prediabetics at baseline and remained PD at first follow up (stable–PD). They were older (49 vs. 52 years) than ND. Leukocyte count (6.9±2.2 vs. 6.1±1.4 GPt/l), FSH (28 vs. 45 U/l) and LDL-C concentration (3.79±1.12 vs. 3.23±0.92 mmol/l) were sign. higher in PD than in ND. Among PD, all women showed normal concentration of serum prolactin whereas among ND and unstable pre-diabetics 32% resp.11% of prolactin concentration were increased. After age-adjustment, baseline HbA1c was significantly directly correlated with leukocyte count, LDL-C, fasting glucose and fructosamin and inversely with Vitamin E and Vitamin A concentration as well as with microalbuminuria. Any significant correlation between prolactin and other risk factors was not demonstrable.
Summary: Among middle-aged women, HbA1c but not prolactin seems to be a useful tool to identify women at increased risk for prediabetes.

P009

Mutation analysis of a single case of thalassemia (Mutationsanalyse eines Einzelfalls für Thalassämie)

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Introduction: The disorder "Thalassemia" is described as a percentage change of globin proteins of the erythrocyte's hemoglobin. Because of the constitution in the human adult body it can be subdivided in alpha-thalassemia and beta-thalassemia. Based on its function to deliver oxygen and carbon dioxide, the hemoglobin's disease can be life-threatening. Here a putative heterozygote thalassemia is examined and identified in a Turkish family.
Methods: Blood samples were taken from the concerned family members (mother, son and father). Hemoglobin alterations were examined by protein electrophoresis. Mutation analysis was done by gene sequencing (HISeq, Illumina).
Results: Overall, five mutations could be identified in the beta-globin gene of the patient (three mutations were heterozygotously transmitted by the father, two mutations by the mother). Two of the three paternal mutations were identified as the trigger for a mild beta-thalassemia minor. One of the mutations is causing a pathological splicing site (IVS-II-745 (C>G)) and the other is located at a promoter like and regulatory region within the 5' UTR (+20 (C>T)). The maternal mutations are connected with the father's ones, but did not show any solitary effect on the mother.
Conclusion: Two single mutations could be identified as reason for the alterations of the functional erythrocyte parameters resulting in the diagnosis of a thalassemia. Therefore, gene sequencing might be an additional parameter for the diagnosis and classification of thalassemia.

P010

Hypothesis: increased consumption of food industry bacterial transglutaminase explains the worldwide surge in celiac disease incidence

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Background: Celiac disease (CD) incidence is increasing alongside the food industry introducing ingredients such as microbial transglutaminase (mTg), acting as a food glue, that revolutionize food qualities. Human tissue transglutaminase (tTg) is the autoantigen of CD. Both enzymes de-or transamidate gluten, the CD environmental inducer.

Results: We suggested that mTg is a new environmental enhancer of CD, based on the following scientific data: It de- or transamidates gluten like the endogenous tTg. Being less substrate sensitive, it is capable of crosslinking many proteins and other macromolecules, changing their antigenicity/physical and chemical characteristics resulting in an increased load on the immune system. It increases stability of protein (including gluten) to proteinases, thus diminishing nutrient digestion and foreign protein elimination. Intestinal permeability is increased in CD where gluten and infections are major contributors and bacterial Tgs are necessary for microbial survival. Gluten is changed and cross-linked to many food constituents by industrial mTg. These mTg-mediated processes open the inter-enterocyte tight junction, allowing more immunogenic foreign molecules to induce CD.
Conclusions: It is hypothesized that the use of food industrial mTg allows celiac pathogenesis to start in the factory, market shelves or intestinal lumen where de-or transamidation and cross-linking starts. Ingestion of mTg manipulated food products induces a surge in CD incidence, enhanced by immunogenic neo-epitopes. If substantiated, it will impact the food industry additive policy, consumer awareness, regulation authorities and public health implementation.
Lerner A, Matthias T. Nutr rev. In press, 2015.

P011

Apolipoproteins and coronary artery disease in the Leipzig Heart Study

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Objectives: Apolipoproteins are promising predictors of cardiovascular disease. However, for the majority of these proteins reliable and cost-efficient, quantitative determination methods are lacking. We analyzed the distribution of apolipoproteins in patients of the Leipzig Heart study undergoing first coronary angiography for suspected coronary artery disease (CAD). Subsequently, the association of these apolipoproteins regarding the prevalence of CAD should be investigated.
Methods: A targeted proteomics approach using stable isotope labeled tryptic peptides as internal standards was developed for the analysis of apolipoproteins A-I, A-II, A-IV, B-100, CI, C-III, E and J. Applying a standardized protocol, only 3 µL of human plasma were required for sample preparation. Micro-liquid chromatography coupled to a triple quadrupole-linear ion trap mass spectrometer (LC-MS/MS) was used for quantification and simultaneous peptide confirmation. The study population consisted of patients with obstructive CAD (≥ 50%, n=530) and subjects with normal angiogram (n=456).
Results: Comparison of the developed method with commercial immunoassays showed good agreements for apolipoproteins A-I (+18%) and B-100 (-1%). Determined plasma apolipoprotein concentrations were in good accordance with reference ranges found in literature. Initial logistic regression analyses revealed apolipoprotein A-IV as a potential risk factor for CAD besides traditional risk factors sex, age and diabetes.
Conclusion: A validated LC-MS/MS method was applied in an epidemiological study to reliably investigate the distribution of eight apolipoproteins and their association with cardiovascular disease.

P012

Quantification of Ethyl Glucuronide in Urine for Monitoring of Recent Alcohol Intake by a Commercial LC-MS/MS Assay

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Background: We verified a commercially available IVD-approved mass spectrometric assay for the quantification of ethyl glucuronide (EtG) and ethyl sulfate (EtS) in urine (RECIPE Chemicals + Instruments GmbH, Munich, Germany) for transplant monitoring of recent alcohol intake.
Material and Method: Sample preparation consisted of a protein precipitation step requiring 50 µL of urine sample which is mixed with an isotope-labeled internal standard solution. After centrifugation, 5 µL of the supernatant was analyzed by LC-MS/MS in a total run time of

3 min. An API 6500 tandem mass spectrometer (AB SCIEX, Toronto, Canada) combined with a Shimadzu UFLC system (Duisburg, Germany) was applied.
Results: We determined 0.07 mg/L for EtG and 0.03 mg/L for EtS for the lower limits of quantification for the commercial assay in urine. The within-day and between-day coefficient of variation for both analytes were below 7% and 15%, respectively. Accuracy ranged between 101 – 144% for samples from an external quality assurance program. The comparison of the commercial test kit and an established LC-MS/MS method showed a very good agreement for EtG (r=0.96) and EtS (r=0.97) over a broad urine concentration range.
Conclusion: These results prove that the commercial IVD-certified LC-MS/MS assay is suitable for the analysis of EtG and EtS in human urine to assess recent alcohol intake in transplant monitoring.

Qualitätssicherung im Labor I/Referenzwerte

P013

Age-specific anti-Müllerian hormone levels in fertile and infertile women

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Background: Anti-Mullerian Hormone (AMH) is the most essential reproductive hormone in women. Two novel fully automated AMH immunoassays have been released in 2014. Despite of the good comparability to the former clinical standard ELISA a de novo evaluation of the reference ranges was essential especially because of the higher analytical sensitivity of the new assays.
Objectives: To describe AMH levels in fertile and infertile women 18-63 years. Subjects were recruited from primary care settings and gynecological hospital departments. Smoking behavior, body mass index, androgen status and taking of oral contraceptives were included in the evaluation as independent variables.
Methods: Serum for AMH levels (ng/mL) were measured using the AMH Access immunoassay on a Beckman Coulter Access2 immunoassay analyzer.
Results: Four-hundred-thirty-one fertile, and 260 infertile subjects were recruited and divided in eight age groups. In the group of fertile women mean AMH levels were 3.76 ng/mL (SD 2.32) for the age group 18-25 years, 2.25 ng/mL (SD 2.23) (26-30y), 1.89 ng/mL (SD 1.67) (31-35y), 1.59 (SD 1.67) (36-40y), 0.78 ng/mL (SD 0.89) (41-45y), (0.23 ng/mL) (SD 0.35) (46-50 y), 0.06 ng/mL (SD 0.09) (51-55y) and 0.01 ng/mL (SD 0.09) (56-63y). Age, BMI, smoking and contraceptives were reciprocally associated with AMH ($p<0.001$, $p<0.001$, $p=0.012$ and $p=0.046$, the androgen status was directly correlated with AMH ($p=0.005$).

P014

Importance of Water Quality to Minimize Impact on Assays and Maximize Clinical Analyzer Uptime and new water systems for clinical analyser feed

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Water is a key components and reagent of the assays on most clinical analyzers today. Selecting the right water solution and maintaining the water quality are parts of the quality management systems in hospital and accredited laboratories. According the CLSI guideline, some aspects of the water quality should be considered in more details, as they can impact patient results, leading to additional assays, loss of time and additional costs. Low bacteria count in pure water is particularly critical in clinical analyzers, because bacteria can generate numerous interferences in biochemistry and immunochemistry assays. The objective here is to describe several of those issues on assays and analyzer maintenance, and provide solutions to avoid bacterial contamination in water supplying the analyzer. Typical impacts of bacterial contamination on assays include unstable calibrations and errors on mean patient values. Those effects generated by the typical bacteria strains identified in clinical analyzers result from proteins and small organic acids released by bacteria. Some of the issues mentioned above and resulting from poor design can be avoided by selecting key purification technologies, which will be discussed. The stability of the blank ensured a much higher reproducibility of the results and reduced the need for frequent calibrations of the assay. Recommendations for the maintenance of the water purification systems are described to ensure a consistent supply of pure water to feed clinical analyzers, in-line with CLSI C3-A4 guideline. New design of water systems for feeding clinical analyzers to overcome issues discussed above will also be discussed.

P015

Performance evaluation of a new particle-enhanced immunoturbidimetric Procalcitonin (PCT) assay on Beckman Coulter AU680 analyzer

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Introduction: The level of PCT rises in response to a pro-inflammatory stimulus, especially of bacterial origin, but not with viral or non-infectious inflammations. For this, PCT is widely used as an early marker to assess the degree of bacterial based sepsis.
Objective: This study was intended to evaluate the analytical performance of a new particle-enhanced immunoturbidimetric PCT assay on Beckman Coulter AU680 analyzer.
Methods: Particle-enhanced immunoturbidimetric PCT assay for use on clinical chemistry analyzers was provided by DiaSys Vertriebs GmbH, Germany. Analytical evaluation included the assessment of limit of Blank (LoB), limit of detection (LoD), precision, linearity and method comparison between PCT test provided by DiaSys and Elecsys PCT (ROCHE) on 99 routine plasma samples.
Results: LoB and LoD were 0.14 ng/mL and 0.17 ng/mL, respectively. The test covers a wide measuring range up to 54 ng/mL. Precision within and between run were determined at 3 concentrations (20 times each). Coefficient of variation (CV) is less than 6.7% for repeatability and less than 4.4% for reproducibility. Good correlation was found between the immunoturbidimetric PCT assay measured on Beckman Coulter AU680 and the Elecsys PCT test in a range of 0.06-33.2 ng/mL PCT (Passing Bablok: $y = 1.0422x + 0.028$, $r = 0.999$).
Conclusion: The new particle-enhanced immunoturbidimetric PCT assay is a reliable assay for diagnosis as well as follow-up of critical patients prone to bacterial infections. The assay proved to be a fast and easy to perform test for daily routine in clinical chemistry.

P016

Procalcitonin measurements in critical ill patients: comparison of a point of care and a core laboratory method

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Introduction: Procalcitonin (PCT) is a valuable marker to guide antibiotic treatment in patients with bacterial infections. Standard laboratories methods used to measure PCT concentration are time consuming since blood samples need to be transported and centrifuged. Measurement of PCT in whole blood using a point of care (POC) method can reduce the time needed to obtain results and therefor bears the potential to improve patient care. In this study we evaluated a novel POC method to measure PCT.
Methods: Blood was drawn into lithium heparin blood collection tubes. PCT concentration was measured in whole blood and plasma using the AQT90 FLEX (Radiometer, Copenhagen, Denmark) as a POC method. As a core laboratory method the ADVIA CENTAUR® (Siemen, Eschborn, Germany) was used to determine PCT concentration in plasma. All measurements were completed within three hours after blood collection.
Results: 118 blood samples were collected from ICU patients at the University Medicine of Greifswald. PCT measurements revealed a median of 0.99 ng/mL (range 0.05-25 ng/mL) in plasma using ADIVA and a median 0.88 (range 0.06-34 ng/mL) in whole blood using the AQT90. A good correlation was observed between both assays ($r=0.95$, $p<0.0001$). When PCT was measured on the AQT90 using plasma ($n=81$), better correlation was observed ($r=0.98$, $p<0.0001$).
Discussion and Conclusion: The use of AQT90 FLEX as a POC method can positively improve the workflow and significantly reduce turnover time. The POC PCT method compares well to the core laboratory PCT method in both, whole blood and plasma samples. This study was conducted with Lithium heparin blood collection gel tubes, which is not yet validated by the manufacturer of the instrument.

P017

Prospective analysis of computerized diagnostics for clinical decision-making in an emergency department: patients with chest pain symptoms

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Objectives: Complex selection criteria of biomarkers and the change from binary patterns of interpretations to multifactorial decision-making need computerized and evidence-based decision algorithms for recording and classification of differentiated patterns.

Methods: The aim of this study was to evaluate the clinical value of computerized diagnostics. 62 patients with chest pain, who presented at the CPU at the RWTH-University Hospital Aachen, were prospectively analyzed to evaluate the correlation between computerized diagnostic pathway, clinical decision and reference diagnosis of a well-experienced cardiologist. Furthermore, clinical symptoms and instrument-based diagnosis were compared to high sensitive Troponin T and biomarkers of cardiac dysfunction, respectively.
Results: The study demonstrates an excellent agreement between computerized diagnostics and reference diagnosis (k=0.849; ICC=0.9358). The decision between inpatient admission or discharge significantly depends on load-dependent chest pain (OR 3.4; p=0.0336) and the combination of more than 3 risk factors (OR 4.7; p=0.0141). Multiple regression analysis demonstrated that high sensitive Troponin T measured after 4-6 hours (p=0.0052), TIMI risk score (p=0.0001) and GDF-15 (p=0.0045) are strong independent predictors for inpatient admission. Patients with suspected heart failure or pulmonary embolism have the lowest concentration of Clusterin while GDF-15, MR-proANP, NT-proBNP, CT-proET1 and CT-proAVP (Copeptin) are elevated.
Conclusion: Our results show computerized clinical diagnosis that is based on symptoms and multiple biomarker patterns can provide guidance for a best possible selection of evidence-based decision-making.

P018

Determination of non-generic calcitonin reference intervals in pediatric population for diagnosis and treatment monitoring of medullary thyroid carcinoma in MEN 2

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Objectives: Calcitonin is known to play a major role in bone metabolism but in clinical practice it is used as a predictive marker of medullary thyroid cancer (MTC) and will be detected especially in patients with MEN 2. The aim of our study was to establish reference intervals of calcitonin for children to enable the diagnosis of MTC as early as possible.
Methods: 975 serum samples were gained from healthy newborns, children and adolescents until an age of 16 years. All subjects were investigated within the Leipzig Research Center for Civilization Diseases (LIFE). Calcitonin concentrations were quantified by the ECLIA system (Roche).
Results: Calcitonin levels of boys were significantly higher than of girls (p<0.001). An overview about the results with regard to age, gender and calcitonin level (ng/L) is shown in the table below:
Conclusion: The distribution of calcitonin levels shows a maximum in the first year of life suggesting that this hormone is distinctly regulated in infants. Cut-points from the 95th percentile of infants are clearly higher as reference intervals of adults which are frequently used for diagnostic use in pediatrics. Thereafter, calcitonin levels decreases continuously until the age of fifteen or sixteen years. Interestingly, the calcitonin value of the 95th percentile from this age group is lower than in adult subjects.

Age (Years)	Boys			Girls		
	Median	n	5th-95thperc.	Median	n	5th–95th perc.
<1	17.7	43	6.1 – 44.2	19.2	23	7.5-27.8
1 – 2	9.3	54	3.5 – 20.7	7.3	34	2.8 – 20.3
3 – 4	6.1	25	2.9 – 13.2	3.65	40	1.05 – 8.65
5 – 6	4.8	39	0.6 – 19.2	3.05	36	0.9 – 9.2
7 – 8	4.1	32	1.0 – 11.0	2.8	45	0.7 – 6.5
9 – 10	5.3	67	1.8 – 10.3	2.7	52	0.5 – 5.9
11 – 12	3.4	75	1.0 – 8.1	1.9	69	0.5 – 7.2
13 – 14	2	72	0.5 – 6.8	0.85	80	0.5 – 4.25
15 – 16	1.6	59	0.5 – 5.4	0.5	64	0.5 – 2.8

P019

First steps towards harmonization in individual bile acids analysis – an inter-laboratory ring trial with a newly developed standardized LC-MS/MS assay

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Background: Bile acids can be used for diagnostic purposes towards for example hepatobiliary diseases and disorders. The reference concentration levels of individual bile acids in published literature are incomplete due to the lack of standardized measurements of absolute concentrations. We introduced an inter-laboratory comparison study of a standardized method for individual bile acids determination. The Bile Acids Kit, based on HPLC-MSMS technology, has been rigorously validated for human and mouse samples.
Methods: 12 laboratories in Europe and North America have participated in the comparison study. These labs have different MS platforms. Each has received a Kit to set up the assay in its own lab. A set of 9 samples (human and mouse) at 3 different concentration levels (endogenous, spiked low and high) was measured in 4 replicates. The acceptance criteria were set as follows: 80% of reported values with accuracy within 70-130% and precision (CV) < 30%.
Results: All 12 participating labs have passed the acceptance criteria. 94% of the overall reported values were within the 70-130% accuracy. When stricter ranges are considers, 79% of reported values were within 80-120% accuracy and 65% within 85-115% accuracy, respectively. The precision criteria of replicate measurements (CV < 30%) has been fulfilled by 99% of the reported values. The percentage of reported values with CV lower than 20%, 15% and 10% were 96%, 91% and 73%, respectively. The average CV of all measurements was 8.3%.
Conclusions: The inter-laboratory ring trial showed very high accuracy and precision of the individual bile acids measurements across a wide variety of LC-MS/MS platforms. Using this Kit the reference range of bile acids in human plasma has been established.

P020

HbA1c at the Point of Care: a method comparison

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Introduction: Rapid measurement of glycated haemoglobin HbA1c at the point of care (POC) enables physicians in ambulatory settings to examine and treat patients at the same visit. Imprecision of the POC methods and the comparability with other routinely used methods should be investigated in view of standardization of patient care. In this study we compared the POC HbA1c analyser 501 to the core laboratory method TOSOH G8.
Materials and Methods: EDTA whole blood samples with a routine requests for HbA1c measurement were analysed using TOSOH corporation, Tokyo, Japan as well as HbA1c analyser 501 (HemoCue AB, Ängelholm, Sweden). Both HbA1c measurements were obtained within 24 hours after blood collection. Prism, Version 5.0 (GraphPad, La Jolla, USA) was used for statistical analysis. Imprecision was obtained from internal quality controls and from double measurements for TOSOH G8 and HbA1c analyser, respectively
Results: 114 samples were included in the study. HbA1c levels range from 30 to 121 mmol/mol (median 46 mmol/mol) on TOSOH G8 and from 27 to 109 mmol/mol (median 41 mmol/mol) on the HbA1c analyser 501. The correlation was found to be $r^2 = 0.907$. Results for HbA1c analyser were slightly lower than for TOSOH G8. The coefficient variation CV was 1.8% and 1.3% for TOSOH G8 and for HbA1c analyser 501, respectively.
Conclusion: Our study demonstrates that the POC device HbA1c analyser 501 shows a good imprecision as well as good correlation with the core laboratory method TOSOH G8 in measurement of HbA1c in EDTA blood samples. Therefor HbA1c analyser 501 is suitable POC device for ambulatory setting.

P021

Reference data for vitamin D binding protein concentrations of children and adolescents are age-dependent

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Objectives: Vitamin D has been linked to several human health conditions and disorders, classically, in connection with bone metabolism and growth but also associated with obesity, cancer, allergy and all cause mortality. In most studies, vitamin D status is limited to the measurement of 25-OH-vitamin D [25(OH)D] level in serum neglecting the fact that vitamin D metabolism is a complex and strictly regulated system which underlies the influence of sun exposure and nutrition. Vitamin D binding protein (VDBP), the major carrier protein for 25(OH)D, is suggested to alter the bioavailability of circulating vitamin D. Aim of the study was to establish age-, gender-, and pubertal stage-dependent reference ranges for serum VDBP.
Subjects and Methods: The LIFE Child study provides data from infancy to early adulthood of a well-characterized cohort recruited from the area of Leipzig since January 2011. VDBP was measured in 2571 blood samples of 1768 children by a turbidimetric immunoassay (Dako, Denmark) on the Cobas 8000 c502 system (Roche Diagnostics, Germany).
Results: Preliminary data showed a VDBP concentration of 358.58 ± 55.43 µg/L (mean ± SD) aged 9 months to 18 years. The 10^{th} and 90^{th} percentile for VDBP were 297.17 µg/L and 429.39 µg/L, respectively. Values decreased slightly after a first peak at the age of 2 years (354.49 ± 32.20 µg/L), remained constant in the age range between 6 and 14 years (354.00 ± 48.85 µg/L) and increased to a maximum until 18 years of age (415.16 ± 110.93 µg/L).
Conclusion: VDBP is age-dependent and increases in adolescence. A comprehensive analysis considering relevant blood parameters, diseases and medication, vitamin D supplementation and seasonal variation is conducted to establish reliable reference data.

P022

Comparison of two automated methods for Procalcitonin determination

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Background: Procalcitonin (PCT) is commonly used for diagnosis and therapeutic monitoring of bacterial sepsis. We evaluated a new PCT assay which was run on an integrated serum platform and compared with an established PCT assay on a stand-alone immunoanalyzer.
Methods: Serum samples of 140 patients from different wards of a tertiary care hospital were analyzed using a new turbidimetric PCT test (A) (Diazym) on an integrated serum analysis system (cobas, *Roche*) and a chemiluminescence immunoassay (CLIA) for PCT (B) (*Brahms*) on an immunoanalyzer (Liaison, *DiaSorin*). For both methods coefficients of variance (CVs) were determined at different PCT concentrations and a linear regression analysis was performed (Passing-Bablock). Some of the discrepant results were clinically evaluated in retrospect.
Results: Interassay CVs were determined for PCT test (A) as 8.2% at 1.22 ng/ml and 8,2% at 13,4 ng/ml and for PCT test (B) as 7,7% at 1.38 ng/ml and 10,6% at 38.9 ng/ml, respectively. Linear regression analysis showed a good agreement of methods with a correlation coefficient r = 0.945 (slope: 0.988, intercept: -0.099). In 44% of cases PCT was below the reference limit of 0.5 ng/ml in both assays, in 42% values were in acceptable agreement (relat. Difference < ± 30%, absol. difference < 2 ng/ml). In the absence of a reference procedure for PCT discrepant PCT results from the remaining patients were evaluated on the basis of medical records. It turned out that at times both PCT tests generate clinically dubious results.
Conclusions: The new turbidimetric PCT test (A) is useful for the diagnosis and therapeutic control of septic patients. It also improves laboratory efficiency when applied on an integrated serum analysis platform.

P023

12 years of an international external quality assessment (EQA) scheme for genotyping: outcome and recommendations

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Background: Suboptimal laboratory procedures resulting in genotyping errors, misdiagnosis or incorrect reporting bear tremendous implications for patient lives, health management and counselling of relatives. External quality assessment (EQA) schemes are an important cornerstone of quality assurance in molecular genetic diagnostics. Therefore the Reference Institute for Bioanalytics (RfB) is organizing an EQA scheme for molecular genetic testing of clinical relevant genotypes since 1998.

Methods: Within an EQA scheme two samples of lyophilized human genomic DNA were provided. Laboratories were asked to use their routine procedures and protocols for genotyping. A panel of assessors reviewed the final returns to assess the quality of genotyping and to evaluate the methods of genotype identification.
Results: Within the last 12 years, 80650 genotypes were evaluated. Since 2002 the number of participants almost doubled from 161 (20 countries) to 401 (35 countries) in 2014. Additionally, the number of parameters offered increased from nine to 50 making the RfB the EQA provider with the largest panel of genotypes offered worldwide. Interestingly, the error rate significantly differs depending on the genotype analysed and the method used.
Conclusion: Our findings reveal a significantly increased error rate for first time offered genotypes, rare sequence variations, certain analytes as well as for certain methods depending on the genotype determined. Even with robust DNA-genotyping methods and the tremendous impact of genetic testing results on an individual's life the overall error rate of this EQA underlies that there is still plenty of room for improvement.

Biobanken/Präanalytik

P024

Technical Enhancement and Cost Optimization of Sample Storage in Vapor Phase Nitrogen

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Damage-free freezing, cold handling and long-term storage of biological samples such as body fluids, cells and tissue are the most important factors of establishing new therapies. The fact that storage of biological samples at most low temperatures positively influences sample quality after thawing is strengthened by an increasing number of publications. Not only the storage temperature, but also the handling temperature significantly influences the sample quality. Second crucial factor in a Biobank is to minimize the storage costs. An effective reduction of costs can be achieved by an increase in capacity of the storage system. However, an increase in capacity by orders of magnitude implies further necessary adaptions of the storage system. This includes the possibility of fully automated management of various sample formats within one storage unit. It is shown how appropriate technologies are used to significantly increase biomaterial quality after thawing. The use of the best possible freezing regime and a well-matched seeding leads to a 30 % higher cell count after recultivation. The warming of a frozen sample occurs 5 times faster during handling in room temperature compared to handling in a -100°C environment. Cyclical rewarming of samples due to handling processes lowers the sample quality by a factor of 20 compared to constant cooling. Furthermore a new automated LIN storage device is presented able to hold up to 750,000 vials. It possesses a free choice of different labware operated fully automated in one storage unit. The handling temperature is at -130°C to avoid migratory crystal growth. The combination of high capacity and fully automated sample processing leads to a significant drop in both acquisition and running costs.

P025

The impact of adherence to correct preanalytics in automated urinalysis

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Background: Due to existing supply structures especially in clinics and outpatient departments, a considerable time gap often occurs between blood and urine sampling and laboratory analysis. No conclusive study has been published to date regarding preanalytical specifications for automated urinalysis.
Aim: Does a critical situation arise if these preanalytical specifications cannot be met in reality?
Methods: In 321 samples, dipstick strip tests and urine particle analysis were performed within 90 min, 120 min and 240 min after urine sampling. Initially, all samples met one of the following criteria: erythrocytes >20/µl, leucocytes >20/µl, bacteria >100/µl or positive for blood/leucocytes/nitrite. Dipstick test strip measurement was carried out with Urisys™ 2400 using Combur[10]Test™strips (Roche Diagnostics, Mannheim, Germany), urine particle analysis with UF-1000*i*™ (Sysmex, Norderstedt, Germany).
Results: A significant increase of conductivity and a decrease in the white blood cell as well as the red blood cell count were found 120 min and 240 min and in casts 240 min after urine sampling. No significant changes were noted for bacteria and epithelial cells. In dipstick strips, a significant increase in specific gravity was noted over time. A significant decrease in pH-value, leukocytes, blood and ketone was noted 120 min and 240 min and for protein 240 min after urine sampling. This was not the case for nitrite, glucose, urobilinogen and bilirubin. Our study stresses the importance of adherence to preanalytical specifications in urinalysis.

P026

Influence of the sample collection techniques on human blood metabolome and mid-term storage stability.

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Introduction: Blood is the most preferred sample type for the pre-clinical research and diagnosis. However, it has been recognized that blood sample preparation procedure can have a major influence on the metabolite profile. In this study the impact of most commonly used blood sampling techniques, including plasma (EDTA, heparin, citrate), serum and dried plasma spots (DBS), on metabolite profile and mid-term storage stability were investigated.
Methods: The blood from a healthy female donor was drawn and processed according to methodology-specific protocols at the same time point for all sample collection techniques. In order to ensure sample homogeneity, the EDTA whole blood was used for a preparation of DBS. The sample aliquots were stored at -80 °C and for DBS also at room temperature for a time period of up to six months. The targeted quantification of more than 180 endogenous metabolites was performed using the AbsoluteIDQ® p180 Kit (BIOCRATES Life Sciences).
Results: The quantitative metabolite profiles obtained from liquid specimens were in good agreement. However, concentration levels of several metabolites in DBS samples were significantly influenced by the on-going enzymatic activity, presence of blood cells and oxidation. Metabolite levels were also influenced by the sample storage time and conditions. In liquid specimens, the metabolite stability was significantly higher in citrate plasma (especially for lipids) and serum. In case of DBS, the storage at the RT had a major influence on amino acid and lipid stability. It was greatly increased when samples were stored at -80 °C instead of RT. As a matter of fact, DBS stored at -80 °C demonstrated superior stability over all investigated sample types for all metabolites.

P027

Identification of confounding factors in clinical chemistry by the determination of serum indices

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Background: Determining the actual value of a concentration or activity in a sample is one of the main objectives in laboratory medicine. Besides the high quality of laboratory analytics the sample quality is of vast importance. Interfering factors such as hemolysis, icterus or lipemia are important intrinsic factors of a blood sample.
Objectives: Determination of benefits and costs for the use of serum indices versus visual evaluation.
Methods: 100 test samples were evaluated by visual evaluation of 12 MTA and by measurement of serum indices in a Roche c501 (cobas® 6000).
Results: The visual evaluation of samples and the automated evaluation by measurement of serum indices showed 87.4% conformity. In detail: 94.9% of hemolytic, 88.1% of lipemic and 90.8% of icteric samples were evaluated correctly by the MTA. 19 of the test samples were not hemolytic, lipemic or icteric, 23.7% of those samples were rejected by the MTA.
Conclusion: The results of our investigation indicate the subjectivity of a solely visual evaluation of samples. Although the majority of samples was evaluated correctly by the laboratory personnel the standardized utilization of serum indices and an assay specific evaluation increases accuracy and ensures the quality of laboratory reports.

P028

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

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Background: Changes in telomere length (TL) are associated with age-related disorders. TL analysis is often performed in DNA isolated from peripheral blood leukocytes, but the influence of preanalytical conditions on TL quantification has never been thoroughly investigated.
Objective: The aim of the current study was to analyze the effect of freezing, degradation and DNA isolation methods on TL quantification.
Methods: Whole blood left-over samples from male and female individuals were pooled according to leukocyte counts (n=4/4) and incubated with and without actinomycin D to induce degradation. DNA was isolated from fresh blood pools or after freezing at -80 °C. Commercially

available DNA isolation kits using beads (Invitrogen), spin columns (Qiagen, Macherey-Nagel and 5prime) or precipitation (Stratec) as well as one published isopropanol precipitation protocol (IPP) were used. A new multiplex qPCR assay was established to simultaneously quantify TL in relation to a single copy gene (SCG).
Results: We show that the newly established TL quantification multiplex qPCR assay was robust and superior with respect to time- and cost-effectiveness compared to previously published assays. Compared to freshly isolated samples, freezing did not change TL, whereas actinomycin D-induced degradation significantly decreased TL. Hands-on time was similar for all used kits. For non-degraded samples, the kits from Invitrogen and Stratec showed the highest TL/SCG ratio, but performed inferior in degraded material. In comparison, using non-degraded, fresh material as a reference, the 5prime kit showed the most consistent results in TL quantification across all conditions tested.

P029

A systematic approach to optimize the preanalytical phase over the last 6 years

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Background: The preanalytical phase is of great importance for correct laboratory testing results. However, the preanalytical phase is complex and difficult to control. We implemented a project to increase awareness and optimize practices.
Objectives: With this study, we strove for quantifying the effect of our systematic approach to optimize practices in a large hospital with high staff turn-over.
Method: In 2008, we analysed the current practices using a BD Preanalytical Review. A communication campaign was launched to create a sense of urgency with the aim to increase willingness to change long standing practices. As next step, we used a cost model to estimate the cost of poor sample quality in the hospital. During the whole period of time, we offered continuous training on the preanalytical phase. To assess changes in behavior, we then developed a customized preanalytical audit methodology.
Results and Discussion: Generally, communication on preanalytical issues have been perceived with great interest by physicians and nurses and have triggered a discussion on the importance of the blood collection process. The cost model revealed that 0.19% of total hospital cost could have been saved would no preanalytical errors happen. There was an improvement in practices over the period: pumping with the fist decreased from 21% to 10%, prolonged trourniquet time from 88% to 32%, and insufficient mixing of tubes from 76% to 46%. Still, obviously our efforts have not completely eliminated errors. Some deviations from SOP may occur for specific reasons. Additionally, the high turn-over of staff, especially of the physicians in training, create a need for continuous re-training and communication.

P030

The effect of preanalytical conditions on human serum N-glycome as judged by MALDI-TOF-MS

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Introduction: Glycosylation is an essential protein post-translational modification in proteins from eukaryotes. Glycans are involved in many biological and cellular processes such as signaling and immune reactions. To date, nothing has been published on the preanalytical conditions of glycan analysis for biomarker studies. The aim of this work is to investigate how preanalytical variables such as prolonged storage prior to centrifugation can influence the results and bring to inaccurate conclusions.
Question: It is likely that the activity of exoglycosidases depends on the time and temperature at which the samples are stored before processing. Additionally, it should be investigated how the composition of the serum/plasma glycome is modified when glycoproteins from cell organelles are released from leukocytes that are undergoing lysis. Thus, preanalytical factors such as the type of blood collection, the sample processing and storage, and the additives in collection tubes are of great importance.
Methods: The samples were collected from 5 healthy donors in 11 blood collection tubes with different additives and processed variously to obtain 15 variables. Glycoproteins from serum/plasma were denatured, reduced and alkylated. N-glycans were released by PNGase F digestion, isolated by C18 micro-columns and purified using carbograph cartridges. The N-glycan pool was permethylated and analyzed by MALDI-TOF-MS in the positive ionization reflectron mode.
Results: We observed that the addition of anticoagulants hinders the permethylation step, but no significant differences were observed in the N-glycome between samples stored at -20°C and -80°C.

P031

Introduction of automated HIL-detection- a field report

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Background: Laboratory results may be influenced by interfering factors such as hemolysis, icterus and lipemia (HIL). Visual inspection of samples to detect HIL-interference is a time consuming and subjectively influenced process. Therefore introduction of automated HIL-detection combined with Laboratory Information System (LIS)-regulated annotations may be a good tool to optimize pre-analytic error-detection.
Question: We aimed to evaluate potential consequences of automated HIL-detection for selected parameters which have very low interference limits for hemolysis (e.g. CK-MB activity), as well as hemolysis in general.
Methods: Semi-quantitative measurement of HIL was applied on a Cobas 8000 c701 platform with Serum Index Gen.2 (Roche Diagnostics Germany) according to the manufacturer's protocol. Interference limits (manufacturer's information) for c-module as well as for HIL-sensitive e-module parameters were programmed in LIS (iSoft) to automatically annotate them if HIL-limits were violated. Hemolysis (Index H) was measured in conventional units.
Results: In 92549 serum samples sent to our laboratory over 3 month Index H was measured. About 1% of samples had an Index H > 100 and just 10 samples had an Index H > 1000. On the other hand only 34% of samples had an Index H < 10, which is the interference limit for hemolysis sensitive parameters such as CK-MB activity and haptoglobin. In consequence, automated HIL-detection resulted in the identification of potential interference for hemolysis sensitive parameters in a majority of serum samples. Further steps for improvement of this pre-analytical error must be investigated.

P032

Serum and whole blood parameters and their time and temperature dependent stability

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Objectives: Centralized and well standardized laboratory analysis is mandatory for epidemiological studies in view of scientific purposes. Pre-analytical effects have to be smaller than the variation caused by different local laboratories and subsequently the different methods applied. In order to ensure reliable comparability of analytical results, serum and whole blood analytes were evaluated according to their temperature dependent long term stability- simulating a prolonged time span between sample taking and analyzing as well as environmental conditions which might occur during over-night sample transport to a core laboratory.
Methods: Venous blood samples of 10 volunteers were collected each encompassing three tubes (BD Vacutainer Serum, 2.5ml; EDTA, 3.0 ml) employed by the the National Cohort. Blood cell count, HbA1c and 22 serum analytes were determined on Sysmex XN 9000, Tosoh G8 and Dimension Vista. Tubes were stored at 4°C, room temperature (RT) and 37°C respectively for up to 120 hours. Every 24 h the samples were re-measured and compared to the base line results.
Results: Hb, RBC and HbA1c proved to be stable for up to 120 h at 4°C, RT and 37°C. WBCs and PLTs were only affected after 72 h at 37°C resulting in a deviation of more than 20% in single samples. 13 serum analytes did not exceed a deviation of 5% for all conditions (e.g. GLU and LDH). The remaining 9 Serum analytes reached a 10% deviation at 72 h.
Conclusion: All investigated analytes proved to be stable for at least up to 72 h, forming a reliable data pool for further epidemiological investigations.

P033

Miniature in-tube logger for quality assurance of single tube pneumatic tube systems

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Objectives and Motivation: Monitoring sample transportation has become an important aspect in quality assurance. For this purpose data logger e.g. for time, temperature and even acceleration are available. Modern pneumatic tubes systems (PTS) allow sending of single samples. Conventional data loggers do not fit in these sample tubes circumventing direct monitoring. Therefore we constructed a fit for purpose data

logger which can be inserted in empty sample tube and collected data to describe transport conditions in a single sample PTS such as time and absolute value and direction of the acceleration.
Methods: A status quo survey of the existing PTS system (TEMPUS600®) in terms of occurring accelerations was performed by a newly developed in-tube data logger. In addition donor blood was drawn in PTS accepted tubes (BD® Vacutainer) and analytes indicating hemolysis (free Hb and LDH) were determined for single and repetitive transport. Furthermore, PTS like accelerations were mimicked by two different mechanical oscillators.
Results and Discussion: The data of our invented in-tube logger reached higher resolution of acceleration data than a conventional product. Due to this, phases such as starting, curve passing and final impact in the laboratory loader could be clearly distinguished. Accelerations of up to 150 *x* g were measured in the PTS. We were able to mimic PTS transport conditions by oscillators and induced hemolysis in blood samples in both systems while recording conditions with our in-tube logger.
Conclusion: We developed an in-tube data logger suitable for monitoring transport conditions even in very small PTS.

P034

Evaluation of 40 different centrifugation conditions with respect to their effect on platelet counts

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Objectives: For aPTT and INR a platelet count (PLT) of 200 10^9/L and for special coagulation tests 10 10^9/L or less are recommended. CLSI guidelines propose centrifugation for 15 min at 1,500 × *g* to obtain plasma with PLT less than 10 10^9/L[1]. Manufacturers of analyzers and assays and laboratory SOPs present widely different recommendations. We investigated 40 combinations of centrifugation force and time to identify those which would satisfy analytical requirements.
Methods: Blood samples from 5 volunteers (40 x 2.7 mL per volunteer) were centrifuged with 5 different *g*-forces between 1,500 x *g* and 3,280 x *g* for 8 periods between 4 and 12 min, thus testing 40 combinations. Plasma PLT (XN-9000, Sysmex, Norderstedt, Germany) and global coagulation tests (BCS XP, Siemens Healthcare Diagnostics, Eschborn) were measured.
Results: PLT of the study samples were between 197 10^9/L and 257 10^9/L. All of the 40 combinations reduced PLT to below 200 10^9/L. The largest gain was obtained between 5 and 6 min and between 1,500 and 2,000 *x g*. Centrifugation at 3,000 x *g* for 12 min as well as at 3280 x *g* for 12 min reduced PLT to 10 10^9/L or less. Results for aPTT and INR were not affected.
Conclusion: By a set of logical combinations we identified centrifugation conditions which would satisfy specifications for coagulation investigations.
References:
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Fortschritte in der Biomarkeranalytik I

P035

Serum concentrations of cysteine-rich angiogenic inducer 61 (CYR61) correlate with tumor stage and clinical-pathological features in patients with 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 ≥10cm 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.

P036

Connective Tissue Growth Factor (CTGF/CCN2) in serum – a new marker of malignant transformation of liver cirrhosis?

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Introduction: Pathophysiological reason and previous studies suggest connective tissue growth factor (CTGF/CCN2), an important downstream mediator of profibrogenic TGF-ß, 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 CYR61 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 cutoff 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.

P037

Human xylosyltransferase-I-a target of fibrosis-associated microRNA-145?

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Objectives: Human xylosyltransferase-I (XT-I) catalyzes the rate-limiting step in proteoglycan glycosylation. In fibrosis, increased *XYLT1* mRNA expression has been linked to abnormal extracellular matrix remodeling. Serum XT activity reflects proteoglycan synthesis rate and is known as fibrosis biomarker. The aim of this study was to analyze whether fibrosis-associated miR-145 might regulate XT-I.
Methods: The expression of miR-145 in response to induction with transforming growth factor-β1 (TGF- β1) was detected by a miRNA PCR-Array in human dermal fibroblasts. To determine potential targets of miRNA-145 and transcription factor binding sites, *in silico* analyses were performed. Fibroblasts were transfected with miRNA-145 and relative mRNA expression was monitored by quantitative real-time PCR. XT activity was analyzed by a radioactive enzymatic assay.
Results: *In silico* analysis revealed that transcription factor krueppel-like factor 4 (KLF4) displays a target of miR-145 while *XYLT1* mRNA is not directly bound. By analyzing transcription factor binding sites, KLF4 was identified to bind to the *XYLT1* promoter region. Transfection of dermal fibroblasts with miRNA-145, which was upregulated by TGF-β1, increased *XYLT1* mRNA expression as well as XT activity. Nevertheless, *KLF4* expression was not regulated on mRNA level. To verify the influence of KLF4 on XT-I a KLF4 specific knockdown by siRNA was performed.
Conclusion: Our data provide first insights into a hitherto unconsidered regulation pathway of XT-I by miRNAs. Further experiments should deepen insights into these complex mechanisms to validate whether an interference with miRNA-145 and XT-I might represent an appropriate anti-fibrotic strategy.

P038

CAAP48, a c-terminal fragment of Serpin A1, contributes to liver dysfunction in sepsis

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Objectives: Sepsis is a leading cause of mortality in the critically ill. Because of its unspecific clinical symptoms diagnosis is often delayed contributing to the high mortality. Understanding the pathophysiology of sepsis may lead to better diagnostics and new therapeutic strategies. In a recent study we identified a proteolytic fragment of α1-antitrypsin (CAAP48) as potential discriminatory sepsis biomarker, which allows differentiation of sepsis from non-infectious etiologies of SIRS with high sensitivity and specificity. Currently we analyze the physiological function of CAAP48 on human hepatocytes.
Methods: A microfluidic-supported organoid that mimics the liver sinusoidal anatomy allows us to study the physiological function of CAAP48 on HepaRG under static or flow conditions. HepaRG were stimulated with synthetic CAAP48 and several control peptides. The functionality of hepatic transporters was determined based on DY-635 and CDFDA staining. Cytokine concentrations were measured using a commercially available cytometric bead array.
Results: We observed a strong reduction of DY-635 and CDFDA excretion after co-incubation with CAAP48. In addition CAAP48 leads to parallel release of pro-inflammatory cytokines (IL-6 and TNFα) presumably by macrophages. In contrast CAAP48 has no effect on the release of IL-10 and IL1-β.
Conclusion: CAAP48 is a diagnostic sepsis marker that actively participates in the progression of sepsis. We have already shown that CAAP48 activates neutrophil granulocytes and induces apoptosis and migration. Now we demonstrate that CAAP48 may be involved in hepatic dysfunction, a very common complication in sepsis, by inhibiting the hepatic transporter MRP2 and induction of IL-6 and TNFα.

P039

Reduced serum lysophosphatidylcholine in patients with more severe liver cirrhosis is related to liver function and ascites

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Background: Recent studies suggest that serum lysophophatidylcholine (LPC) is reduced in patients with liver cirrhosis and hepatocellular carcinoma.
Aim: It was analyzed whether LPC is associated with residual liver function and complications of liver cirrhosis.
Methods: LPC species were quantified by direct flow injection electrospray ionization tandem mass spectrometry (ESI-MS/MS) in serum of 44 patients with mainly alcoholic liver cirrhosis.
Results: Systemic saturated and total LPC decline in patients with more advanced liver injury defined by the CHILD-PUGH score. Subsequently, these LPC species negatively correlate with the MELD score. Serum markers of liver function and CRP are negatively associated with saturated and total LPC. Ascites and varices are secondary complications of liver cirrhosis. Although these lipids do not correlate with hepatic venous pressure gradient, levels are reduced in patients with little compared to no ascites but do not further decline in those with modest / massive ascites. There is no change with increasing variceal size. Phosphatidylcholine is not altered in patients with little ascites or worse liver function suggesting that phospholipase A2 and lecithin cholesterol acyltransferase catalyzed synthesis is not reduced. Portal venous serum (PVS) LPC was lower compared to systemic venous serum (SVS) and hepatic venous serum (HVS) levels. Current data suggest that reduced hepatic release of distinct lysoPC species contributes to lower serum levels in patients with more advanced liver injury.

P040

Ferromagnetic particles as a rapid and robust sample preparation for absolute quantification of eicosanoids

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Background: Arachidonic acid and its metabolites, referred to as eicosanoids, are important lipid mediators. Among other body functions they have a substantial influence on inflammation, coagulation, immune response, and smooth muscle tonus.
Aim: Development of an UHPLC-MS/MS method to quantify seven eicosanoids of particular interest (TXB_2, PGE_2, PGD_2, 5-HETE, 11-HETE, 12-HETE, and arachidonic acid) with a sample preparation suitable for large study cohorts.

Method: For the sample preparation we employed the innovative approach of "ferromagnetic particle enhanced deproteination": in brief, 100 µL of human plasma were mixed with 25 µL of the internal standard solution, afterwards 40 µL magnetic particle suspension and 300 µl acetonitrile were added. The samples were placed on a magnetic separator generating a clear supernatant. Further clean-up was performed using "on-line solid phase extraction" with a trapping column included in the UHPLC-MS/MS system. The chromatographic separation was achieved in a total run time of 7.5 min and negative electrospray ionisation was used.
Results: The method was evaluated thoroughly with a protocol based on the EMA guideline for bioanalytical method validation. The results were very satisfying: Imprecision ranged between 3-14% for ethanolic QC and 6-15% for authentic matrix controls (depending on the analyte). Accuracy was 105-110% (only determined for ethanolic QCs). We were able to show the feasibility of "ferromagnetic particle enhanced deproteination" as a rapid and robust way of sample preparation for quantitative LC-MS/MS analyses of plasma samples. This novel technique might be an attractive and generic tool smoothing the trail to automation in LC-MS/MS.

P041

Estimated indocyanine green plasma disappearance rate – a novel liver function parameter in intensive care?

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Background: Indocyanine green (ICG) plasma disappearance rate (PDR) plays an important role in ICUs as a dynamic, sensitive and prognostic liver function test. To test the ICG-PDR by LIMON technology, ICG is administered intravenously. ICG is selectively taken up by hepatocytes, eliminated into the bile unchanged and the ICG-PDR is monitored transcutaneously. Limitations of this important test are especially the the substantial costs and the laborious manual procedure.
Objectives: We aimed to develop formulae that allow predicting the ICG-PDR using conventional laboratory parameters.
Methods: In 69 patients (18 patients with continuous renal replacement therapy (CRRT), 51 patients without CRRT) of a prospective observational study, ICG-PDR and numerous different clinical and laboratory parameters were determined. Parameters were correlated with ICG-PDR in a multivariate linear regression analysis to find independent covariates correlating significantly with ICG-PDR.
Results: In CRRT-patients, only total bilirubin (tBil) was an independent covariate (Spearman $r_s = -0.94$; $p < 0.0001$). Values of the estimated $ICG\text{-}PDR_{CRRT}$ (eICG-PDR) agreed well with real ICG-PDR ($r^2 = 0.84$; $p < 0.0001$). In Non-CRRT-patients, independent variables were aPTT ($r_s = -0.44$; $p = 0.001$), 24-hour urine volume ($r_s = 0.52$; $p < 0.001$) and tBil ($r_s = -0.38$; $p = 0.006$). Using these variables, the adapted formula for $eICG\text{-}PDR_{non\text{-}CRRT}$ for non-CRRT-patients moderately agreed with ICG-PDR ($r^2 = 0.56$; $p < 0.0001$). These results indicate that the laborious determination of ICG-PDR by LIMON technology has no advantage over tBil in critically ill CRRT-patients.

P042

Clinical Metabolomics: advantages of global (non-targeted) or targeted phospholipid analysis

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For metabolomics analysis targeted or non-targeted approaches based on Triple Quadrupole or QToF mass spectrometry are used to reveal significant differences between sets of samples (e.g., healthy vs. disease; before vs. after treatment) in the search for (bio)markers. While QTof scan an entire mass range of small molecules in very short time intervals and at high mass resolution, triple quads are more sensitive and specific mass transitions to quantify more reproducible. Here we present a comparison of high-end QToF and Triple Quadrupole analysis in terms of their ability to distinguish between sample sets. We focused in our comparison on phospholipids.

P043

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

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Background: 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.
Methods: 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 than 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 leukocyte changes in sepsis and SIRS.
Results and Discussion: We are currently exploring the feasibility of this approach as diagnostic and prognostic in a cohort of patients.

P044

Joint database mining and fragmentation simulation facilitates identification of unknown metabolites by UPLC-QTOF-MS

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Non-targeted metabolic profiling based on ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) offers new chances for biomarker discovery. Although standardized UPLC-MS assays for measuring clinical samples are well established, extracting valuable information of these large datasets is still challenging. To facilitate structural identification of metabolites in complex datasets, we developed a bioinformatic pipeline that mines databases and performs in silico fragmentation using MetFrag. We first searched discriminative metabolites between disease and control samples against HMDB, KEGG, Reactome and WikiPathways to identify specific pathways or molecule classes differentially regulated among sample groups. To further increase chances of identification, we fragmented compounds in PubChem in silico using MetFrag. By combining our bioinformatics tools with Progenesis QI into a workflow pipeline, we demonstrate that our approach reinforces the identification of features of interest.

P045

Expanding the human metabolome coverage by combining HILIC and reversed-phase UHPLC-MS

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Background: Non-targeted metabolic profiling studies based on ultra-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-MS) aim to achieve broad metabolome coverage by simultaneously measuring differential levels of multiple metabolites in biological matrices. The broad range of physicochemical properties of metabolites is challenging for UHPLC-MS and stipulates multi-method approaches for the discovery of diagnostic signatures and candidate biomarkers in clinical samples.
Aim: The aim of this work was to expand the metabolome coverage of clinical plasma samples by combining the commonly used reversed-phase (RP) chromatography with the complementary hydrophilic interaction liquid chromatography (HILIC) separation for improved non-targeted UHPLC-MS metabolomics analysis.
Methods: Non-targeted metabolomics analysis of plasma samples was performed on a UHPLC-QTOF MS system using both HILIC and RP chromatography. Using our in-house developed bioinformatics pipeline, the list of detected metabolites identified was searched against HMDB, KEGG, Reactome and WikiPathways to assess specific pathways or molecule classes.
Results: By combining two complementary LC methods, an improved metabolome coverage of compounds with diverse physicochemical properties was achieved, compared to the traditionally used RP chromatography. The combination of both methods led to an in-depth characterization of pathways that are likely to be differentially regulated in comparative metabolic studies.

P046

Fischer's ratio predicts outcome in patients with end-stage liver disease

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Objectives: In progression of liver cirrhosis, metabolic balances of amino acids are disturbed as the liver is a main metabolizing organ. Plasma concentrations of the branched-chain amino acids (BCAA: valine, leucine and isoleucine) decrease whereas concentrations of the aromatic amino acids (AAA: phenylalanine, tyrosine and tryptophan) increase. Fischer's ratio, defined as BCAA to AAA ratio, combines several pathways. This is the first study evaluating the prognostic role of Fischer's ratio in patients with end-stage liver disease.
Methods: We included 166 patients who were evaluated for liver transplantation. Patients were not standardized according to food intake. Events were censored at time of transplantation or end of follow-up time. Maximum follow-up time was 24 months. Amino acid plasma concentrations were measured by LC-MS/MS.
Results: 33 (19.9%) patients died during follow-up time without receiving a liver transplant. 18 (10.8%) patients were transplanted and 115 (69.3%) survived without transplantation. Low values of Fischer's ratio were highly significantly associated with a higher mortality rate ($p<0.001$). Below the median of Fischer's ratio (<1.33), patients had a hazard ratio of 2.35 (95% CI, 1.12 to 4.94; $p=0.02$) for death in comparison to patients above the median in our study population.
Conclusion: Fischer's ratio is a strong predictor of mortality in end-stage liver disease. It may serve as a diagnostic instrument to improve the clinically established MELD (Model for end-stage liver disease) score. Furthermore, regarding to individual values, Fischer's ratio could lead to innovative treatment and dietary concepts.

Endokrinologie

P047

Analytical and Clinical performance of the new the Fujirebio Lumipulse® G 25-OH Vitamin D assay; a comparison with liquid chromatography-tandem mass spectrometry (LC-MS/MS) and 3 other automated assays

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Background and Aim: We evaluated the analytical and clinical performance of the new Lumipulse *G* 25-OH Vitamin D assay from Fujirebio, and compared it to a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method and 3 other commercial automated assays.
Methods: Total 25 hydroxy Vitamin D (25(OH)D) levels were measured in 100 selected serum samples from our routine analysis with Fujirebio 25(OH)D assay. The results were compared with those obtained with LC-MS/MS and 3 other automated 25(OH)D assays (Beckman, Abbott and Roche). The accuracy of each assay tested was evaluated against a Labquality reference serum panel for 25(OH)D (Ref!25OHD; University of Ghent).
Results: Intra- and inter-day imprecision of the Fujirebio 25(OH)D assay was less than 5%. Fujirebio 25(OH)D assay showed the highest correlation among the assays tested to the LC-MS/MS method ($r = 0.986$). The mean relative bias obtained was -15.59% (Fujirebio), -12.68% (Beckman), -2.06% (Abbott) and 9.72% (Roche) as compared to LC-MS/MS method. Comparison with the Labquality certified reference serum panel yielded a mean bias of -11.83% (Fujirebio), -14.13% (Beckman), 4.37% (Abbott) and 3.18% (Roche), respectively. Compared to LC-MS/MS, the sensitivity of different methods in detecting vitamin D deficiency (<50 nmol/l) varied from 100% for the Fujirebio assay to 72.73% for Roche, and specificity ranged from 94.38% for Roche to 87.64% for Beckman.
Conclusion: The Lumipulse *G* 25-OH Vitamin D assay from Fujirebio demonstrated a good correlation with the LC-MS/MS method and some of immunoassays. The performance of the assay is well-suited for routine 25(OH)D measurement in clinical serum samples.

P048

Multicenter performance evaluation of a second generation cortisol immunoassay on roche diagnostics cobas® systems

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Objectives: To assess the analytical performance of a new Elecsys® Cortisol Generation II (Cort II) assay (Roche Diagnostics) and to compare it to the Elecsys® Cortisol Generation I (Cort I) assay, liquid chromatography-mass spectrometry (LC-MS/MS) and other immunometric methods.
Methods: The Cort II assay is a fully automated competitive electrochemiluminescence immunoassay using 10µl serum/plasma or saliva and is traceable to IFCC 451 Panel (ID-GC/MS). For precision experiments, **cobas e** 411 analyzers were used according to CLSI EP05-A3 guidelines, and each study site used identical native and spiked samples covering the measuring range (1.70–1735 nmol/L).

Results: Standard deviations (SDs) for intermediate precision were ≤1.42 nmol/L at cortisol concentrations of 7.03–8.55 nmol/L and coefficients of variation (CVs) were ≤5.75% for cortisol concentrations of 94.0□1660 nmol/L. For the reproducibility study, total SD was 0.96 nmol/L at 8.44 nmol/L, and CVs were 6.8□9.5% at serum cortisol concentrations of 99.7, 482, 966 and 1611 nmol/L. A method comparison (Passing/Bablok regression) yielded the following results (n = 256□541): Cort II(y) vs. Cort I(x) y = 0.76x + 10.27 nmol/L, r = 0.968 for serum and y = 1.21x-5.50 nmol/L, r = 0.992 for saliva samples; Cort II(y) vs. LC-MS/MS y = 1.02x + 4.47 nmol/L, r = 0.986 for serum and y = 1.13x + 0.83 nmol/L, r = 0.993 for saliva samples; Cort II(y) vs. Abbott Architect y = 1.16x-24.50 nmol/L, r = 0.971 for serum; Cort II(y) vs. Siemens Centaur y = 0.92x-4.06 nmol/L, r = 0.832 for serum.
Conclusion: The Elecsys® Cortisol II assay had good precision over the entire measuring range, and with excellent correlation to LC-MS/MS. The test was found suitable for routine diagnostic application.

P049

Quantitation of steroid hormones in different biological matrices – one method to rule them all

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Objectives: The increasing interest in quantitative steroid analysis is hindered by the many different biological matrices, their different concentration ranges and particular pre-analytical requirements. Hence, there is an apparent need for different and suitable analytical methods. Our aim was to forge one single, unifying method for the quantitation of 17-OHP, Aldosterone (A), Androstenedione (A), Cortisol (F), Cortisone (E), DHEAS, Estradiol (E2), Progesterone (P), and Testosterone (T) that is easily applied to different matrices.
Methods: Plasma, serum, saliva, and urine samples as well as hair and dried blood extracts were prepared by dilution with precipitating agent including internal standards. Online solid phase extraction for sample cleanup and analyte enrichment was combined with rapid reverse phase liquid chromatography via automatic column switching. Detection by tandem mass spectrometry was performed on an AB SCIEX QTRAP® 6500 applying ESI, MRM and MS^3.
Results: With a total run time of 4.0 min the lower limits of quantification ranged from 10 pg/ml for E2 up to 1 ng/ml for DHEAS. The overall imprecision was 2.9-15.3%. Accuracy in plasma/serum was 89.1-100.8% for 17-OHP, A, AE, F, E, E2, P and T and 103.5-130.1% for DHEAS. Method comparisons to routine immunoassays showed good correlation. The issue of very low endogenous concentrations for 17-OHP, A and E2 in saliva and/or plasma as well as the impairment of chromatographic quality in hair analysis due to pre-analytical factors could be overcome utilizing MS^3.
Conclusion: We present a reliable and highly adaptive method for the quantitation of certain steroid hormones in plasma, serum, saliva, urine, hair, and dried blood.

P050

Comparison of indirect and direct determinants of placental 11beta-hydroxysteroid dehydrogenase 2 (11bHSD2) activity using LC-MS/MS method

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Background: The placental enzyme 11bHSD2 mediates the conversion of cortisol to its inactive 11-ketoform cortisone, thereby shielding the human fetus from maternal glucocorticoid excess. Malfunction of this barrier has detrimental effects on intrauterine and postnatal development. So far, the majority of studies on the assessment of 11bHSD2 function are based on the determination of parameters that reflect the indirect activity of 11bHSD2.
Aim: It remains elusive, whether these indirect measurements correlate with the actual enzymatic function of 11bHSD2. Hence, we set out to compare parameters of indirect tissue activity (mRNA expression, cortisol-to-cortisone-ratio) with the direct enzymatic 11bHSD2 turnover-rate in the same placental tissue sample.
Materials and Methods: 3 chorionic and basal placental samples were taken from term placentas (n=10) in relation to their proximity to the umbilical cord. For comparison analysis, qPCR and LC-MS/MS, as well as microsomal extraction for direct in vitro analysis of 11bHSD2 activity were conducted in the same placental sample.
Results: Indirect determination of placental 11bHSD2 activity via measurement of steroid isoforms by LC-MS/MS correlates with the results obtained from the same sample by direct enzymatic assay in vitro, using LC-MS/MS. While this observation seemed independent from the sampling side, a strong influence of the mode of delivery on tissue steroids was observed: The mRNA expression of 11bHSD2 correlated with indirect and direct LC-MS/MS cortisol turnover-rates in C-section placentas only. CRH, cortisol and cortisone levels were significantly increased in placental samples following spontaneous birth.

P051

Does the use of a high sensitive fully automated AMH assay improve the prediction of menopause?

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Background: Anti-Mullerian hormone (AMH) has emerged as a marker of ovarian reserve and a possible surrogate measure of reproductive aging.
Objectives: The aim of the study was to evaluate the predictive value of AMH levels in determining the median time to menopause for late reproductive age women.
Methods: A 9-yr follow-up, 2006–2015, was conducted in a population of 77 late reproductive age women. Observed time to menopause was measured. Serum for AMH levels (ng/mL) were measured using the AMH Access immunoassay on a Beckman Coulter Access2 immunoassay analyzer and standard deviation scores (SDS) were calculated.
Results: All participants were premenopausal, with a mean (SD) age of 48.57 (3.11) yr and a median AMH level of 0.28 ng/ml at baseline. AMH strongly predicted time to menopause; age further improved predictions. Among women with an AMH SDS below -1.0, the median time to menopause was 1.21 yr [95% confidence interval (CI), 0.20–2.33] in the 50- to 55-yr age group and 4.94 yr (95% CI, 1.81–6.63) in the 45- to 50-yr age group. With higher AMH SDS above 0 the median time to menopause was 2.33 yr in the older age group and more than 6.07 yr in the younger age group. Smoking significantly reduced the time to menopause (hazard ratio, 1.67; 95% CI, 1.21–2.24; p= 0.004).
Conclusion: AMH is a strong predictor of medium time to menopause in late reproductive age women. Age and smoking are significant and independent contributors to the predictions of AMH.

P052

Measurement of 1,25 (OH)2 Vitamin D and 25-OH Vitamin D in hemodialysis patients

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Background: Vitamin D, whether absorbed from the intestines through diet or synthesized subcutaneously, is hydroxylated in the liver to 25-hydroxyvitamin D. A second hydroxylation step occurs in the kidney by the 25(OH) D-1α hydroxylase which is tightly regulated by hormonal control loop consisting primarily of serum calcium, phosphorus and PTH. The product of the hydroxylation is the 1,25 $(OH)_2$ Vitamin D, the active metabolite of Vitamin D.
Aim: The aim of the study was to analyse and evaluate vitamin D status in the group of hemodialysis patients.
Methods: The analyses were realized in 478 CKD patients on hemodialysis with an age range of 22-94 years. 1,25 $(OH)_2$ Vitamin D was performed on LIAISON®XL (DiaSorin) analyzer. GFR was determined by the MDR formular based in serum creatinine (Roche Cobas 8000), age, sex and race. 25-OH Vitamin D was measured with the LIAISON®XL analyzer and the Roche E170 System.
Results: In all 478 patients creatinine based GFR-MDR was below 30 mL/min/1,73m^2. Median 1,25$(OH)_2$ Vitamin D was 15,4 ng/L (range 5,0-72,0 ng/L, 2,5-97,5th percentile **5,0-46,6** ng/L) which is lower than the reference range in healthy adults aged 21-75 years (2,5-97,5thpercentile **19,9-79,3** ng/L). Comparison of the 25-OH Vitamin D measurements revealed differences expressed in the following equation:
LIAISON®XL = 0,73 (Roche E170) + 6,6 r=0,856.
Conclusion: Our results demonstrate that 1,25 $(OH)_2$ Vitamin D values in hemodialysis patients, measured with the new LIAISON®XL 1,25 $(OH)_2$ Vitamin D assay, are below the reference range in healthy individuals. These results are consistent with results of other studies.

P053

Biochemical diagnosis of pheochromocytoma by measurements of overnight excretion levels of catecholamines and metabolites as a simplified alternative to 24-hour collections

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Objective: To evaluate differences in day and overnight collections of urinary catecholamines and their free O-methylated metabolites.

Methods: We compared urinary free metanephrines and catecholamines collected separately during day and overnight in subjects with (n=38) and without pheochromocytoma (PCC) (n=677; 260 healthy normo- and hypertensive volunteers, 417 patients in whom PCC was tested and excluded). Levels were determined by liquid chromatography tandem mass spectrometry and expressed as µmol/mol creatinine.
Results: Among volunteers, urinary outputs of normetanephrine, metanephrine, norepinephrine and epinephrine were respectively 49%, 12%, 97% and 327% higher ($p<0.001$) for daytime than overnight urine collections. Similarly, among patients tested for PCC but without tumors, urinary outputs of normetanephrine, metanephrine, norepinephrine and epinephrine were 41%, 5%, 63% and 183% higher ($p \leq 0.006$) for daytime than overnight collections. In contrast, there were no differences in urinary excretion of metanephrines between daytime and overnight collections for patients with PCC. ROC curve derived diagnostic power, evaluated by comparisons of areas under curves (AUC), showed similar AUC using overnight excretion of metanephrines corrected by creatinine and for total 24h excretion levels of metanephrines without correction for creatinine (0.987 vs. 0.982 respectively).
Conclusions: Overnight collections of urine for measurements of free metanephrines corrected for creatinine provide similar diagnostic efficacy to standard measurements in 24h collections, but offer a simplified alternative collection method without being compromised by daytime increases in sympathoadrenal activity.

P054

Comparison of two automated assays for the determination of circulating 1,25 (OH)2 Vitamin D levels

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Background: Due to the very low concentrations of 1,25 (OH)2 Vitamin D (1,25(OH)2VitD) in the blood and its lipophilic character, the measurement of this parameter is analytically challenging. Current assays require a previous manual purification step. To facilitate this extraction step, new tests have been developed, which include an automated extraction followed by the measurement of 1,25(OH)2VitD by immunoassay. Today, two such tests are available: one assay from DiaSorin for measurement on the immunoassay analyzer LIAISON and another assay from IDS for measurement on the IDS iSYS immunoanalyzer.
Method: We compared the performance of both tests for quantitative determination of 1,25(OH)2VitD. Serum concentrations were measured in patient samples sent to our institute for determination of this parameter.
Results: Measured values ranged between 5.0-126 pg/mL and 7.5-202 pg/mL for the Diasorin and the IDS iSYS tests, respectively. Intra-assay and inter-assay precision was similar for both methods. We found a good correlation between 1,25(OH)2VitD concentrations determined with both methods even though the DiaSorin test showed a tendency to provide lower values for 1,25(OH)2VitD compared to the iSYS test. To check for accuracy of the tests, we are going to compares the results with those obtained via LC-MS/MS as the reference method.
Conclusion: In summary, both tests allow for a reliable measurement of 1,25(OH)2VitD with good sensitivity. The automated extraction step facilitates the determination of this parameter within a shorter time frame in clinical routine, which is essential given the increase in 1,25(OH)2VitD analysis orders in recent years.

P055

Tauroursodeoxycholic acid (TUDCA) ameliorates both tubular and glomerular injury in diabetic nephropathy, thus providing an added value to ACE-inhibition

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Background: Therapeutic inhibition of the Renin-Angiotensin Aldosterone System (RAAS) is firmly established in diabetic nephropathy (dNP). Despite efficient RAAS inhibition dNP frequently progresses to end-stage renal disease, necessitating the need of additional and mechanistically distinct therapeutic approaches. We have recently demonstrated that amelioration of endoplasmic reticulum stress using TUDCA protects mice from dNP (Madhusudhan, NatComm 2015). To foster clinical evaluation of TUDCA, which is approved for other medical indications, we determined the efficacy of TUDCA in db/db mice in addition to ACE-inhibition (Enalapril).
Methods: 16 weeks old db/db mice with established albuminuria were randomly assigned to control (PBS), Enalapril (50mg/L, drinking water), TUDCA (150mg/Kg, i.p. daily), or combined Enalapril and TUDCA treatment. Mice were analyzed after 6 weeks of treatment. Albuminuria, glomerular and tubular damage (PAS-staining, electron microscopy, marker proteins), and markers of ER-stress were analyzed.
Results: Both agents (Enalapril and TUDCA) resulted in a significant reduction of UACR, glomerular hypertrophy, and FMA (fractional mesangial area). The combined treatment was more efficient with regard to UACR reduction, but similarly protective against glomerular sclerosis. Unlike Enalapril, TUDCA conveyed additional tubular protection, which was associated with reduced ER-stress (e.g. nuclear ATF6) in the tubular compartment.
Conclusion: A combined therapy of TUDCA and Enalapril is more efficient than Enalapril alone in preventing the progression of dNP in db/db mice. These results should foster translational efforts evaluating TUDCA in patients with dNP.

P056

Inhibition of caspase-1, but not of caspase-3, ameliorates diabetic nephropathy: Does apoptosis play a role in diabetic kidney disease?

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Background: Glomerular apoptosis is thought to contribute to diabetic nephropathy (dNP), but insights into its pathogenetic relevance in dNP are incomplete.
Methods: Here we employed two partially distinct caspase inhibitors in db/db mice: M-920 (inhibiting caspases-1,-3,-4,-5,-6,-7,-8) and CIX (inhibiting caspases-3,-6,-7,-8,-10). The nephromine database was interrogated for glomerular expression of apoptosis and inflammasome markers in human dNP. *In vitro* glucose stimulated podocytes were used and caspase-1 and caspase-3 deficiency was analysed in diabetic mice *in vivo*.
Results: Both M-920 and CIX reduced glomerular cell death and caspase-3 and -7 activity, but only M-920 ameliorated dNP. Nephroprotection by M-920 was associated with reduced renal caspase-1 and inflammasome activity. Glomerular expression of inflammasome markers (NLRP3, CASP1, IL18, NLRP3), but not of apoptosis markers (CASP3, CASP7, PARP1), was significantly elevated in patients with dNP compared to non-diabetic controls without dNP. Markers of inflammasome activation (Nlrp3, caspase-1 cleavage) precede those of apoptosis activation (caspase-3,-7, and PARP cleavage) in glucose stressed podocytes. Finally, caspase-3 deficiency in mice does not protect from dNP, while both homozygous and hemizygous caspase-1 deficiency is protective.
Conclusion: Taken together, manifestation of diabetic nephropathy is independent of caspase-3, but requires caspase-1 in mice, suggesting a pivotal role of caspase-1 dependent inflammasome activation in dNP.

P057

Activated protein C epigenetically regulates the metabolically memorized p21 expression in tubular epithelial cells and protects against dNP

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Tubular injury is a frequent but ill-defined consequence of diabetic nephropathy (dNP). Interestingly, tubular senescence is enhanced in dNP. One of the regulators contributing to cellular senescence is the cyclin-dependent kinase (CDK) inhibitor p21. However, the mechanistic relevance of p21 for tubular senescence and damage remains unknown.To address this question, murine models of type-1 (streptozotocin) diabetes were analyzed. dNP was validated based on albuminuria and histological injury. A subset of mice received a SGLT-2 inhibitor, aPC, or 5aza-deoxycitidiene alone or in combination for 6 weeks.mRNA profiling showed that p21 was most prominent gene differentially expressed in dNP. This was confirmed by immunoblotting and immunohistochemistry. In glucose stressed HEK-239 cells p21 expression was increased in a time dependent manner and it remained high even if normoglycemia was restored, suggesting that p21 expression is epigenetically regulated. This was confirmed by methylation specific PCR, showed hypomethylation of the p21 promoter in hyperglycemic condition, which was sustained when normoglycemia was restored. Absence of p21 prevented tubular senescence and injury in diabetic mice. Intriguingly, the cytoprotective protease aPC induced hypermethylation of the p21 promoter and suppressed its expression. *In vivo* aPC treatment abolished hyperglycemia induced sustained p21 expression and protected from tubular damage and senescence. The 5-aza-deoxycidiene abolished the suppressive effect of aPC. These data establish a mechanistic relevance of p21-induced tubular senescence for tubulointerstitial damage and albuminuria in dNP. The nephroprotective protease aPC can revers these p21 dependent changes.

P058

Evaluation of the Greiner Bio-One saliva collection device for the analysis of Cortisol with UPLC-MS/MS

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Introduction: Salivary cortisol (C) reflects unbound (free) serum C concentration and has therefore become a valuable diagnostic tool in endocrinology. However, collecting oral fluid (OF) from xerostomic individuals with adsorption-based systems like the Salivette Cortisol (Sarstedt (SA)) can be cumbersome. In this study we compared the stimulating liquid-based pH 4.2 Saliva Collection System (SCS; Greiner Bio-One (GBO)) to the SA applying a sensitive UPLC-MS/MS method for C quantification.

Methods: OF collection devices were used as described by the manufacturer. OF concentrations in SCS spls. were determined on an Olympus AU680 using the GBO saliva quantification kit. C was quantified on a Waters Acquity/Xevo TQ-S UPLC-MS/MS. Calibration range was from 0.025 to 20 ng/mL (n = 16) with the internal standard C-D4 at 0.5 ng/mL neat OF. Twenty healthy volunteers (20-35 years, 12 males, 8 females) took part in 2 series of OF collection: series A: 10 volunteers each collected 3 consecutive OF spls. using the same device (n = 60 samples); series B: all 20 individuals collected 4 consecutive OF spls. using the 2 different devices in different order (n = 80 spls.). In both series collection time did not exceed 20 min.
Results: In series A the difference between lowest and highest C value from the mean was < 15% for both devices. Repetitive, consecutive sampling of OF resulted in similar C concentrations. Therefore the C data from series B could be correlated. Agreement of C mean value in OF from SCS and SA: slope = 0.978, r^2 = 0.992 (n = 20).
Conclusion: Consecutive OF sampling with the same collection device in an individual resulted in similar C concentrations. Using both devices in an individual revealed good agreement of the C concentration for the SCS and SA spls..

P059

Comparison of three procedures for glucose determination in a hospital setting

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Background: Preanalytical glucose degradation due to ex-vivo glycolysis is commonly thought to be eliminated by use of NaF-EDTA plasma instead of serum for glucose analyses. We examined whether this is true and whether acidic citrate-buffered plasma provides a better alternative for plausible glucose results.
Methods: Glucose was determined in patients (n=100) of a metabolic ward within less than two hours using the glucose hexokinase method on a routine analyzer (*Cobas, Roche*). Measurements were done in triplets based on serum, NaF-EDTA plasma and citrate plasma, pH 5,5 (specified sample tubes from *Sarstedt*). Paired values were compared by linear regression and differential plots and reclassified in line with established reference values.
Results: Serum glucose was determined in the range of 3.6-26.6 mmol/l with a mean of 9.0 mmol/l. Glucose in serum and in NaF-EDTA plasma was indistinguishable with mean differences between paired values of 0,0 ± 0,2 mmol/l. In contrast, glucose in citrate-buffered plasma was on average 0,4 ± 0,4 mmol/l (5.2%) higher than either in NaF-EDTA or in serum. When standard reference values were applied, the use of citrate-buffered plasma diminished the number of normal (< 5.5 mmol/l) by 27% and increased the number of diabetic results (> 7.0 mmol/l) by 8% compared to NaF-EDTA plasma (39% and 10% respectively, compared to serum).
Conclusions: In a hospital setting the use of NaF-EDTA plasma for glucose analyses has no measurable advantage compared to serum. Citrate-buffered plasma provides higher values resulting in a potentially relevant clinical reclassification of patients as normal or diabetic.

Lipidstoffwechsel

P060

Improvement of LDL-C Determination by Roche LDL-C Generation 3 Direct Assay compared to Friedewald Formula

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Medical Background: The LDL-Cholesterol (LDL-C) concentration is the most powerful clinical predictor with respect to coronary atherosclerosis, and, therefore, it is used for coronary heart disease (CHD) risk assessment and monitoring of lipid lowering therapies. LDL-C is the primary target of lipid lowering therapy. The Friedewald formula is still widely used in the laboratory routine although its limitations are well documented in the literature. It is not recommended for use in non-fasting blood samples, in presence of hypertriglyceridemia > 2.0 mmol/L (177 mg/dL) or type III hyperlipoproteinemia and diabetes. Due to new studies there is a 30 – 40% difference in LDL-C results between Friedewald versus direct measurement if triglyceride levels are ≥2.0 mmol/L (177 mg/dL). As 20% of triglycerides results are ≥ 2.00 mmol/L (177 mg/dL), and the Friedewald equation tends to underestimate CHD risk in 20% of patients. Direct methods for LDL-C determination overcome the limitations of the Friedewald formula. Roche Diagnostics improved the specificity of LDL-C Gen.3 assay (LDLC3), a highly specific assay for direct measurement of LDL-C in serum and plasma. The new method shows low interference of endogenous metabolites (hemoglobin, bilirubin, and turbidity) tested according Glick. The LDLC3assay is capable of using non-fasting samples.

Development Goals LDL-Cholesterol Gen.3:
- Traceability to reference method (Beta Quantification method)
- Improvement of specificity for LDL-C
- Reduction of interference of remnants and of VLDL fractions
- Improvement of turbidity interference (L-Index 1000)

The development goals for the new LDLC3 assay were met in internal and external evaluation studies.

P061

Dysregulation of Lysophospholipid and Sphingolipid Signaling in Diabesity and Vascular Disease

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We performed morbidity(MB) and mortality(MT)studies in Diabesity, quantifying lipid species by ESI-MS/MS. Plasma lysophosphatidylcholine (LPC) levels revealed a decrease of most LPC species in Diabesity and negative correlations with BMI, CRP, IL-6 and HbA1c levels. Correlating BMI ratios before and after >10% weight loss with the ratios of total LPC vs. individual LPC species,showed significant negative relationship of LPC ratios with BMI ratios. While obesity was associated with decreased plasma LPC, hypertension without obesity correlated with elevated LPC levels. Sphingosylphosphorylcholine(SPC) levels,together with soluble CD163 (Hb/Haptoglobin receptors) were significantly higher in Diabesity patients. Association of plasma lipid species with total- and cardiovascular MT, revealed protective effects of LPC species together with PUFA-phosphatidylcholine(PUFA) species and long chain sphingomyelin (SM) and ceramide (Cer) species. In contrast, SAFA- and MUFA-PC species (e.g. PC32:0), and 16:0- and24:1-containing SM and Cer species showed strongest positive association with MT. A ratio of the sums of the six most protective species and the six species with the strongest positive MT associated, indicated an almost 3-fold risk for MT, which was higher than the hazard ratio for known risk factors. Among blood cells, LPC16:0 and LPC18:0 predominate in red blood cells and platelets, and are low in monocytes, where LPC20:4, LPC22:5 and LPC22:6 predominate. LPC18:0 is high in granulocytes(PMN). Cer 16:0 is high in in PMN, while Cer20:0 and Cer22:0 predominate in platelets. Regulatory lipid species in plasma and cells may be valuable biomarkers for stratification, management and outcome prediction of diabesity patients.

P062

Pitfalls in the high resolution analysis of mitochondria by subcellular lipidomics

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Background: Investigations of mitochondria by metabolomics or lipidomics approaches to answer (patho)physiological questions are getting more and more into the focus. Key for valid metabolipidomics results is the purity of isolated mitochondria and a sophisticated UPLC-MS analysis. However, the existence of a considerable number of mitochondria isolation procedures makes a decision for the most suitable strategy quite challenging.
Objectives: Development and optimization of a robust and practical strategy for the high resolution analyses of pure mitochondrial lipid profiles for functional subcellular lipidomics studies.
Methods: Three different isolation methods (differential centrifugation (DC); DC followed by ultracentrifugation (UC); magnetic-bead assisted method (MACS)) were compared using HepG2 cells and liver tissue. Organelle-specific markers for mitochondria as well as for impurities (ER, peroxisomes, nuclei, lysosomes and lipid droplets) were investigated by western blot analysis. Non-targeted lipidomics was performed using HR-UHPLC-LTQ-Orbitrap-MS.
Results: The frequently used DC, showed major organellar contaminations (ER, nuclei, etc.). Purification by UC showed the highest purity. A total of 393 lipid species, including 32 cardiolipins, could be detected in only 100 µg of hepatic mitochondria applying our optimized strategy for the sensitive, comprehensive and reproducible investigation of the mitochondrial lipidome.
Conclusions: Subcellular metabolipidomics provide a promising research perspective in medical research. Impurities caused by contaminations with other organelles are a major pitfall possibly resulting in misleading, incomparable or misinterpreted analytical findings.

P063

Normal functionality of high-density lipoproteins in children with type 1 diabetes mellitus

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Background: Type 1 diabetes mellitus (T1DM) in children is associated with endothelial dysfunction. High-density lipoproteins (HDL) have multiple endothelial-protective and antidiabetic functions that are impaired in adults with diabetes mellitus.
Aim: To assess whether the functional properties of HDL are changed in children with T1DM.
Methods: HDL was isolated from 19 children with T1DM and 27 non-diabetic control children who were hospitalized because of non-severe illnesses. The endothelial-protective and antidiabetic effects as well as the efflux capacity of HDL were examined in vitro.
Results: The HDL-induced increase in endothelial nitric oxide production tended to be attenuated in children with T1DM, whereas the inhibitory effect of HDL on cytokine-induced adhesion molecule expression in endothelial cells was not altered. In addition, the ability of HDL to protect endothelial cells from starvation-induced apoptosis was not changed in children with T1DM. Similarly, there was no difference in the protective effect of HDL against thapsigargin-induced pancreatic beta cell apoptosis between T1DM children and controls. Although there was a tendency for a decrease in ATP-binding cassette transporter A1 (ABCA1)-independent cholesterol efflux from macrophages toward HDL isolated from children with T1DM, total cholesterol efflux from ABCA1-expressing macrophages was not significantly different between the groups.
Conclusion: These data indicate that HDL function is not significantly impaired in children with T1DM as compared to non-diabetic children without any severe illness. To prove normal HDL functionality, the study should be re-done by comparison of T1DM children with children from the population.

P064

Trib1 deficient mice show increased insulin sensitivity and resistance to diet-induced obesity

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Background: Genetic studies identified *TRIB1* to be associated with plasma lipids and the risk of CHD. We recently demonstrated that Trib1 deficiency in mice on chow diet increases hepatic lipogenesis and VLDL production, leading to significant elevations of plasma cholesterol and triglycerides. In the present study, we aimed to investigate whether Trib1 is also involved in regulating energy homeostasis.
Material/Methods: Trib1-/- mice and littermate controls were fed a high fat diet (60% kcal/fat) for 16 weeks with weekly recording of body weight. NMR measurements of body composition, glucose and insulin tolerance tests were performed. Food intake, energy expenditure and activity were analyzed in metabolic cages and euglycemic-hyperinsulinemic clamp experiments were carried out. In addition, *TRIB1* mRNA expression was determined in human white adipose tissue samples.
Results: Trib1-/- mice on high-fat diet remained significantly leaner than controls. Concomitantly, we observed better glycemic control in Trib1-/- mice, as determined by glucose tolerance- and insulin tolerance assays. Euglycemic-hyperinsulinemic clamps revealed an enhanced whole-body insulin action in Trib1-/- mice. While we did not observe differences in body temperature or less food intake, voluntary physical activity was decreased in Trib1-/- mice. In human adipose tissue, higher *TRIB1* mRNA expression was associated with increased body weight and percentage of body fat.
Conclusions: Our data strongly indicate that Trib1 contributes to multiple metabolic pathways involved in the regulation of whole body energy homeostasis in mice and humans. Further analyses are needed to determine the underlying molecular mechanisms of Trib1 in energy metabolism.

P065

Simultaneous Identification and Quantification of Triacyglycerol Species in Human Plasma by Flow Injection Electrospray Ionization Tandem Mass Spectrometry

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Objectives: Civilization diseases like atherosclerosis and type II diabetes are associated with elevated triacylglycerol (TG) levels. Fatty acyl (FA) residue 16:0 had shown to have atherogen potential. TGs containing FA residue 20:4 were associated with a decreased risk in the development of diabetes mellitus. With increasing knowledge of the effects of FA distribution in TGs, it is necessary to study the TG molecular species. Using conventional enzymatic methods, the TG molecular species cannot be differentiated.
Methods: Flow injection analysis coupled to tandem mass spectrometry was performed on an AB Sciex API 4000 with positive electrospray ionization. Ammoniated precursor ions of 19 TG species were studied by combination of 9 neutral loss (NL) experiments. Sample preparation was carried out by simple toluene/methanol (1:1 v/v) protein precipitation. The deuterated internal standard d_5-TG 50:0 had been used for quantification.
Results: The method was validated including linearity, coefficient of variation, lowest detectable concentration, lower limit of quantification and recovery. The predominant TG species in human plasma are 52:2 and 52:3. The most abundant FA residues in plasma TGs have been found to be 16:0, 18:1, 18:2 and 20:4. Inter-individual differences in composition of TG molecular species could be identified. Good correlation for total TG concentration between the conventional photometric and MS/MS method had been obtained. Significant differences between fasting and non-fasting subjects were obtained.
Conclusion: The developed MS/MS method can be applied to get knowledge of the normal physiological distribution of TG molecular species in human plasma in patients with coronary heart disease and diabetes.

P066

Metabolomic characterization of human lipoprotein fractions and plasma in patients with coronary artery disease

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Objective: The development of atherosclerosis is remarkably influenced by the lipoprotein metabolism. While the proatherogenic LDL is thought to be oxidized *in vivo* and promotes foam cell formation, HDL acts anti-atherogenic through reverse cholesterol transport. We aimed to characterize the lipid and apolipoprotein composition of plasma and lipoproteins in patients with and without coronary atherosclerosis.
Methods: Applying tandem mass spectrometry sphingolipids, polyunsaturated fatty acids, sterols and cholesterolesters, triacylglyceride species as well as 9 apolipoproteins were analyzed in plasma and lipoproteins (VLDL+chylomicrones, LDL, and HDL) of 17 coronary healthy patients and 22 patients with coronary artery disease (i.e. ≥ coronary stenosis ≥ 50%). Lipoproteins were isolated by density-gradient ultracentrifugation.
Results: Significant differences were found regarding the composition of plasma and lipoproteins. The distribution of sphingolipids revealed the highest content of sphingosine-1-phosphate in HDL with 86% compared to 73-78% in VLDL, LDL, and plasma. Interestingly, VLDL seems to have the lowest esterification rate of sterols. For cholesterol, the esterification rate was 61%, 79%, 85%, and 76% for VLDL, LDL, HDL, and plasma, respectively. Further, apolipoprotein M amounted to 9% in LDL relative to the other lipoproteins, while it amounted to 1-2% in the other fractions and plasma. Concerning triglycerides, 46:1 and 46:0 could be detected in each VLDL, but only in single LDL and HDL fractions.
Conclusion: These results might indicate the benefit of analyzing lipoproteins and plasma instead of plasma alone, where differences between patients with and without atherosclerosis might be concealed.

P067

The genetic variant I148M in PNPLA3 is associated with increased hepatic retinyl-palmitate storage in human subjects

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Objectives: Previous studies revealed that the common sequence variant I148M in patatin-like phospholipase domain-containing protein 3 (PNPLA3) is associated with liver fat content and liver diseases but not with insulin resistance. Recent data suggests that the PNPLA3 I148M variant has reduced retinyl-palmitate lipase activity in hepatic stellate cells. We hypothesized that the PNPLA3 I148M variant is associated with elevated retinyl-palmitate storage in human liver as potential link to the clinical pathology.
Methods: Using high pressure liquid chromatography (HPLC) we quantified the retinoid metabolites in tissue extracts obtained from liver biopsies from 42 human subjects including 13 heterozygous and 6 homozygous carriers of the minor PNPLA3 I148M variant.

Results: The PNPLA3 I148M variant was associated with a significant increase (1.4-fold) in liver fat. The content of retinyl-palmitate was elevated and the ratio of retinol/retinyl-palmitate was reduced in liver extracts obtained from homozygous PNPLA3 I148M minor allele carriers.The minor retinyl-fatty acid esters were similarly increased in homozygous PNPLA3 I148M carriers. In a multivariate model including liver fat content, these differences remained significant independent of liver fat content.
Conclusion: The increased content of hepatic retinyl-palmitate and the reduced ratio of retinol/retinyl-palmitate in PNPLA3 I148M minor allele carriers support in vitro findings of an altered retinyl-palmitate lipase activity and provide a possible link to chronic liver disease.

P068

Heterozygous deficiency of Tribbles homolog-1 gene (Trib1) increases atherosclerotic lesions in ApoE-knockout mice

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Background: We have previously identified Trib1 as a novel regulator of plasma cholesterol and triglycerides in mice. In the present study, we used heterozygous Trib1-deficient mice on the atherosclerosis prone apolipoprotein E knockout background (Trib1+/-ApoE-/-) to study the role of Trib1 in atherosclerosis development.
Methods: Trib1+/-ApoE-/- and Trib1+/+ApoE-/- control mice were fed a low-fat semisynthetic AIN76 diet (0.02% cholesterol) for 14 weeks or a high-fat diet for 16 weeks. Triglyceride and cholesterol concentrations were determined in plasma and isolated lipoproteins. Atherosclerotic lesions were analyzed at the aortic root by oil-red-O staining. For mice on high-fat diet, body weight was recorded weekly and glucose and insulin tolerance tests were performed. In addition, foam cell formation and expression of scavenger receptors was studied in Trib1-deficient bone marrow derived macrophages.
Results and Conclusion: Trib1+/-ApoE-/- mice showed significantly elevated plasma total cholesterol and non-HDL cholesterol levels on both diets, but no differences in triglycerides. In addition, Trib1+/-ApoE-/- mice on high-fat diet were significantly lighter and showed greater glucose tolerance and insulin sensitivity. However, Trib1+/-ApoE-/- mice displayed significantly larger atherosclerotic lesions at the aortic root under both dietary conditions. Furthermore, uptake of acetylated-LDL was increased in Trib1-deficient macrophages, which was accompanied by higher mRNA expression of several scavenger receptors. In conclusion, we demonstrate that heterozygous Trib1 deficiency is sufficient to impair lipid metabolism and increase atherosclerotic lesion formation in ApoE-/- mice.

P069

Heritability of cholesterol synthesis: A German twin study

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The aim of the study was to investigate the heritability of cholesterol synthesis in healthy humans under special consideration of dietary intervention. 46 healthy pairs of twins (34 monozygotic, 12 dizygotic) were investigated for 12 weeks. An isocaloric diet rich in carbohydrates (LF) was applied for 6 weeks, followed by an isocaloric diet rich in saturated fat (HF) for another 6 weeks. Lipids including total cholesterol, HDL, LDL and the cholesterol synthesis markers lanosterol, desmosterol and lathosterol were measured in serum at study entry, after the LF period, after one week and at the end of the HF period.We estimated the proportion of additive genetic variance from a model comprising additive genetic influence (A), environmental effect common to cotwins (B) and individually unique environmental (E) influence (ACE model). Serum levels of non-cholesterol sterols like lanosterol, desmosterol and lathosterol are an indicator of whole-body cholesterol synthesis. We found a high genetic influence of all cholesterol synthesis markers (ratio to total cholesterol) based on the measurements at study entry. After 6 weeks of an isocaloric diet rich in carbohydrates the genetic heritability was still high for all cholesterol precursors and after 6 weeks of an isocaloric diet rich in saturated fat the lathosterol/cholesterol ratio still showed moderate genetic heritability. Moreover, the absolute differences of serum concentrations of cholesterol precursors between study entry and 6 weeks of an isocaloric LF and 6 weeks of an isocaloric HF diet were genetically determined. In summary, not only the whole-body cholesterol synthesis but also its increase or decrease following nutritional intervention is moderately to highly heritable.

P070

AXINON® lipoFIT®-S100: an in-vitro diagnostic (IVD) test system for high-throughput lipoprotein profiling

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Background: To date, the widespread application of lipoprotein profiling for cardiovascular risk prediction has been hindered by the relatively laborious and time-consuming nature of existing measurement methods. Being mainly used as a research tool so far, NMR spectroscopy now becomes available for routine diagnostics in Europe.
Aim: This study aimed at evaluating the performance characteristics of *AXINON® lipoFIT®-S100*, an NMR-based IVD test system intended for quantification of lipoprotein particle and cholesterol concentrations in lipoprotein (sub)classes, mean particle sizes as well as the concentrations of total cholesterol, triglycerides, LDL-C, HDL-C, glucose, lactate, alanine, valine, leucine and isoleucine in human serum.
Methods: Assay performance was evaluated based on Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: Linearity was shown for all 29 analytes. Trueness experiments revealed excellent correlation for lipoprotein particle concentrations and sizes, as well as amino acids ($r \geq 0.99$). Correlation coefficients ranging from 0.82 to 1.00 were observed for standard lipid parameters, glucose and lactate. Coefficients of variation ranged between 0.1 % and 4.5 % (within-run), 0.0 % and 4.9 % (between-run), 0.1 % and 4.0 % (between-day) and 0.0 % and 5.1 % (between-site), while the mean total imprecision between 3 different systems was 3.7%, demonstrating an overall excellent precision.
Conclusion: The analytical performance characteristics of the CE-labelled IVD test system *AXINON® lipoFIT®-S100* and its throughput of up to 300 samples per day suggest that it is fit for both, diagnostic and research use, especially for studies with a large number of samples.

P071

Changes of fat and monocyte microRNA expression in patients with metabolic syndrome following lifestyle-induced weight loss

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Objectives: microRNAs (miRNA) regulate biological processes by translational repression of specific target genes. Tissue specific changes of miRNAs are common in metabolic diseases like obesity or type 2 diabetes mellitus as they regulate fat metabolism and insulin action but also obesity-related inflammation. Aim of this study was to identify how weight loss affects miRNA expression in fat tissue and monocytes from patients with metabolic syndrome (MetS).
Methods: 74 non-smoking men (45-55 yr) with MetS were randomized to a lifestyle-induced weight loss program or to a control arm. Before and after a 6 months intervention period subcutaneous fat tissue and CD14+ monocytes were obtained. Initially, RNA was isolated and expression of 1100 miRNAs was analyzed by quantitative PCR in a subgroup of 6 participants of each arm. Clinical and laboratory parameters as well as body composition were determined.
Results: Weight loss (-13,1%) was mainly attributable to a reduction of individual body fat mass (-23,5%) and was associated with an increasing number of differently expressed miRNAs in fat tissue (before: 272, after: 465) and monocytes (before: 157, after: 457). Following weight loss a subset of 280 miRNAs was found to be differentially regulated in both sample types.
Conclusion: Current results indicate that lifestyle-induced weight loss is associated with distinct alterations of miRNA expression in fat tissue and monocytes. Next, differently expressed miRNAs will be validated in the whole study population by miRNA-array. Results of this study, which may indicate distinct miRNAs as potential diagnostics or therapeutic targets for obesity-related diabetes and inflammation, will be presented and discussed.

P072

Analysis of Molecular Mechanisms Linking Obesity And Breast Cancer Progression

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Objective: Obesity is not only a risk factor for metabolic and cardiovascular diseases but recently emerged as an independent negative prognostic factor for breast cancer. New evidence suggests that tumor-associated adipocytes contribute to tumor progression. However, the molecular mechanisms that underlie this cross-talk remain elusive. In our work, we aim to analyze the interactions between adipocytes and human breast cancer cells that drive tumor progression by using a 2D co-culture system.
Methods: To analyze the interactions between mature adipocytes and human breast cancer cell lines (MDA-MB-231/436) we set up a 2D co-culture system. Furthermore, we performed cell imaging, migration assays, microarray and quantitative RT-PCR analyses on breast cancer cells to determine morphologic and molecular changes upon co-culture with adipocytes.

Results: We demonstrate that human breast tumor cells show enhanced migratory capabilities upon co-culture with mature adipocytes. Additionally, microarray and quantitative RT-PCR analyses of co-cultured breast cancer cells revealed activation of inflammatory signaling pathways (e.g. NfκB-signaling) known to be involved in breast cancer progression
Conclusion: Our preliminary data indicate that adipocytes promote breast cancer progression *in vitro*, most likely by activation of inflammatory signaling pathways. Future studies focus on analyzing the molecular mechanisms by which adipocytes enhance aggressive behavior of breast cancer cells in more detail. Achieving a better understanding of this adipocyte-cancer cell interaction may help to identify novel targets for cancer therapies.

Infektiologie

P073

Mass Spectrometry Protease Profiling for Laboratory based Diagnosis of Invasive Aspergillosis

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Background: Patients with invasive aspergillosis (IA) are classified as proven, probable or possible according to the EORTC/MSG criteria. The majority of patients are classified as possible whereas probable and proven cases remain rare. However, results showed large differences between ante- and post-mortem classifications with more proven cases classified post mortem. Hence, we applied here mass spectrometry based protease profiling to build a classification model in order to reclassify possible IA samples by monitoring the protease activity of the fungal diseases in serum as potential biomarker for IA diagnosis.
Methods: Reporter peptides were spiked in serum of patients with proven IA (n = 9) and in serum of healthy controls (n = 94) under standardized conditions. Signal intensities obtained from LCMS analysis of relevant proteolytic fragments were used to build a classification model based on support vector machines. This classification model was used to re-classify samples from possible invasive aspergillosis patients (n = 188).
Results: Reporter peptides spiking and the subsequent data analysis with support vector machines revealed 5 reporter peptides that are able to correctly differentiate between sera of healthy individuals and proven invasive aspergillosis patients. Re-classification of possible IA samples resulted in the following results: 15% (28/188) of samples were reclassified as proven IA whereas 85% (160/188) were reclassified as healthy.
Conclusion: Applying protease profiling using exogenous reporter peptides might improve the timely diagnosis of IA in the future.

P074

Performance comparison between two chemiluminescent immunoassay systems for different serological infectious disease parameters

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Infectious diseases not only are a tremendous medical burden but also testing accuracy and precision of results remain challenging for laboratories. Various automated chemiluminescent immunassays (CLIAs) from different manufacturers are available. In this study we compared the performance of 11 serological infectious diseases parameters (anti-HIV/ p24 Ag, anti-HCV, anti-HAV, anti-HAV IgM, anti-HBs, HBsAg quantitative, HBsAg qualitative, anti-HBc, anti-HBc IgM, anti-HBe, HBeAg) on cobas e602 (Roche Diagnostics Deutschland) and Architect i2000SR (Abbott Diagnostics Deutschland). The precision and accuracy of the Roche assays were evaluated using control samples and human serum samples. To determine sensitivity and specificity for the assays on both systems routine serum samples (range n=130 to n=320) as well as sera from pregnant women (n=258, for anti-HIV/p24 Ag and Anti-HCV only) were compared. Discrepant results were either resolved using PCR or Immunoblot. The majority of the Roche assays showed a better inter- and intraassay precision than stated in the instructions for use. In general, both cobas e602 and Architect i2000SR discriminated positive and negative samples with good concordance (Cohen's kappa ranged from 0,66 to 1,0) and are statistically equivalent. For anti-HIV/p24 Ag, three of the Architect reactive and two of the cobas reactive results could not be confirmed. For anti-HCV, 13 of the Architect reactive and four of the cobas reactive results could not be confirmed (either Immunoblot or PCR negative). In conclusion, the cobas e602 and the Architect i2000SR demonstrated comparable performance. A better specificity for anti-HIV/p24 Ag and anti-HCV was observed using cobas e602.

P075

Serological analysis of Epstein-Barr Virus (EBV) infection with Liaison® by DiaSorin

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Background: Epstein-Barr virus (EBV) is one of the most common human viruses. The seroprevalence for people older than 30 years is more than 90%.
Objectives: Definite confirmation or exclusion of an acute primary infection with EBV is of great importance in differential diagnosis.
Material and Methods: The analyses were performed at the Oberlausitz-Kliniken gGmbH in the hospital Bautzen. Quantitative automated luminometric immunoassays for the determination of EA-IgG, EBV-IgM, VCA-IgG and EBNA-IgG were used at LIAISON XL (DiaSorin Deutschland GmbH). The results were interpreted according to the "Liaison® EBV Guide to the interpretation of the results". Additionally EA-IgG status was evaluated. We analyzed 406 sera from 208 male and 198 female patients.
Results: 19% of patients tested had an EBV-negative serology, 15% displayed the pattern of an acute/recent infection and in 58% an infection in the past was ascertained. 8% of the tests revealed an unresolved pattern and required repetition.Testing of the three parameters EBV-IgM, VCA-IgG and EBNA-IgG without EA-IgG is sufficient for efficient EBV serology.

P076

A microarray gene expression study of collagen-binding of Streptococcus gallolyticus subsp. gallolyticus

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Introduction: *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG) is a pathogen in about 20% of streptococcal-caused infective endocarditis (IE) cases. It was postulated that collagen-binding ability is the key virulence feature of SGG in humans. For a better understanding of this host-SGG interaction, changes in the transcriptome of SGG, related to the binding to collagen matrix, were analyzed.
Methods: Binding of SGG to collagen was measured after two hours of incubation. For transcriptome analysis, RNA was extracted from two SGG strains in BHI medium in solution or bound to collagen and analyzed by microarray.
Results: The binding-ability of SGG to collagen is strain-dependent. Strain A (non-invasive) shows a weak binding-ability whereas strain B (isolated from an IE patient, invasive) strongly binds to collagen. When strain A is bound to collagen two regions in the genome were upregulated. One Region contains genes which are related to the streptococcal phage P9 and the other one is a TnGBS-related integrative and conjugative element. Binding to collagen resulted in strain B in regulation of 78 genes, 48 targets down- and 30 upregulated. Especially genes which are related to carbohydrate metabolisms are downregulated. In contrast, genes of diverse transport proteins are upregulated along with others (e.g. murein hydrolase, peptidase).
Conclusion: The expression of phage and transposon proteins in strain A indicates that the cells start to build a biofilm and provide conjugation. Downregulation of carbohydrate metabolism in strain B and expression of transport molecules shows a switch to a new energy source. Expression of peptidase and lipase by strain B might indicate a potential virulence association.

P077

MALDI-TOF MS based carbapenemase detection from solid culture media isolates and positive blood culture vials

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Background: Antibiotic resistance of bacteria leads to massive health problems. The incidence of carbapenem and multidrug resistance in Gram-negative bacterial strains are increasing globally and turn out to be a very urgent challenge in health care. The resistant bacteria play an important clinical role in outbreak situations in hospitals as well as in sepsis. Rapid diagnostic tests are necessary to provide immediate information for antimicrobial treatment and infection control measures.
Methods: Our mass spectrometry based assay was validated on 63 carbapenemase-producing Gram-negative bacterial isolates, and 35 carbapenem-resistant Gram-negative species with no carbapenemase production. These were analyzed from solid culture media and positive blood culture vials. After 4 h of incubation the carbapenemase products were analyzed by use of the MALDI-TOF MS All the isolates were genotyped for carbapenemase genes by PCR and sequencing.

Results: For culture isolates the concordance of hydrolysis assay to genetic results was 98% for OXA variants, KPC, VIM, IMP, GIM, and NDM. In contrast, only 14 of 29 *A. baumannii* isolates carrying the OXA and NDM genes could be identified from blood culture. However, from blood culture vials our method allowed the detection of carbapenemases in 98% of *Pseudomonas* and *Enterobacteriaceae* isolates harboring different genes.
Conclusions: This MALDI-TOF MS–based assay permitted the detection of carbapenemases either from solid culture media (98%) or blood culture vials (96%) for all non-*A. baumannii* isolates within 4 hours. In case of *A. baumannii*isolates the assay was highly sensitive for the detection of carbapenemases directly from solid culture media.

P078

Confirmation of genotypic detection of carbapenemase genes in Gram-negative bacteria by use of biochemical-based Carba NP assay

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Background: The emergence and dissemination of carbapenemase-producing Gram-negative bacteria is a worldwide emerging public health threat. Moreover, the accurate detection of carbapenem-resistant Gram-negative bacteria is clinically relevant for better therapy options and infection control measures. This study aimed to compare the rapid biochemical method Carba NP test with the PCR and sequence analysis of carbapenemase genes in Gram-negative rods.
Methods: 44 carbapenem-resistant *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates were collected during 1-year period. PCR was performed to detect the carbapenemase genes and Carba NP assay for the phenotypic detection of carbapenemase activity.
Results: The analysed bacterial isolates were PCR positive for KPC, IMP, VIM, NDM or OXA variants. The Carba NP assay detected the activity of VIM, IMP, KPC and NDM of *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii*isolates. However, we observed consistent problems with the detection of carbapenemases in 10 (25%) of the OXA-23 and one (2.3%) of the OXA-51 producing *A. baumannii* isolates.
Conclusion: The biochemical-based assay Carba NP test is cost-effective and affordable technique and offers a potential alternative for the rapid detection of carbapenemase production in *Enterobacteriaceae* and *P. aeruginosa*isolates, but has limitation in the detection of OXA-type carbapenemases in *A. baumannii* isolates. However, we observed no difficulty of detecting the enzyme activity when two or more carbapenemase types were produced within the same carbapenem-resistant bacterial isolate.

P079

Genotypic analysis and comparison of carbapenemase genes and virulence factor genes in clinical Acinetobacter baumannii isolates

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Background: *Acinetobacter baumannii* is a significant nosocomial pathogen due to the dissemination of highly multidrug resistant isolates. Previous genetic analysis revealed that *A. baumannii* is diverse, which correlates with major variations seen at the phenotypic level. In the current study we aimed to elucidate the genetic characteristics of nosocomial *A. baumannii* isolates and compared the antibiotic resistance and virulence genotype of these isolates.
Methods: 44 cabapenem-resistant and 18 -susceptible *A. baumannii* isolates were collected during 2014 and 2015 and PCR was performed to detect the carbapenemase genes (KPC, NDM, OXA) and the virulence factor genes*csuE* (biofilm associated factor) and *ompA* (porin loss).
Results: The carbapenem-resistant *A. baumannii* isolates were predominantly positive for the cabapenemase genes of OXA-variants (OXA-23, -51, -72) followed by NDM. All carbapenem-resistant and -susceptible *A. baumannii*isolates harboured the *ompA* gene, responsible for the porin loss. We detected in 42 (95.5%) of 44 carbapnem-resistant isolates *csuE* whereas 13 (72.2%) of the carbapenem-susceptible isolates were positive for *csuE* gene.
Conclusion: The correlation of the carbapenemase genes and the virulence factor genes *csuE* and *ompA* indicates the the pathogenic potential of such nosocomial isolates. However, the carbapenem-susceptible *A. baumannii*isolates may possess the ability to become carbapenem-resistant due to loss of outer membrane proteins (OMPs) or porins, especially in prolonged treatment with carbapenems. Overall, the results emphasize our understanding of *A. baumannii* pathogenicity and will assist in future studies determining the significance of further virulence factor genes.

P080

Routine Hepatitis E virus RNA screening of blood donors – knowledge means safety

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Background: Hepatitis E virus (HEV) infection is recognized as an emerging and often undiagnosed disease in industrialized countries, actually with asymptomatic infections occurring in blood donors of various European countries. The clinical relevance of transfusion-associated HEV infection is insufficiently understood with an ongoing debate on the impact of blood safety. Actually, we present the successful implementation of a 100% routine screening of therapeutic blood products for HEV RNA.
Methods: From January to June 2015, 44,666 donations from 22,965 individual donors (whole blood, platelets) were routinely screened for the presence of HEV RNA using the RealStar HEV RT-PCR assay (Altona Diagnostics GmbH). Nucleic acids were extracted from 4.8 ml plasma using the Chemagen MSM-I extractor (Viral 5k, Perkin Elmer Chemagen GmbH). The presence of HEV-specific IgM and IgG antibodies was determined using the anti-HEV IgM/IgG ELISA (Euroimmun, Luebeck). HEV RNA concentrations were quantified with the 1st WHO international Standard for hepatitis E Virus RNA (NAT-based assays).
Results: Screening was additionally performed to our routine screening procedure for HAV, HBV, HCV, HIV and Parvovirus B19 in a pool size of 96 samples without secondary pooling or extraction efforts. The 95% LOD of the assay was determined to 4.66 IU/ml (447 IU/ml per single donation). In total, 29 HEV RNA positive donors were identified (incidence: 0.13%). Only 7 donors already showed reactive IgM and/or IgG antibody titers (IgM+/IgG-, IgM+/IgG+, IgM-/IgG+).
Conclusion: HEV NAT screening is currently the most efficient option to improve blood safety. Therefore, we implemented a routine sensitive NAT-screening method for the detection of HEV in blood donors.

P081

Microbiological sterility control of cellular products: sense or nonsense of a dual-temperature setting for bacterial screening of platelet concentrates

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Background: An experimental study by the Paul-Ehrlich institute (PEI) demonstrated that temperatures between 35-37°C are too high for the growth of some bacterial strains (e.g. *Pseudomonas fluorescens*). Therefore, the PEI passed a statement including the requirement of a dual-temperature microbiological control of haematopeotic stem cell preparations. Here, we analyzed a potential relevance for the microbiological sterility control of platelet concentrates (PCs) as another cellular blood product.
Methods: PCs were inoculated with 37 bacterial strains (3-6 donors per strain) from different origins (PC isolates, reference strains) and stored for 3 days at 20–22°C under constant agitation. Subsequently, PCs were split to inoculate aerobic and anaerobic culture bottles (BacT/Alert AST/NST, 5 ml each), and culture bottles were incubated at 25 °C and 35 °C using the automated BacT/Alert Dual temperature system.
Results: Tested strains of *Staphylococcus* spp. (n=10), *Streptococcus* spp. (n=4), *Bacillus* spp. (n=4) and *Pseudomonas aeruginosa* (n=5) revealed a faster growth kinetic at 35 °C. Tested *P. putida* (n=3) strains showed a noticeable reduced capability to grow in PCs. Nonetheless, those having a growth capability revealed faster growth kinetics at of 35 °C. Exclusively *P. fluorescens* strain ATCC 13525 was able to grow in PCs with a faster growth kinetic at 25 °C but also detection at 35 °C.
Conclusion: Bacteria commonly involved in bacterial contamination of PCs were clearly detectable at 35°C incubation, only one *P. fluorescens* strain showed a superior growth kinetic at 25°C. Therefore screening of PCs using a dual-temperature setting for the microbiological control seems to be not necessary at the moment.

P082

Rapid detection of multidrug-resistant Escherichia coli by LC-MS

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Objectives: Antibiotic resistance is an unsolved healthcare problem with increasing impact on patient management in the last years. In particular, multidrug resistance among gram-negative bacterial strains has become the most pressing challenge.Therefore we have developed a mass spectrometry-based assay for the rapid determination of ampicillin and cefotaxime resistance.
Methods: MS-based assays for susceptibility testing are based upon the monitoring of the microbial biotransformation of antibiotics. The assay quantifies beta-lactamase activities towards ampicillin and cefotaxime within a turnaround time of 150 minutes, which is substantially faster than classical susceptibility testing. The incubation time for the detection of CTX hydrolysis was set to 2 h and to 5 h additionally at a temperature of 37°C. CTX and the internal standard SPZ were simultaneously quantified in the supernatant by LC-MS within a 10-minute HPLC-gradient and the ratio of CTX and SPZ was calculated.
Results: Using the MAAST (mass-spectrometry-based antibiotic susceptibility testing) protocol with 120 minutes incubation time we found a sensitivity (resistant-tested among resistant) of 92.4% and a specificity (susceptible-tested among susceptible) of 97.4% (results compared to Vitek 2)
Conclusion: The high precision of the LC-MS/MS-based quantification of antibiotics and metabolites with a coefficient of variation of <15% is the key advantage of this method compared to the qualitative MS-based AST-assays. The rapid detection of resistance should significantly improve the patient outcome and the management of outbreaks of nosocomial infections.

P083

Automated Isolation of HBV-DNA and HCV-RNA for quantitative Virus Detection from Human Serum Samples Using the MagNA Pure Compact System

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To reduce the hands on time and to improve the workflow efficiency for HBV-DNA and HCV-RNA purification from human serum samples we aimed at changing the currently used manual IVD certified HighPure System Viral Nucleic Acid Kit protocol into an automated purification protocol with the MagNa Pure Compact System (MPC; ROCHE, Mannheim).
Materials and Methods: Routinely tested samples for quantitative HBV or HCV PCR were collected and measured after automated preparation with the MPC in addition to the manual method. The virus load of the samples ranged between HCV (2,9E+02 – 5,6E+06 IU/mL)) and HBV (6,0E+00 – 1,2E+06 IU/mL). Additionally, reference samples from interlaboratory surveys (INSTAND e.V.) were probed for standardized evaluation. The manually specimen preparation was carried out with the HP-System Viral Nucleic Acid Kit recommended by the manufacturer guidelines. The automated purification was performed with the MPC- Nucleic Acid Isolation Kit I- Large Volume applying the purification protocol: Total_NA_Plasma_1000. For viral load quantification we used the COBAS® TaqMan® HCV Test v2.0 for Hepatitis C virus (HCV) and COBAS® TaqMan® HBV Test for Hepatitis B virus (HBV).
Results and Conclusion: Our findings provide evidence that the PCR results obtained from manually isolated HCV-RNA (HBV-DNA) samples and samples processed by MPC are congruent, precise, reproducible, and diagnostically reliable (correlation of the manual and automated processed sample results for the log titer IU/mL HCV R^2= 0,99 and for HBV R^2=0,98). Consequently automated isolation can support the workflow efficiently without losing accuracy in regard to the determination of the viral load for diagnosis and follow up in patients with HBV- and HCV-infection.

P084

Evaluation of the automated StaphSR-PCR test for pre-admission MRSA screening

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Background: Methicillin-resistant Staphyllococcus aureus (MRSA) is an important cause of hospital-acquired infections. Spread of MRSA can be avoided by isolation of asymptomatic MRSA carriers which requires rapid screening for MRSA at the time of hospital admission.
Methods: Risk of MRSA colonization was assessed using a standard questionnaire. Nasal swabs from patients with increased risk were analyzed by the StaphSR-PCR test (BD Diagnostics) detecting the MREJ and mecA/C gene loci. Positive results were validated by bacterial culture using chromogenic media and selective enrichment bouillion (Oxoid). A subset of positive StaphSR-PCR results was re-examined by a second PCR test (MRSA-PCR, same manufacturer) based on amplification of the main MREJ variants only.
Results: Of 11.500 admitted patients 3.750 were at increased MRSA risk and 5,3% of the latter (n=198) were StaphSR-positive for MRSA. Of these only 55 were confirmed by a positive MRSA bacterial culture, the remaining 143 patients being isolated unnecessarily after admission because of a false-positive PCR screening result (PPV: 27,8%, NPV: 100%). A subset of StaphSR-PCR positive samples (n=100) was re-examined

with the MRSA-PCR test. Only 35 turned out to be positive, 25 of these were confirmed positive by MRSA culture. Of the 65 MRSA-PCR-negatives all but one were also culture negative (PPV: 68,6%, NPV: 97,9%).
Conclusions: The StaphSR-PCR test is unsuitable for MRSA admission screening due to an unacceptably low positive predictive value resulting in unnecessary patient isolations. The performance of the MRSA-PCR test is in far better agreement with the reference method of MRSA bacterial culture although single false-negative results may occur.

P085

Suitability of a novel urine collection tube for microbial testing

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Background: Reliable urine testing results are of utmost importance for diagnosis, monitoring and therapy of patients with urinary tract diseases. Particularly with regard to delays in delivery to the laboratory, an increase in microbial counts due to a missing preservative or too high transport temperatures may lead to false results. The VACUETTE® Urine CCM Tube contains a novel preservative stabilizing urine samples at room temperatures (20-25°C) for up to 48 hours in order to offer a urine tube for collection, transport, storage and urine culture in the laboratory.
Materials: A study was designed to evaluate urine samples (total n= 170, partly spiked) from clinically inconspicuous as well as conspicuous (nitrite and leucocyte positive with dipstick urinalysis) urine specimens. Those samples were collected in the new VACUETTE® Urine CCM Tube. The microbiological cultures for bacterial counting were generated at the same day within 2 h after sample tube filling, after 24 h and 48 h. All specimens were stored at room temperature (20 – 25°C) between the sampling time points. The samples were tested regarding stability of the following pathogenic organisms: Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Proteus mirabilis, Candida albicans.
Results: According to the performance criteria, that the starting values do not differ significantly from the reference tube and the results after storage for 48 hours at room temperature do not differ significantly (one log step) from the 0-2 hour results, the stability of the pathogens could be demonstrated without significant differences in comparison to the reference tube.
Conclusion: On the basis of these results, the suitability of the VACUETTE® Urine CCM Tubes for microbial testing has been demonstrated. This tube stabilizes the tested organisms being responsible for urinary tract infections for 48h at room temperature. The VACUETTE® Urine CCM tube is a urine sampling and transport system suitable for microbiologic diagnostics and is found to be useful in improving preanalytics in urine culture testing.

Qualitätssicherung im Labor II

P086

Method -comparison of Immunturbidimetry, Immunnephelometry and Cation –exchange -chromatography

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The measurement of glycated hemoglobin is a routine parameter for monitoring Diabetes (Niederau and Reinauer, 1993). Hemoglobin A1c is a component of erythrocytes finding in adults, whose concentration can increase to the double in patients with diabetes mellitus (Koenig et al., 1977). The concentration of HbA1c in blood reflects the glycaemic status (Little et al., 2011) over a period of last three months, which is why it is served as an indicator and also as aim for the therapy of diabetes (Araki et al., 2011). Therefore the identification of the HbA1c value through a fast, precise device can improve the treatment of diabetes (Lemke and Matthaei, 2009). The measurement of immunglobuline G, A, M and albumine in liquor cerebrospinalis measured by a fast multianalyser in the daily labouratory routine has got plenty of advantages respective to the deployment and the rapidly provision of results. I'll will present to you the results of the method comparison of immuneturbidimetry, immunnephelometrie und cation exchange chromatography to you in the following presentation. For the analytes glycosylated Hemoglobin A1c the methods HPLC and immuneturbidimetry will be compared. Immunglobuline G,A,M in liquor cerebrospinalis will be likened to immunturbidimetry and immunnephelometry. For the diagnosis of a neurological disease the analysis of intrathecal antibody synthesis against a specific antibody can be supportive (Jacobi et al., 2007). The criteria of method comparison were, amongst others, the measurement of 50 patient samples, recovery rate, accuracy and linearity. The statistical evaluation for the results were, among others, made by Passing Bablok Regression and Bland Altman Plot.

P087

New Abbott methotrexate immunoassay is suitable for the follow up of high dose methotrexate therapy

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Objectives: Methotrexate (MTX) is a niche drug for the treatment of several oncologic and non-oncologic conditions. Especially high-dose MTX (HDMTX) is the vital therapy component for some malignant diseases, e.g. acute lymphoblastic leukemia, and is usually combined with leucovorin (LV) rescue therapy to reduce the MTX toxicity. Monitoring of the MTX concentration is essential during HDMTX because of potential individual differences in MTX metabolism and elimination as well as LV administration should be continued until low MTX concentration was reached.
Design and Methods: Patient samples and external quality assurance (EQA) samples were assayed by a new Abbott immunoassay (CMIA) and by Siemens *Emit*® MTX Assay using Architect System *ci*16200™, and by LC-MS/MS. Immediately after analyses patient samples were stored at 4°C (24 h) and -20°C (4 and 8 weeks), and stability testing of MTX were performed. CMIA imprecision studies were also performed.
Results: CMIA and *Emit*® reveal a positive bias compared to LC-MS/MS, respectively, when patient samples were measured, while no bias between methods was detected by measurements of EQA samples. In contrast to *Emit*® the positive bias of CMIA was notably lower. There were no differences in MTX concentration after storage. Linearity was validated between 0,015 and 1,500 µmol/l and Limit of Quantification was established at 0,013 µmol/l for CMIA.
Conclusions: A positive bias of immunoassays is presumably due to presence of MTX metabolites in patient samples, while CMIA seems to be more reliable method. MTX can be safely measured after one day in samples stored at 4°C. The results obtained present CMIA as an accurate, precise, sensitive and rapid MTX assay for use in clinical routine diagnostics.

P088

Comparability of co-oximetry and SLS hemoglobin determination for anemia diagnosis in emergency patients at a supra-maximal care hospital

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Background: In emergency departments, determination of Hb concentration for anemia diagnosis is usually performed with POCT devices. As many hospitals in Germany no longer avail of hematology analyzers at a central laboratory for routine analysis, nursing staff must be sufficiently skilled in preanalytics and management of POCT. Thus, a POCT concept in accordance with RiliBÄK was developed at University Clinics Bonn.
Aim: Comparison of anemia diagnosis (according to WHO definition) performed at the emergency department using POCT devices and at the central laboratory as part of routine diagnosis with SLS detection.
Method: In a retrospective analysis, we evaluated data from 2549 patients and a subcollective of 190 supra-geriatric patients (age > 85 yrs.) in whom Hb concentrations were determined at the emergency department (THb) by POCT co-oximetry (RapidPoint 1265, Siemens HealthcareDiagnostics, Germany) as well as SLS detection (XN1000, Sysmex, Germany) during routine diagnosis at the central laboratory (HbZL).
Results: On average, HbZL values were 13.06 g/dl ±2.23 (SD) and THb values 13.51 g/dl ± 2.28 (SD) [$r = 0.96$; $p < 0.001$]. Regarding diagnosis of anemia, there were significant differences between THb and HbZL in the whole collective as well as between male and female patients (in each case $p < 0.001$). In the supra-geriatric patients, this difference was also found in the whole collective ($p < 0.001$), and in male ($p < 0.01$) and female patients ($p < 0.02$). Applying the HbZL values, anemia would have been diagnosed significantly more often than with THb values. In 26 cases, the difference in results between the two measurement methods was > 2.5 g/dl.

P089

Comparison of al fully automated human placental alkaline phosphatase immunoassay with the clinical standard ELISA

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Background: An immunoassay was developed for the quantitative determination of human placental alkaline phosphatase (PLAP) in cerebrospinal fluid for the Fujurebio Lumipulse immunoassay systems. PLAP is used a tumor marker in diagnosis and monitoring of primary testicular cancer especially seminomas.

Objectives: To evaluate the application of the new fully automated PLAP assay in serum and plasma and to compare it with the clinical standard assay, the INNOTEST PLAP ELISA.
Methods: Serum levels of PLAP [pg/mL] were measured using the Fujirebio Lumipulse G1200 immunoassay analyzer. Reproducibility and linearity in dilution were determined. A method comparison with the clinical standard assay was performed and a reference range was established for healthy men and smokers to compare it with samples of patients with testicular tumors.
Results: The coefficients of variation of the within run and between run imprecision were between 3.9 and 7.8%. The Spearman's coefficient of correlation for the linearity study was 0.986. The Passing Bablok regression resulted in a coefficient of correlation of 0.998 ($p<0.001$). The equitation was PLAP (Lumipulse) = 12.29 x (ELISA) – 50.4. In the group of healthy men PLAP levels were 51.4 pg/mL (SD 12.1), 171.6 pg/mL (SD 91.3) in the smoker group and 2852.2 pg/mL (SD 4244.2) in patients with seminomas.
Conclusion: The Lumipulse PLAP assay can be used as a tumor marker in diagnosis and monitoring of testicular tumors.

P090

Using the platelet volume for the quality assessment of apheresis procedures (Verwendung des sog. Parameters "MPV" zur Qualitätsbeurteilung von Apheresеprozeduren)

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Introduction: Referring to current standards the quality of an apheresis procedure is estimated by the quantity of collected cells. Nowadays a new kind of quality measurement could be found in the detection of cell volumina. Recent diagnostics have shown that stem cells and platelets-when separated-are likely to appear in a higher volume inside the cell product. Therefore, in this study the question should be discussed wether platelets of higher volume are more likely to be separated than platelets showing a lesser volume.
Methods: Blood samples of three different apheresis procedures could be observed: allogenic platelet donations (n=5) (Trima, Terumo), autologous (n=5) and allogenic stem cell donations (n=5) (Cobe Spectra, Terumo). To examine the blood samples the Sysmex hematology analyser (XT-2000) has been used.
Results: The volume of the separated platelets was 1.2fold increased compared to the platelet volume in the peripheral blood before separation. Before apheresis the mean platelet volume in the peripheral blood was found to be 6,28 fl, after apheresis 6,13 fl and inside the platelet concentrate 7,46 fl. The platelet number in the peripheral blood was also significantly decreased (before separation 186.1/nl and after separation 137.8/nl). In the blood products the concentration of platelets was 7,62fold higher than in the peripheral blood before separation.
Conclusion: Overall, the observed apheresis procedures are more likely to separate platelets showing a higher voulme than common in the peripheral blood. This might indicate that not only the amount of separated cells reflects the quality of the apheresis procedure but also that the volume of the separated cells can be used as a parameter for quality assessment.

P091

Frequency of pseudonormonatraemia in ICU patients using indirect ion-selective electrodes

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Background: Sodium concentration (Na) can be measured by ion-selective electrodes (ISE) either indirectly (I-ISE) or a directly (D-ISE). I-ISE is sensitive to extreme protein concentrations with a tendency to give increased Na in relation to the D-ISE, thus erroneously indicating hypernatremia in for instance ICU patients. We investigated the frequency of all discordant classifications of Na between the two methods in a large sample of ICU patients.
Method: Na results from patients admitted to ICU at the University Medicine of Greifswald from 2011 to 2014 were retrospectively collected. Patients were included if Na was measured by D-ISE (Li-heparin whole blood, blood gas syringes; ABL90 flex Radiometer, Copenhagen, Denmark) and I-ISE methods (Li-heparin plasma, BD vacutainer; Dimension Vista, Siemens Healthcare Diagnostics, Eschborn, Germany) within 3 hours and results of total protein concentration were available. The D-ISE was used for classifying patients as hypo-, normo- or hypernatraemic based on the reference interval 135-145 mmol/L.
Results and Discussion: The study comprised 7,690 sample pairs. Discordant I-ISE and D-ISE results were found in1,616 (22%) patients. In this group, 8.5% were classified as pseudohypernatremic (Na >145 mmol/L), 11.7% pseudonormonatremic and 0.8% pseudohyponatremic (Na <135 mmol/L). Among the pseudohypernatremic and pseudonormonatremic patients 88% and 64%, respectively, were found to have protein concentrations below the reference interval (65-85 g/L).
Conclusion: In ICU patients with very low protein concentrations I-ISE indicated pseudonormonatreamia more frequently than previously reported pseudohypernatraemia.

P092

Comparison of the reliability of celiac disease serology to reflect intestinal damage

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Objectives: In view of increasing importance of serological biomarkers for screening and diagnosis of celiac disease (CD)-their differential performance and lack of head to head comparison-the reliability of those isolated or combined antibodies to reflect the intestinal damage in CD children was evaluated. The aim is to compare CD serological markers for intestinal histological damage.
Methods: 95 pediatric CD patients (mean age 8.3), 45 nonspecific abdominal pain children (AP) (mean age 7.3), 99 normal children (NC) (mean age 8.5) and 79 normal adults (NA) (mean age 28) were tested by the following ELISAs, detecting IgA, IgG or both, IgA and IgG: AESKULISA® Gliadin (AGA), AESKULISA® tTg (tTG; RUO), AESKULISA® DGP (DGP) and AESKULISA® tTg New Generation (Neo-epitope tTg complexed to gliadin=tTg-neo). The results were compared to the degree of intestinal injury, using revised Marsh criteria. Scatter diagrams and regression analysis comparing the 12 antibodies' optical density (OD) activities to the degree of the intestinal damage were correlated.
Results: Most of the assays were able to differentiate patients with low and high degree of intestinal damage. Comparing the different correlations between CD associated IgA and IgG antibodies' isotypes, the tTg neo IgA (r2=0.968, p<0.0025) and tTg-neo/DGP IgGs (r2=0.989, p<0.0001/ r20.985, p<0.0001, respectively) stood out as the best indicators of the intestinal damage in CD. The highest OD values (medium 2.94±1.2, p<0.0001) were achieved by using the tTg-neo IgA ELISA in patients with Marsh 3c.
Conclusion: It is suggested that tTg-neo IgA/IgG antibodies should be preferably used to reflect intestinal damage during screening, diagnosing and monitoring compliance in childhood CD.

P093

Clinical performance of LOCI™-based assays for tumor markers CEA, CA 19-9, CA 15-3, CA 125 and AFP in gastrointestinal cancer

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Background: There are only few data available on the clinical performance of LOCI™-based tumor marker assays for gastrointestinal (GI) cancers.
Aim: Here, we investigated the diagnostic power of these markers in patients with diverse GI cancers in comparison with appropriate control groups.
Patients and Materials: CEA, CA 19-9, CA 15-3, CA 125 and AFP were analysed in sera of 107 patients with GI cancers (35 colorectal, 33 pancreatic/gall bladder, 19 liver, 20 esophageal/gastric), 73 patients with benign GI diseases and 24 healthy controls on the Dimension™ Vista 1500 analyser (Siemens Healthcare Diagnostics, Eschborn, Germany). Discriminative power between groups was calculated by Wilcoxon test, AUCs in ROC-curves and sensitivities at a fixed specificity of 95%.
Results: Many markers discriminated well between cancers and healthy controls, but only tumor-type associated markers between cancers and benign controls. In colorectal cancer, CEA and CA 19-9 were significantly higher in malignant than in benign patients. For this comparison, CEA achieved the best AUC (0.84) and highest sensitivity (51.7%) at 95% specificity. The same markers differentiated well between pancreatic and gall bladder cancer and benign controls. However, CA 19-9 was the best marker reaching an AUC of 0.85 and a sensitivity of 60.6% at 95% specificity. No marker was found to be of value for esophageal and gastric cancer, whereas AFP was the most relevant marker in liver cancer achieving the highest AUC (0.87) and sensitivity (68.4%) at 95% specificity vs. benigns.
Conclusion: Our study confirms the high diagnostic performance of well-known biomarkers for diverse GI cancers using LOCI™-technology.

P094

Validation of a turbidimetric immunoassay for fecal Calprotectin

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Objectives: Calprotectin is a multifunctional protein that plays an important role in the diagnosis and follow-up of inflammatory bowel disease (IBD). High levels of calprotectin in stool samples are associated with inflammation of the intestinal tract. We evaluated the analytical performance of a new particle enhanced turbidimetric immunoassay (PETIA) on the clinical chemistry analyser BS-380 (MINDRAY) including linearity, security zone, precision and correlation to BÜHLMANN fCAL® ELISA.

Methods: The new latex based turbidimetric calprotectin assay BÜHLMANN fCAL® turbo from BÜHLMANN Laboratories AG, Switzerland applies particles coated with anti-human calprotectin (MRP8/14) antibodies: the agglutination is proportional to the calprotectin concentration. Extracts of 60 fecal patient samples were analysed on the BS-380 and compared with the results generated with the BÜHLMANN fCAL® ELISA.
Results: The assay has been tested to be linear in the range from 9 to 2059 µg/g calprotectin in stool. Security zone: Samples up to 8'000 µg/g results in concentrations above the upper assay limit of 2000 µg/g. The intra- and inter-assay precision (CV) were ≤ 4.5%. Passing and Bablok regression analysis revealed an intercept of -5.2 (-14 to 2) µg/g (95% CI), a slope of 1.04 (0.96 to 1.12) (95% CI), and a regression coefficient (r) of 0.95, suggesting that the new PETIA method showed a good correlation compared to matched ELISA assay.
Conclusions: The new latex turbidimetric procedure for determining calprotectin is an attractive alternative to ELISA allowing random access and full automation of fecal calprotectin quantitation. Moreover, it represents an accurate and precise method to determine calprotectin levels in fecal extracts.

P095

Impact of software updates on performance and workflow of laboratory automation systems

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Introduction: State of the art in laboratory automation systems (LAS) improves fast. Advanced analyzing instruments and supportive equipment like pipetting units or vision detection modules (VDM) replace hand-made processes and turnaround times. Software updates are necessary tools to keep this technology at its most potential. Within a month approximately 54.000 samples are routed automatically to clinical chemistry, coagulation and hematologic analyzers by the LAS. VDMs gather information on sample identification number (SID) and cap color by taking multiple pictures. Samples flagged with an error (e.g. unreadable SID) are sorted out for a control by personnel.
Methods: Error rates for defined use-cases were obtained from the LAS software (FlexLab, SIEMENS Healthcare Diagnostics, Eschborn, Germany) before and after an update of the LAS.
Results: A monthly rate of 2.6% (mean daily rate: 1.0 – 3.9%) of unreadable SID and 0.2% (daily rate: 0.0 – 1.1%) of false material was recorded before the update. After the software update which involved new features for VDM, the rate of unreadable SID doubled to 5.2% (daily rate: 3.3 – 7.6%) and inconsistent cap type significantly increased to 0.8% (daily rate: 0.0 – 2.0%), within 2 weeks of close monitoring. The VDM performed less efficient, many samples were falsely sorted out.
Conclusion: LAS is vulnerable to changes which might be imposed by a software update. It is necessary to carefully judge software release notes and monitor the update process. In addition changes of the established workflows must be avoided by close monitoring of important functions and applying use-cases before and after the update.

Fortschritte in der Biomarkeranalytik II

P096

Semaphorin-3C inhibits pathological angiogenesis in an animal model for retinopathy of prematurity

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Background: Preterm born babies who receive intensive neonatal care therapy with increased oxygen supply are at high risk of developing retinopathy of prematurity (ROP). This is the major ocular disorder of the neonate and the dominant cause of visual impairment in childhood. Relative hyperoxia leads to vessel regression which is followed by destructive neo-angiogenesis and growth of vessels into the vitreous humor.
Question: This study was aimed at analyzing if local delivery of recombinant Semaphorin-3C (Sema3C) suppresses pathological retinal angiogenesis.
Results: Sema3C exerted potent inhibiting effects in cellular models of angiogenesis. In an endothelial cell xenotransplantation assay Sema3C acted primarily on immature microvessels by inducing endothelial cell apoptosis. Intravitreal administration of recombinant Sema3C disrupted vessel sprouts and endothelial cell-cell contacts, which led to decreased vascular bed expansion and vessel branching in the growing retinal vasculature of newborn mice, while not affecting mature vessels in the adult retina. Sema3C administration strongly inhibited the formation of pathological pre-retinal vascular tufts during oxygen-induced retinopathy. Mechanistically, Sema3C signaled through the receptors Neuropilin-1 and PlexinD1, which were strongly expressed on vascular tufts, induced VE-cadherin internalization and abrogated VEGF-induced activation of the kinases AKT, FAK and p38MAPK. This disrupted endothelial cell junctions, focal adhesions and cytoskeleton assembly resulting in decreased cell migration and survival.
Conclusion: This study identified Sema3C as a potent and selective inhibitor of pathological retinal angiogenesis (EMBO Mol Med, 2015).

P097

Old Biomarkers (oBMs) are new Biomarkers (nBMs) of Cerebrospinal-fluid (CSF) Diagnostics, revealed with Marburg CSF Model and healthy humans.

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Leukocyte counts in CSF are oBMs of CSF cell normality: 0-1 leukocyte /µl in ventricle (V-)CSF, 0-3 leukocytes /µl in cisternal (C-)CSF, 1-5 leukocytes /µl in lumbar (L-)CSF. The oBMs represent nBMs: Leukocytes in V-CSF and C-CSF are nBMs evaluating how many leukocytes (mainly blood lymphocytes) are pressed through leaky circumventricular organs (CVOs) into V-CSF by blood pressure. nBMs 'lymphocytes in L-CSF' evaluate how many lymphocytes, refluxed from thoracic duct lymph into L-CSF, are modified with the blood lymphocytes in C-CSF (sum of 4 V-CSF), drained from Vs into spinal space. oBMs declare spinal CSF flow rate as modifier of lumbar CSF proteins, e.g. of albumin, IgG, IgA, IgM (H. Reiber, CCA 2001;310:173-186). nBMs 'V-CSF proteins' evaluate how many blood proteins are filtered through molecular sieves of choroid plexus into V-CSF: albumin >IgG >IgA >IgM. nBMs 'proteins in L-CSF' reveal how the blood proteins in V-CSF, flowed into spinal CSF space, drain out along spinal nerves: Small proteins (albumin) drain out easier than large ones (IgM). nBMs for spinal proteins summarize protein draining capacity, renewing the sink effect of CSF. Participation of blood proteins, pressed through CVOs, and of proteins of refluxed lymph makes minor contributions to total CSF proteins in Vs and spinal CSF. The nBMs renew CSF pathobiochemistry and improve human CSF diagnostics, revealed with the Marburg CSF Model.

P098

Hereditary spherocytosis-evaluation of Acidified Glycerol Lysis Time and Eosin-5-Maleimid-binding-Assay.

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Hereditary spherocytosis (HS) is the most frequent form of haemolytic anaemia in middle- and northern Europe. The prevalence in the German population is estimated at about 1:2000 to 1:2500. The diagnostic strategy can not focus on a single laboratory test. According to the recommendations of DGHO it should include both the Acidified Glycerol Lysis Time (AGLT) and Eosin-5-Maleimid-binding-Assay (EMA) in parallel. The present investigation focused on the validation of AGLT and EMA as the proposed dual-test strategy for regular routine procedure as well as the definition of Cut-Off values for both assays. 141 samples were evaluated including 16 HS-positive cases. The AGLT was performed according to the standard protocol (1). The EMA was performed as reported by King (2). The relative reduction of the mean fluorescence intensity was calculated applying two samples of non-HS-controls for each assay. The results of the EMA-assay confirm the published and widely discussed Cut-Off of 11% for the relative reduction of the EMA-binding (3).With the AGLT-assay data we were able to define a new Cut-Off of 78 seconds. The combination of AGLT and EMA supports the differentiation of a HS diagnosis with a sensitivity of 100% and a negative-predictive value of 100%. Combined with a specificity of 96% and a positive-predictive value of 76% these results are concordant with those published previously (4). Taken together, the data confirm the relevance of the approach applying AGLT and EMA in parallel.

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P099

The ascites N-glycome of epithelial ovarian cancer patients

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Background: Epithelial ovarian cancer (EOC) is worldwide the sixth most lethal form of cancer occurring in women. Early-stage EOC is usually asymptotic and specific symptoms arise after the cancer has already metastasized. In a majority of cases an advanced-stage EOC remains incurable because most patients develop resistance to chemotherapy, which results in disease recurrence. One of the causes for

recurrence of the disease is the presence of malignant ascites, an accumulation of fluid in the peritoneal cavity. Although its effect on tumor cell microenvironment remains poorly understood, its presence is correlated with bad diagnosis.
Methods: N-Glycans were digested from equivalent amount of ascites and serum from 18 primary EOC patients and from serum of 20 age-matched controls and measured by MALDI-TOF-MS, followed by statistical evaluation of N-glycan pattern among all sample cohorts.
Results: Ascites showed qualitative as well as quantitatively different N-glycosylation pattern compared to healthy serum. Overall, increased antennarity, branching, sialylation and Lewis^x motives were observed in ascites samples. Indeed, different intensities of N-glycans were detected especially for the highly branched N-glycans. The serum profile showed increased intensities of these structures compared with ascetic fluid.
Summary: We reported for the first time the N-glycome of ascetic fluid and showed that the glycome modulations, previously detected in EOC serum were also present in ascites. Both serum and ascetic fluid from EOC patients exhibited typical features of inflammatory conditions, when compared with healthy serum.

P100

Screening for synthetic cannabinoids in urine by immunoassay versus LC-MS/MS – an evaluation of the diagnostic efficiency

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Synthetic cannabinoids (SC) are important designer drugs. The demand for reliable screening methods is constantly increasing. Immunoassays (IA) targeting SC metabolites are available. However, due to the structural diversity of these substances and the highly dynamic changes of the drug market it seems questionable if the applied antibodies show sufficient cross reactivity for all relevant substructures. Two commercially available IA for urine were evaluated regarding their suitability for detecting the use of currently prevalent substances. 100 consecutive negative samples and samples positive for metabolites of only one SC (as assessed by LC-MS/MS) each were selected from a pool of authentic urine samples collected from January to June 2015. The up-to-date LC-MS/MS method covered the main metabolites of 45 synthetic cannabinoids. The samples were blinded and reanalysed using 2 homogeneous enzyme IA (SC kit 1 and 2, Immunalysis, Pomona, CA, USA). Using the cut-offs as recommended by the manufacturer, the combination of the two IA led to a sensitivity of 2% and an accuracy of 51%. The samples tested positive by IA "SC 1" were positive for THJ-018 metabolites (LC-MS/MS), which are structural similar to JWH-018. Samples containing metabolites of AB CHMINACA, AB FUBINACA, ADB CHMINACA, AM 2201, MDMB-CHMICA or 5F PB 22 were not detected by both IA when using manufacturer cut-offs. These results can be explained by an insufficient cross reactivity of the antibodies for 'new generation' SC and/or the generally low analyte concentrations in urine and a lower sensitivity of the HEIA tests. Halving the cut-offs led to a sensitivity of 7% but did not improve the overall diagnostic efficiency.

P101

Characterization of CDA activity in healthy volunteers using Roche/Hitachi analyzers

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Background: The enzyme cytidine deaminase (CDA) is involved in the metabolism of many antimetabolite drugs. Mutations in the CDA gene (*CDA*) were suspected to explain therapy-related toxicities in some cases. Direct measurement of CDA activity in plasma provides an alternative approach to investigate its role in antimetabolite-related toxicities.
Aims: Our aim was to study the variability of CDA activity in healthy volunteers and to evaluate genetic variants in *CDA*, associated with differences in activity.
Methods: CDA activity was quantified in plasma samples from 300 healthy blood donors by measuring the amount of ammonia generated during the conversion of cytidine to uridine, using the Roche/Hitachi Modular P800 and compared to the commonly used Berthelot method. Coding, promoter and exon-flanking intronic region of *CDA* were sequenced in 10 and 13 individuals with very low or high CDA activity, respectively, and 10 controls with average CDA activity.
Results: Both, the Roche/Hitachi assay and the Berthelot method yielded comparable results r^2=0.89. Passing-Bablok analysis showed a small but tolerable systematic bias between the methods: y = 1.01x -2.35 (95% CI: 0.86x-1.18x and-4.5 to -0.96), suggesting that the automated method is a suitable alternative to previously reported protocols. Plasma CDA activity in healthy volunteers showed wide variability (range: 1.2-52.8 U) and significantly higher activity in men compared to women (P=0.005). Genetic analysis revealed no variants associated with extreme CDA phenotypes.
Conclusions: These results show that CDA activity is very variable and its regulation is not explained by *CDA* genotype, and that other factors are the main regulators of CDA activity.

P102

Unraveling the IgG glycome of primary ovarian cancer patients

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Introduction: Over the years ovarian cancer remains one of the most common causes of cancer deaths in woman, particularly due to poor specificity of the currently used biomarker CA125. We recently defined a GLYCOV score calculated on the basis of glycome modulations observed in total serum N-glycome of ovarian cancer patients. GLYCOV displays much better diagnostic performance especially in the early stage of the disease. Preliminary studies carried out in our group showed, however, that changes in glycosylation of immunoglobulin G, the most abundant class of glycoproteins in blood, can be totally different than those observed in total serum.
Issue: The aim of the project is therefore to study the glycosylation subtype and status of IgG of ovarian cancer patients on the glycopeptide and N-glycan level, and eventually to combine obtained data into a novel glycan score which, originating solely from IgG, would be complementary but independent from CA125 and GLYCOV.
Methods: IgG subclasses were purified from serum of ovarian cancer patients and healthy controls by incubation with Protein A Sepharose, which captures IgG 1, 2 and 4. Unbound fraction was then subjected to Protein G Sepharose, which binds remaining IgG 3. Purified IgGs were subsequently denatured, reduced and alkylated prior to digestion with trypsin. Resulting glycopeptides were purified using cotton-HLIC columns and analyzed by MALDI-TOF-MS in the negative ion mode.
Results: Our first results prove that ovarian cancer related changes in IgG glycosylation are different than those observed in total serum. We observed alterations of galactosylation and sialylation levels in ovarian cancer patients.

P103

Standard and ultra high-field NMR as screening tools for human blood samples

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Background: Nuclear Magnetic Resonance (NMR) spectroscopy is a standard tool in structural biology, chemistry, and materials science. More recently, NMR spectroscopy proved valuable for non-targeted analyses and quality control of foods and medical samples.
Problem: For standard analyses of blood composition in order to detect deviations from normal that are caused, e.g., by diseases or doping in high-performance sports with known or unknown drugs, a general approach is needed to screen samples efficiently. We expect NMR spectroscopy to provide such a highly efficient, general screening method that is not only able to detect known substances, but also can provide information on appropriate changes of blood composition due to drugs.
Method: NMR spectroscopy at various frequencies from 400 MHz to 900 MHz were employed to test plasma, serum, and urine samples that were collected under standard conditions.
Results: We started a project to study differences of composition of plasmas, sera, and urine under various test person conditions. Initially we compared spectra of test persons with different erythropoietin blood concentrations at 600 MHz and 900 MHz field strength. Here we present preliminary data to discuss advantages of NMR-spectrometers of field strength 900 MHz and above in routine and non-routine blood analysis, and we show that the new 900 MHz, single story AEON type magnets are well-suited to study blood samples. We will outline a project directed at creation of a blood spectral data bank with the ultimate goal of screening unknown samples for illegal drugs

P104

Validation of a homogenous immunoassay for Buprenorphine testing in oral fluid samples

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Introduction: Oral fluid (OF) gains increasing interest in drugs of abuse testing of patients in opiates maintenance therapy because of ease of collection and less risk of adulteration. Regarding compliance testing in Buprenorphine (B) substitution therapy false negative results for

the substitution drug must be avoided. In this study the performance of a homogenous EIA for B in OF was evaluated with B patient samples (spls) (>0.1 ng/mL) selected with a sensitive UPLC-MS/MS method.
Methods: 500 OF positive (pos) and 50 negative (neg) spls from patients in B maintenance therapy (dose range 0.6 to 28 mg/d) were collected with the Greiner Bio-One SCS pH 4.2 device. B was quantified on a Waters Acquity/Xevo TQ-S UPLC-MS/MS. Calibration range was from 0.025 to 20 ng/mL (n = 16) and the internal standard B-D4 was at 0.5 ng/mL neat OF. OF spls were measured with CEDIA Buprenorphine Assay (Thermo Fisher Scientific) on an Olympus AU680 with calibrators at 0.0, 0.2, 0.5, 1.0, 1.5, 2.0 and 4.0 ng/mL.
Results: For the 500 pos OF spls the following B concentration distribution was received from UPLC-MS/MS: 0.1 to <1.0 ng/mL: 90 spls, >1.0 to <4.0 ng/mL: 115 spls, >4.0 to 20 ng/mL: 96 spls and >20 ng/mL: 199 spls. At the 1 ng/mL UPLC-MS/MS cutoff (410 true pos, 90 true neg spls) the EIA found 363 true pos spls (88.5%). 3 spls were false pos (3.3% of the true neg). When the EIA cutoff was lowered to 0.5 ng/mL the true pos rate improved to 95.6% (392 spls) and the false pos rate increased to 24.4% (22 spls). However, these false pos spls contained B between 0.1 and <1.0 ng/mL. For the 50 neg OF spls 49 were true neg and 1 false pos (2%).
Conclusion: Initial testing for B abuse and compliance in OF can be performed with the evaluated EIA at a cutoff of 0.5 ng/mL to spot B concentrations >1.0 ng/mL.

P105

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

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Objectives: Carbohydrate specific antibodies play an important role in human disease: Antibodies against glycan epitopes are formed for example during bacterial infections, in autoimmune diseases, and heparin-induced thrombocytopenia. Microarrays with immobilized carbohydrates could provide a new means of detecting these antibodies. Our aim was the development of a microarray platform, which enables the detection of carbohydrate specific antibodies in human serum samples.
Methods: A selection of carbohydrates was printed on N-hydroxysuccinimide activated Codelink glass slides with a sciFLEXARRAYER S11 and incubated with human serum samples. After incubation with anti-human-IgG-Cy3, anti-human-IgM-Cy5, and anti-human-IgA-Alexa Fluor 594 secondary antibodies, carbohydrate specific antibodies were detected with a Tecan LS Reloaded scanner.
Results: With the microarray, we were able to detect several carbohydrate specific antibodies in human serum samples. We found 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. Blood group antigen specific antibodies bound to the microarray according to the patients' blood group.
Conclusion: We created a functional microarray for the detection of carbohydrate specific antibodies. Based on this microarray, it would be possible to establish carbohydrate microarrays for *in vitro* diagnostics in the future.

P106

UPLC-UV method for Vitamin A and E within one minute

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Objectives: Are UPLC-UV methods still an alternative to fast lc-ms/ms methods? The major reasons using liquid chromatography tandem mass spectrometry (lc-ms/ms) instead of liquid chromatography ultraviolet detection (hplc-uv) are short sample preparation, small sample volume and short run time. Therefore most people changed to lc-ms/ms. The Aim of our study was, to develope an UPLC-UV method for Vitamin A and E with a one minute run time.
Methods: Liquid liquid extraction was performed using Vitamin A-Acetat as internal standard. After votex and centrifugation the upper phase was injected into the UPLC-UV system. A H-Class Aquity UPLC from Waters (Milford, MA, USA) equipped with a HSST3 1.7um column was used. Patient result, external qc-samples as well as run times were compared with our in house hplc-uv method.
Results: Method comparison Vitamin A: y=3,98+0,956*x (n=21, r=0.99) Method comparison Vitamin E: y=-0,317+1,059*x (n=21, r=0,961)
Conclusion: UPLC-UV is a costeffective alternative to time consuming hplc uv methods.Sample volume and run times could be dramatically reduced.

Gerinnung und vaskuläres System

P107

Soluble Notch ligand and receptor peptides act antagonistically during angiogenesis

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Background: Notch signaling is essential for blood vessel formation. During angiogenesis, the Notch ligand DLL4 on the leading tip cell activates Notch receptors on the adjacent stalk cells. DLL4-Notch signaling is impaired by the Notch ligand JAG1 in endothelial cells. Thereby the DSL domain of the Notch ligands binds to the EGF-like repeats 11–13 of the Notch receptor..
Question: This study aimed to elucidate how soluble proteins containing these short domains interfere with Notch signaling during angiogenesis.
Results: Adenoviral vectors were generated to express the DSL domains of DLL1, DLL4, JAG1, and the Notch1 EGF-like repeats 11–13 fused to immunoglobulin-G heavy chain. These soluble ligand peptides inhibited Notch signaling in endothelial cells and this caused excessive blood vessel formation and branching in cellular assays and in the neonatal mouse retina. The soluble Notch receptor peptides bound stronger to the inhibitory JAG1 than the stimulating DLL4 ligands, resulting in increased Notch signaling activity. This led to impaired blood vessel formation and branching in the mouse retina.
Conclusion: This study identified short peptides that interfere selectively with Notch signaling to either promote or inhibit blood vessel formation (Cardiovasc Res. 2015, ePub).

P108

Cerebral Cavernous Malformation-1 Protein controls Notch signaling between the endothelium and pericytes

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Background: Cerebral cavernous malformation (CCM) is a neurovascular dysplasia characterized by conglomerates of enlarged endothelial channels in the central nervous system, which are almost devoid of pericytes or smooth muscle cells. This disease is caused by mutations in CCM1, CCM2, or CCM3 genes in endothelial cells, making blood vessels highly susceptible to angiogenic stimuli. CCM1-silenced endothelial cells have a reduced expression of the Notch ligand Delta-like 4 (DLL4) resulting in impaired Notch signaling and irregular sprouting angiogenesis.
Question: This study aimed to address if DLL4, which is exclusively expressed on endothelial cells, may influence interactions of endothelial cells with pericytes, which express Notch3 as the predominant Notch receptor.
Results: Endothelial cell–specific ablation of Ccm1 and Ccm2 in mouse models led to the formation of CCM-like lesions, which were poorly covered by pericytes. CCM1 silencing in endothelial cells caused decreased Notch3 activity in co-cultured pericytes. DLL4 proteins stimulated Notch3 receptors on human brain pericytes. Active Notch3 induced expression of PDGFRB2, N-Cadherin, HBEGF, TGFB1, NG2, and S1P genes. Notch3 signaling in pericytes enhanced the adhesion strength of pericytes to endothelial cells, limited their migratory and invasive behavior, and enhanced their antiangiogenic function. Pericytes silenced for Notch3 expression were more motile and could not efficiently repress angiogenesis.
Conclusion: The data suggest that Notch signaling in pericytes is important to maintain the quiescent vascular phenotype. Deregulated Notch signaling may, therefore, contribute to the pathogenesis of CCM. (Stroke. 2015;46:1337-1343.)

P109

Hydroxychloroquine blockiert die proinflammatorischen und procoagulanten Eigenschaften von Antiphospholipid Antikörpern.

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Background and Aim of Study: Antiphospholipid Syndrome (APS) is associated with thrombosis and recurrent abortions during autoimmune pathologies. Until now, APS has no cure. However, medicines can help prevent complications. For instance, it has been shown that

hydrocychloroquine (HCQ), an antimalarial compound, can reduce the risk of thrombosis significantly. However, the exact mechanism of action of this drug remain elusive. In this study we demonstrate how HCQ blocks the procoagulant and proinflammatory activity of antiphospholipid antibodies (aPL).
Methods: NADPH oxidase (NOX) activation was measured using FACS. The localization of Gp91phox (catalytic subunit of NOX2) and Toll-like receptor (TLR) 7 and 8 was demonstrated by confocal laser scan microscopy. QRT-PCR was used to detect Tissue Factor (TF) mRNA up-regulation.
Results: Recently, we could verify that aPL develop their pathologic properties by activating endosomal NOX after internalization in monocytes. The generation of superoxide leads to rapid translocation of TLR7/8 from the endoplasmic reticulum in the endosome. As a consequence, cells are dramatically sensitized to ligands for TLR7/8. At the same time endosomal NOX activation resulted in a strong upregulation of TF expression and the activation of NLRP3 inflammasome. We now succeeded in showing that HCQ can completely prevent the activation of endosomal NOX although HCQ had no effect on aPL endocytosis. Thereby HCQ inhibited NOX activation by blocking the translocation of the catalytic subunit of NOX into the endosome. This resulted in the prevention of TF induction, inflammasome activation and TLR sensitization. As a consequence, the risk of thrombosis can be remarkably decreased.

P110

A case report of unexpected low plasma levels of the direct oral anticoagulant dabigatran etexilate

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Background: Direct oral anticoagulants (DOACs) such as dabigatran, apixaban and rivaroxaban are used to prevent stroke and systemic embolism in patients suffering from atrial fibrillation. However, plasma levels of DOACs are reported to vary continuously. Due to their pharmacological profiles, they can be taken without routine monitoring. Furthermore, the lack of specific tests leads to having rare data of the concentration of DOACs when bleeding or thromboembolic events occur. We report about a patient, who was reliably stable on dabigatran therapy for at least six weeks.
Methods and Results: The dabigatran plasma levels were measured using the assay from CoaChrom Diagnostica (Maria Enzersdorf, Austria) as well as mass spectrometry. The dabigatran plasma trough level has been assessed immediately before routine uptake (0 hours) and 3 hours, 6 hours and 9 hours after drug intake. At 0 hours the result of the measurement was 41 ng/mL with Hemoclot® assay and 39 ng/mL with mass spectrometry. 3 hours and 6 hours after drug intake, the dabigatran plasma level decreased but astonishingly after 9 hours increasing concentrations (53 ng/mL (Hemoclot®), 52 ng/mL (mass spectrometry)) could be detected. Our results are leading to the assumption of a persistently low peak dabigatran level. Furthermore the increase of drug concentration, which was detected 9 hours after medication, showed a different pharmacokinetic compared to published data of peak levels after 2-4 hours. The described case has made us think about slightly different management over the course of long-term DOAC anticoagulation. Maybe measurements-e.g. once in steady state and during bleeding/thromboembolic events – during long-term drug use should be recommended.

P111

Antibodies against f VIII, heparin and ADAMTS 13-is there a connection between all of them?

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Background: Antibodies against f VIII, heparin and ADAMTS 13 are measured by different methods, as antibodies in ELISA systems and as functional assay based on mixtures with normal plasma. Whereas HIPA test and PF4 antibodies are established methods, for f VIII inhibitors the Bethesda (BE) assay is gold standard and immunological methods are seldom; in ADAMTS 13 deficiency a functional test is not established.
Aim: All patients with pathological ADATMS 13 activity were analysed in the classical inhibitor ELISA and additionally with the Bethesda (BE) method as functional test.
Methods: ADAMTS 13 parameter (activity, antigen and inhibitor) were analysed with test systems from Technoclone GmbH Vienna (Austria). In cases of pathological activity the inhibitor was measured in the ELISA and additionally with the BE based on activity measurement after incubation over two hours.
Results: There is a discrepancy between inhibitors measured in the ELISA and BE method with only 50% of ELISA positive in the BE assay. That means: not all inhibitors are neutralizing and maybe not all of them have diagnostic importance. There is no correlation between inhibitor titre in the ELISA and BE assay.
Conclusions: Inhibitor in ELISA and activity based inhibitors as functional test measure not the same. Are only inhibitors which also inhibit the protease in normal plasma of diagnostic relevance or is the BE too insensitive to recognize inhibitors against ADAMTS 13?
We see a connection between HIT II, f VIII inhibitors and ADAMTS 13. ELISA methods without any functional assay overestimate the incidence of inhibitors. The design of an assay determines the results; this should be taken in consideration in clinical studies.

P112

Identification of miRNAs involved in the higher level network control of the plasmatic coagulation system

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MicroRNAs (miRNA) are small non-coding RNAs controlling the regulation of biological functions on a post-transcriptional level. Importantly, deregulated miRNA expression can result in various disorders. This can occur via aberrant post-transcriptional fine-tuning of the expression of individual target genes or deregulation of entire molecular networks constituting biological meaningful pathways. Interestingly, despite the ubiquitous involvement of miRNAs in almost all biological processes, their role in the hemostatic control has remained poorly defined. Here, we set out to comprehensively identify miRNAs regulating pro- and anticoagulatory components of the hemostatic system in an unbiased manner. In contrast to widely applied association and *in silico* studies, we made use of an integrative assay approach that combines functional aspects of miRNA silencing with unbiased physical interaction screens based on RNA pull downs coupled to next generation sequencing and finally validation by luciferase reporter experiments. This revealed a number of both gene-specific and broadly-targeting miRNAs to interact with transcripts encoding components of the plasmatic coagulation system. These results suggest that the coagulation system is controlled both by miRNAs targeting specific genes which may act as regulatory target hubs within the pathway, as well as by individual miRNAs more broadly targeting a large number of components of the pathway. These findings will assist in deciphering the role of miRNAs in the systemic control of blood coagulation and provide a foundation for the development of the diagnostic and therapeutic potential of miRNAs in the context of de-regulated hemostasis.

P113

Circulating extracellular DNA indicates disease extent and predicts mortality in patients with venous thromboembolism

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Objectives: Venous thromboembolism (VTE) is the combined disease entity of deep vein thrombosis (DVT) and pulmonary embolism (PE). VTE affects up to 5% of the population during their lifetimes and the incidence of VTE rises exponentially with age. Extracellular DNA enhances the activity of clotting factors and may provide a target to improve the diagnosis and therapy of VTE. We have previously identified elevated levels of extracellular DNA in the circulation of patients with DVT. The diagnostic and prognostic value of circulating extracellular DNA in VTE is not known. We hypothesized that the concentration of extracellular DNA in circulation correlates with the extent of VTE at diagnosis and is indicative of the clinical outcome.
Methods: To test our hypotheses we analyzed 863 patients of the SWITCO65+ cohort, a multicenter cohort study that prospectively enrolled consecutive patients aged ≥ 65 years with acute, symptomatic VTE. We quantified extracellular DNA in plasma using specific probes and antibodies.
Results: Plasma DNA was positively correlated with the extent of VTE at enrollment ($p < 0.001$). Median DNA levels increased with VTE extent as indicated by distal DVT (141 ng/ml; n = 71), proximal DVT (171 ng/ml; n = 195), non-massive PE (200 ng/ml; n = 589) and massive PE (220 ng/ml, n = 8). Plasma DNA was not associated with the clinical outcomes VTE recurrence or major bleeding, but DNA levels were predictive for mortality within 6 months post VTE diagnosis ($p < 0.001$).
Conclusion: Increased levels of extracellular DNA in circulation are associated with the extent of VTE at time of diagnosis. Quantification of extracellular DNA may help identifying patients at risk of dying within months after acute VTE.

P114

Developmental Endothelial Locus-1, expressed by endothelial cells, as a novel factor of hematopoietic stem cell niche.

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Objectives: Endothelial cells are a main cell component of the hematopoietic stem/progenitor cell (HSPC) niche, having critical role in the maintenance of hematopoiesis. Developmental endothelial locus-1 (Del-1) has been previously identified as a ligand for the integrins LFA-1 and αvβ3. Herein, we study the role of Del-1 in the regulation of hematopoiesis.
Methods: The effect of Del-1 on hematopoiesis was assessed using Del-1$^{-/-}$ mice and mice with endothelial specific overexpression of Del-1. HSPC numbers and proliferation potential in bone marrow (BM)was analyzed by FACS. Stress hematopoiesis after transplantation or administration of LPS or G-CSF was also assessed. *In vitro* culture of HSPC on Del-1 was used to test the direct effect of Del-1 on HPSC cell cycle.
Results: We identified endothelial cells as the population that expresses Del-1 in the BM. Increased quiescence of HSCs from Del-1$^{-/-}$ mice is observed under homeostasis and in conditions that mimic stress hematopoiesis, including LPS and G-CSF administration, as shown by cell cycle analysis, impairing the proper expansion of HSPC pool. Endothelial overexpression of Del-1 resulted in the expansion of HSPC pool. Transplantation of cells from wild type mice to Del-1$^{-/-}$ mice has further shown that Del-1 deficiency impairs myeloid lineage development. Downregulation of cyclins D1, G1 and G2 was correlated with the decreased proliferation potential of HSPC in Del-1$^{-/-}$mice. The direct interaction between HSPCs and Del-1 affects cell cycle and cyclin D1 expression and is mediated by αvβ3 integrin.
Conclusion: Del-1 emerges as a novel niche factor in the regulation of HSC, promoting HSPC proliferation and myeloid lineage development under stress hematopoiesis.

P115

D-Insight-a novel genetic model to dissect the expression and secretion dynamics of F2 real-time in vivo

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The proper coordination of protein biosynthesis and delivery to the final destination critically determines the integrity of cells and fate of multicellular organisms. Although numerous diseases illustrate the fatal dimension of disordered protein homeostasis, the coordinated dynamics of gene expression, protein biosynthesis and protein secretion are less well understood. Here, we set out to generate an experimental model system, which allows the "visualization" of regulated gene expression and protein secretion, and the coupling between these processes real-time in a living context. By applying a multimodal tagging strategy, we generated a novel knock-in mouse model based on fluorescence and luminescence reporters separated by P2A peptides for tailored multicistronic expression. This model, termed D-Insight, enables to interrogate the expression and secretion dynamics of prothrombin (F2) in its natural context in a living animal with non-invasive optical imaging. Complementary, we generated primary hepatic cell lines derived from this mouse model. This cell line permits the deconvolution of gene expression, protein biosynthesis and secretion in high resolution, and in a scalable high-throughput format ex vivo with bioluminometry and fluorescence microscopy. We confirm that this experimental setup faithfully recapitulates established modifiers of F2 expression, making this model widely applicable to identify physiologically relevant rheostats involved in the cross-talk between gene expression and protein biosynthesis and secretion. This model will also help deciphering underlying mechanisms of disordered hemostasis i.e. in response to environmental (patho)physiological cues and/or other perturbations.

P116

A scientific Approach to Factor VIII Inhibitor Testing, Interpretation and Standardization

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Objective: The foundation for inhibitor testing was laid in the early 1970s. At this time, neither the biological nature of FVIII nor the mechanisms of inhibition by antibodies were known. Assays were designed assuming a bimolecular reaction in which FVIII is destroyed. Inhibitors against other coagulation factors are tested in a similar approach. The relationship between inhibitor properties and assay results must be fully understood to allow valid interpretation or clinical decisions. This was not achieved yet.
Methods: The influence of assay variables on inhibitor testing was tested and interpreted on the base of the more appropiate receptor-ligand model. This includes antigen concentration, antibody dilution, incubation time and the affinity of the interaction. The influence of VWF was investigated.
Results: For non-FVIII inhibitors the Bethesda/Nijmegen assay is a valid approach to quantify inhibitor potency. The affinity of the antibody-antigen interaction plays an insignificant role. It is even possible to simplify the calculation of inhibitor potency. The uniqueness of FVIII inhibitors, besides its interaction with VWF, lies in the low concentration of FVIII. The antibody-antigen affinity plays a major role as low-affinity antibodies show a significant different behavior as medium- or high-affinity antibodies.
Conclusion: While inhibitor testing for non-FVIII antibodies bases on a straightforward relationship between inhibitor potency and antibody concentration, this does not hold true for FVIII inhibitors. Interpretation of anti-FVIII inhibitor results must be seen as an educated guess. Due to the influence of assay-related variables on inhibitor testing, standardization requires strict conditions.

P117

Determination of platelet parameters: Is magnesium sulphate an alternative anticoagulant?

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Objectives: There are conflicting reports on reliable determination of platelet count and mean platelet volume. Magnesium sulphate, recently shown to be effective as anticoagulant in EDTA-induced pseudo-thrombocytopenia might be an alternative to EDTA. We therefore conducted a comparative study to evaluate $MgSO_4$ as in vitro anticoagulant for platelet count and MPV.
Methods: Platelets were counted by the XE 5000 impedance or fluorescence optical technique. Whole blood from volunteer donors was anti-coagulated by EDTA, citrate or $MgSO_4$.
Results: The mean impedance platelet count was 227.7*10ˆ9/l in EDTA, 197.0*10ˆ9/l in citrate and 201.1*10ˆ9/l in $MgSO_4$. Using the fluorescence optical method the mean platelet count in EDTA and MgSO4 anticoagulated blood differ only marginally (228.3*10ˆ9/l and 222.8*10ˆ9/l), whereas the platelet count in citrate blood remains lower (204.1*10ˆ9/l). The MPV in EDTA anti-coagulated blood was 10.4 fl whereas the MPV in citrate and magnesium anti-coagulated blood was markedly lower (9.5 fl and 9.3 fl, respectively). In EDTA blood the MPV increased by 6.9% within 3 hours. Cell swelling was markedly less when citrate or $MgSO_4$ anticoagulation was used.
Conclusions: MPV in magnesium anti-coagulated blood is 1 fl lower compared to EDTA, but is not associated with relevant cell swelling and might therefore be advantageous for reliable MPV measurement. The platelet count is only underestimated when MgSO4 anti-coagulated blood is measured by impedance, whereas lower platelet counts are measured in citrate anti-coagulated blood independent from the method used.

P118

The effect of pneumatic tube system (PTS) on platelet function Tests

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Objectives: The PTS transport may lead to spurious results for platelet function. Here we evaluate the effect of PTS transport on the platelet function measured by **Mutilplate** electrode aggregometry (Roche) and platelet function analyser **PFA-100** (Siemens).
Methods: Paired blood samples were collected into vacutainer (Citrate 3,8% for PFA, Citarat 3,2% and Hirudin for multiplate). The samples were either hand delivered to the laboratory or transported through the PTS. Then they were analysed with PFA (COL/EPI, COL/ADP, and P2Y), and with Multiplate (ADP-, COL-, ASPI-, and TRAP-test).
Results: Multiplate: In citrate samples we found no significant differences in all tests (ADP, COL, ASPI, and TRAP). In the hirudin samples there were significant differences in all tests. We found that these differences were due to the fill status of these tubes (with normal use they will be only 60% filled). There were no significant differences (in all tests) after the complete filling of these tubes. PFA-100: We found no statistically significant differences in the COL/EPI and P2Y tests. In the COL/ADP test there was significant difference. However this difference was not bigger than the difference between the duplicate measurements without PTS transport.
Conclusion: The PTS transport of properly prepared blood samples for platelet function assays (PFA-100 and Multiplate) can be done in our hospital.

Immunologie

P119

Correlation between cold ischemia time and Monokine induced by IFN-? (MIG) in kidney transplantion

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Introduction: In addition to other causes, the duration of cold ischemia is hold responsible for the extent of the ischemia-reperfusion injury of the graft. We investigated if there are parameters which are correlated with the cold ischemia time (CIT), and accordingly allow conclusions about the quality of the graft.

Materials and Methods: In 34 patients, who received a kidney transplant at our hospital, the pro-inflammatory urinary cytokines interleukin 6 (IL6), interleukin 8 (IL8) and the chemokine MIG were measured both before and after surgery. The CIT was less than 10 hours in 20 patients (group 1) and longer than 10 hours in 14 patients (group 2).
Results: The median values of the cytokine levels of the patients in group 2 (> 10h CIT) are higher than the ones in group 1 (< 10h CIT). The same conclusion applies the glomerular filtration rate, measured 3 months and 1 year following surgery. These differences in the groups are only significant for MIG, measured in the first urine after transplantation (p=0,012, Mann-Whitney U-Test). A significant positive correlation was detected between the duration of cold ischemia time and urinary MIG (r=0,449, p=0,011, Spearman rank correlation).
Conclusion: Urinary MIG is correlated with the duration of the CIT and could possibly help to provide information regarding the extent of ischemia-reperfusion injury.

P120

Limited Value for Antibody Detection in a Polyreactive Serum of a 24 year-old Man with Congenital Aortic Stenosis/ Aneurysm of the Ascending Aorta and Replacement with a Mechanical Valve and Graft.

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Suspected Diagnosis: Goodpasture syndrome GS referring to clinically evident pulmonary haemorrhage.
Differential Diagnosis: Pulmonary and/or renal manifestations can be encountered in various conditions, such as antineutrophilic cytoplasmic antibody (ANCA)–positive vasculitis. As a consequence, the identification of anti-GBM antibodies in the patients serum is of paramount importance in the diagnosis of GS. Confirmation of the diagnosis occurred through detection of antibodies against the alpha-3 chain of type IV collagen.
Results: anti-GBM 200 U/ml. Notable was that PR3- und MPO were also highly elevated. Because of this mismatch an analysis was made with another producer of reagents and all the results were negative, consequently the first results were false positive and the therapy based on these results inadequate.
Conclusion: Polyspecific serum appears to have reacted with a component used by the first producer, probably against the blocking agent. To prevent nonspecific binding of the antibodies in the sampling preparation the remaining binding surface must be blocked before using antibodies to detect proteins that have been dotted or transferred to a membrane**.** Otherwise, the antibodies or other detection reagents will bind to any remaining sites that initially served to immobilise the proteins of interest. Any protein that does not have binding affinity for the target or probe components in the assay can be used for blocking so every manufacturer uses another mixture. It is a rare instance that this usual approach of the assays test performance causes an unwanted side-effect, i.e. to trigger a reaction instead of preventing it. Possibly the antibodies of the patient were triggered by the device of the implanted graft/ valve.

P121

IGA Subtypes: a supplement to M-protein quantification by electrophoretic methods in monitoring patients with multiple myeloma (MM)

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Objectives: For monitoring patients with multiple Myeloma (MM), the quantification of the M-protein is recommended by electrophoretic methods. Monoclonal IgA (mIgA) often migrates into the β-fraction, leading to difficulties in determining the M-gradient. We investigated whether the quantification of mIgA by standard electrophoretic method can be improved by determination of the IgA subtypes (IgAκ/IgAλ).
Methods: In sera from 88 patients with IgA monoclonal gammopathy the monoclonal component was determined using electrophoretic tests (SPEP, serum protein electrophoresis; CE, capillary electrophoresis; IFE, immunofixation; Sebia, Lisses, France), Freelite® (FLC, free light chains) and Hevylite® (HLC, IgAκ/IgAλ; The Binding Site, Schwetzingen).In a IgAλ-MM patient SPEP, CE, IFE, FLC and HLC were examined during therapy (n=18). The mIgAλ was calculated [mIgAλ=IgAλ-(IgAκ/1.18)] and compared to the quantified M-protein (SPEP).
Results: In the 88 patients with IgA monoclonal gammopathy the M-protein was detectable in 69.3% and 87.5% using SPEP and CE, respectively. Free light chains ratio was abnormal in 68.2%. In contrast IgAκ/IgAλ ratio was abnormal in 93.2%. Combination of the IgAκ/IgAλ ratio and SPEP or CE increased the sensitivity to 96.5%.The M-protein from the patient with MM ranged from 0.8-37.1g/L during monitoring of the therapy. The calculated mIgAλ correlated with the M-Protein during the entire treatment period (r^2=0.997).
Conclusion: The IgAκ/IgAλ ratio might become important for the detection of monoclonal gammopathies. The calculated monoclonal IgA using IgA subtypes is an alternative to the quantification of M-protein using SPEP or CE, especially for M-proteins migrating into the β-fraction.

P122

Investigation of Chitinase-3 like 1 Protein as marker of acute pulmonal inflammation

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Background: Pulmonary inflammatory diseases such as pneumonia and COPD are among the leading causes of death in industrialized countries. New and more specific inflammation markers could aid diagnosis and monitoring of these diseases. Chitinase-3-like 1 (Chi3l1 or YKL40) is a 40 kDa-sized chitin- and heparin-binding lectin with a proposed role in inflammatory disease. It is secreted by macrophages and epithelial cells directly in the inflamed tissue. Some findings indicate it may have special relevance in pulmonary disease.
Subject: Estimating the diagnostic relevance of YKL40 compared CRP concentrations in sera from patients suffering acute pneumonia.
Methods: Measurement of CRP (Cobas) and YKL40 (R&D Systems) serum concentrations in three collectives: a) Healthy blood donors (n=100); b) Patients with non-pulmonary inflammatory disease (n=30); c) Patients with radiologically confirmed pneumonia (n=28). The study was approved by the local ethics committee, and all patients gave informed consent.
Results: The inflammatory and pneumonia collectives did not differ significantly in gender (37% female vs. 43% female), age (66 ± 13.4 a vs. 70 ± 12.7 a; p = 0.488) or CRP serum concentration (11.6 ± 11.4 mg/dL vs. 13.2 ± 8.5 mg/dL; p = 0.86). The collectives, however, differ significantly with respect to the YKL40 Serum concentrations (213 ± 426 vs. 405 ± 301 ng/mL; p < 0,005). Patients with inflammatory disease had approximately 10 times elevated YKL40 compared to healthy controls (27.8 vs. 213 ng/mL), but pneumonia patients had the highest YKL40 serum values (405 ng/mL). YKL40, but not CRP can distinguish between other inflammatory disease and pneumonia (AUC = 0.73 vs. 0.51).

P123

The prospective multicentre trial of antibody diagnostics in paediatric coeliac disease (AbCD)-blinded status report after registration of 774 patients

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Diagnosis of coeliac disease (CD) is based on assessment of a highly variable clinical status, antibodies, histology of intestinal biopsies, and response to gluten-free diet (GFD). We hypothesize that for a significant fraction of paediatric patients a definitive diagnosis is possible solely by means of antibody assays. Thus, the invasive biopsy technique would no longer be necessary to confirm the diagnosis. A prospective international multicentre biopsy-controlled trial on antibody diagnostics in paediatric CD (AbCD) was started (first participant included Oct 18, 2012) to assess sensitivity, specificity, and predictive values of antibody tests (IgA and IgG antibodies against tissue transglutaminase, deamidated gliadin peptides, and endomysium) of EUROIMMUN (Lübeck). Children and adolescents (age 6 months to < 18 years) scheduled for duodenal biopsy as by standard clinical practice with primary aim to confirm or refute CD, are recruited by 13 European trial sites. Routine histological assessment is followed by central reference histology. After 3 months, follow-up under GFD is documented. In case of inconclusive results, there is another follow-up after 6 months. A status report will be presented (update Jul 01, 2015). The analysis will be blinded (data on symptoms, antibodies, histology not correlated with diagnoses). Information will be provided on number of patients registered/ on GFD/ with final diagnoses (CD or no CD) / reasons (symptoms, previous antibody measurements) for inclusion patients / correlation of local routine with final trial biopsy evaluation. Last patients are expected to be recruited until end of this year.

P124

Developmental endothelial locus-1 modulates platelet-monocyte interactions and thrombo-inflammatory reactions in islet transplantation.

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Platelet-monocyte interactions are implicated in thrombo-inflammatory injury by actively contributing to intravascular inflammation, leukocyte recruitment to inflamed sites, and the amplification of the procoagulant response. Instant blood mediated inflammatory reaction (IBMIR) represents thrombo-inflammatory injury elicited upon islet transplantation (islet-Tx), thereby dramatically affecting transplant survival and function. Developmental endothelial locus-1 (Del-1) is a functionally versatile endothelial cell-derived homeostatic factor with anti-inflammatory properties, but its potential role in IBMIR has not been previously addressed. Here, we establish Del-1 as a novel inhibitor of IBMIR using a whole blood–islet model and a syngeneic murine transplantation model. Del-1 pretreatment of blood before addition of islets diminished coagulation activation and islet damage. Mechanistically, Del-1 decreased platelet-monocyte aggregate formation, by blocking the interaction between monocyte Mac-1-integrin and platelet GPIb. Consistently, islet-Tx in transgenic mice with endothelial cell-specific overexpression of Del-1 resulted in a decrease of monocytes and platelet-monocyte aggregates in the transplanted tissues, relative to those in wild-type recipients. Our findings reveal a hitherto unknown role of Del-1 in the regulation of platelet-monocyte interplay and the subsequent heterotypic aggregate formation. Therefore, Del-1 may represent a novel approach to prevent or mitigate the adverse reactions mediated through thrombo-inflammatory pathways in islet-Tx and perhaps other inflammatory disorders involving platelet-leukocyte aggregate formation.

P125

CD63 and CD203c expression during specific immunotherapy (SIT) for wasp venom allergy using Basophile Activation Test (BAT): 2-years and first 3-years followup results

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Background: SIT is an established therapy for wasp venom allergy. The aim of our work is to investigate theprogression of surface antigen CD63 and CD203c expression during SIT using BAT.
Material: We included 71 patients in our study (61 wasp,10 honey bee; 17 aborted). The study was approved by the institutional ethical review board. Here we report on patients with SIT against wasp venom in course of 2 year (40 patients) and 3 years (9 patients). Blood samples were collected before and 3 days (3d), 2 weeks (2w) and 6 months (6m) after SIT start. Further blood samples were repeatedly collected every 6 months until 3 years. For all samples we determined CD63 and CD203c expression using BAT after stimulation with various wasp venom concentrations. We evaluated the relative proportion of activated basophile granulocytes at 57µg/l venom concentration (a2) and the calculated concentration c50 to stimulate 50% of total activatable basophile granulocytes.
Results: CD63 expression (and inversely c50) at 2y/3y (CD63 nonresponder: 5) decreased in 24/4 and increased in 5/0 patients, while it was constant in 6/3 cases. Median changes to baseline at 2y/3y were a2=-49% ($p< 0.01$)/-78% ($p< 0.05$) and c50=576% ($p< 0.01$)/761% ($p=0.24$). CD203c expression (and inversely c50) at 2y/3y (no CD203c nonresponder) decreased in 17/5, increased in 11/1 and did not change in 12/3 patients. Median changes to baseline at 2y/3y were a2=-16% ($p= 0.01$)/-56% ($p=0.08$) and c50=208% ($p< 0.01$)/426% ($p< 0.01$).
Conclusion: Statistically significant differences can be demonstrated for CD63 and CD203c expression after 2 y; for the evaluation after 3y we need more results. Further work is required to gain inside into long-term stimulation behavior in BAT and correlation with sting challenge.

P126

Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs

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Introduction: Under inflammatory conditions, T cell-dependent (TD) protein antigens induce pro-inflammatory T and B cell responses. In contrast, tolerance induction by TD antigens without co-stimulation triggers development of regulatory T cells. Under both conditions, IgG antibodies are generated, but whether they have different immunoregulatory functions remains elusive.
Question: We sought to examine the Fc glycosylation and anti-inflammatory quality of IgG molecules formed upon TD tolerance induction.
Methods: After purification, IgG samples were digested with EndoS. N-linked glycans were purified, permethylated and analyzed by MALDI-TOF mass spectrometry.
Results: Stimulation with TD antigens under inflammatory conditions induces plasma cells (PCs) expressing low levels of alpha2,6-sialyltransferase (AST) and producing de-sialylated IgGs. In contrast, PCs induced upon tolerance induction failed to downregulate AST expression

and secreted immunosuppressive sialylated IgGs that were sufficient to block antigen-specific T and B cell responses, DC maturation and allergic airway inflammation. Importantly, successful allergen-specific immunotherapy in allergic patients also induced sialylated allergen-specific IgGs. Our data show a novel antigen-specific immunoregulatory mechanism mediated by anti-inflammatory sialylated IgGs that are formed upon TD tolerance induction. Our data further show that monoclonal antigen-specific sialylated IgGs are sufficient to suppress pro-inflammatory immune responses in an antigen-specific manner. These findings may help to develop novel antigen-specific therapies for the treatment of allergy and autoimmunity

P127

Investigation of uremia-induced neuroinflammation

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The pathophysiologic interaction between the diseased kidney and the brain is complex, but of high clinical relevance. Cognitive impairment and dementia are strikingly increased in **chronic kidney disease (CKD)** patients of all stages compared to the general population. To reveal potential mechanisms impairing cerebral function in CKD patients, we ask whether the induction or enhancement of neuroinflammation may be involved. To answer that question, we investigated the effect of CKD on microglial activation *in vivo*. To induce and model CKD, we subjected mice to 5/6 nephrectomy. 8 Weeks after operation, mice showed significantly increased blood level of urea and creatinine, indicating reduced filtration rate. Brains from CKD-mice contained increased number of activated microglia. Interestingly, this activation coincided with a disruption of intracellular K+ homeostasis. Further investigations aim to reveal how K+ dyshomeostasis and inflammation interact; what process represents the starting point; and what cellular structures are involved. Understanding the pathophysiologic interactions between renal impairment and brain function in CKD patients is important in order to minimize the risk for cognitive impairment in these patients and to design diagnostic approaches to this systemic disorder as well as new therapeutic or – even better – preventive means.

P128

Structural requirements of mono- and multivalent L-selectin blocking aptamers for enhanced receptor inhibition in vitro and in vivo

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L-selectin mediates extravasation of leukocytes from the blood into the surrounding tissue during inflammation and is therefore a therapeutical target in certain overwhelming immune reactions. In this study, we characterized an L-selectin specific blocking DNA aptamer with respect to nucleotide composition and target binding. Introduction of deletions and nucleotide exchanges resulted in an optimized DNA sequence but preservation of the IC_{50} in the low nanomolar range. The inhibitory potential was significantly increased when the aptamer was displayed as a di- and trimer connected via appropriate linker length. Similar to monoclonal antibodies, trimer yielded picomolar IC_{50} values in a competitive binding assay. In comparison to the monovalent aptamer, the trivalent assembly reduced lymphocyte interactions to L-selectin ligands 77-fold under shear and exerted superior inhibition of lymphocyte rolling*in vivo*. In conclusion, our work demonstrates the feasibility of optimizing aptamer sequences and shows that multivalent ligand presentation enables superior adhesion receptor targeting.

Immunologie

P129

The industrial food additive microbial transglutaminase is immunogenic in children with celiac disease

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Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules. It is a family member of human tissue transglutaminase (tTg), involved in celiac disease (CD). Despite declarations of mTg safety, direct evidence for immunogenicity of the enzyme is lacking. The

serological activity of mTg, tTg, gliadin complexed mTg (mTg neo-epitope) and gliadin complexed tTg (tTg neo-epitope) were studied in: 95 pediatric celiac patients (CD), 99 normal children (NC) and 79 normal adults (NA). Sera were tested by ELISAs, detecting IgA, IgG or both IgA and IgG: AESKULISA® tTg (tTg), AESKULISA® tTg New Generation (tTg neo-epitope (tTg-neo)), microbial transglutaminase (mTg) and mTg neo-epitope (mTg-neo). Marsh criteria was used for the degree of intestinal injury. Comparing pediatric CD patients with the 2 normal groups: mTg-neo IgA, IgG and IgA+IgG antibody activities exceed the comparable mTg ones ($p<0.0001$). All mTg-neo and tTg-neo levels were higher ($p<0.001$). tTg IgA and IgG+IgA were higher than mTg IgA and IgA+IgG ($p<0.0001$). The levels of tTg-neo IgA/IgG were higher than tTg IgA/IgG ($p<0.0001$). The sequential antibody activities, reflecting best the increased intestinal damage, going from M0 to M3c were: tTg-neo IgG ≥ mTg-neo IgG > mTg-neo IgA+IgG > tTg-neo IgA. Taken together, mTg-neo IgG and tTg-neo IgG correlated best with intestinal pathology ($r2=0.989$, $r2=0.989$, $p<0.0001$, $p<0.0001$, respectively). mTg is immunogenic in children with CD and by complexing to gliadin its immunogenicity is enhanced. Anti-neo-epitope mTg antibodies correlate with intestinal damage to the same degree as anti-tTg. Further studies are needed to explore the pathogenic potential of anti-mTg antibodies in CD.

P130

The break in intestinal tight junction permeability by industrial food additives

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Autoimmune disease (AD) incidence increases alongside the food additives added to the industrial food processing. The intercellular tight junction (TJ), controls the equilibrium between tolerance and autoimmunity. Neo-linked, transformed molecules, represent mucosal load with altered immunogenic properties. It is hypothesized that commonly used industrial food additives abrogate human epithelial barrier function, thus, increasing intestinal permeability, resulting in activation of the autoimmune cascade. Glucose, salt, organic solvents, emulsifiers, gluten, microbial transglutaminase, and nanoparticles are being exponentially used by food industries to improve the qualities of the food. All those food additives increase intestinal permeability by opening TJ paracellular transfer by the following described mechanisms: Rearrangement, disturbance and destabilization of TJ protein (Zonulin-1, E-cadherin, catenin, actine, occludin, claudin), contraction of the perijunctional actomyosin ring, decrease in the hydrophobicity of the mucus layer, dissociation of the PTP1B-E-cadherin-beta-catenin complex, induction of actin disbandment and structural separation of TJ. In fact, in multiple AD, a breach in TJ integrity and function was observed. Future research on food additive exposures on intestinal permeability and autoimmunity interplay will enhance our knowledge of the common environmental mechanisms associated with AD. As a corollary, individuals with non-modifiable risk factors (i.e., familial autoimmunity or carrying shared autoimmune genes) should consider decreased exposure to some food additives in order to avoid increasing AD risk.Lerner A, Matthias t. Autoimmun Rev. 2015;14:479-89.

P131

Autoantibodies against CD74 – A new diagnostic marker for Spondyloarthritis (SpA)

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Objective: Spondyloarthritis (SpA) is a common debilitating inflammatory disorder. The pathogenesis of axial SpA (axSpA) including ankylosing spondylitis (AS) is still largely unclear. Diagnosis is difficult, since abnormalities in conventional X-rays develop with a latency of several years and only HLA-B27 is used as a laboratory marker. Additionally, the presence of radiographic sacroiliitis is now essential for the diagnosis of SpA. To prevent destructive effects early diagnosis and intervention in SpA patients may be important. The aim of our study was to evaluate antibodies to the human leukocyte antigen class II-associated invariant chain peptide (anti-CD74) as a diagnostic marker of SpA.
Methods: Sera of 117 patients with axial SpA and 38 non-SpA patients were analyzed for IgA and IgG antibodies against CD74 by ELISA. HLA-B27 status was available in 112 patients. All donors provided informed consent for the study approved by the local ethics committee (project number 4928).
Results: Anti-CD74 antibodies were detected in 85.1% of the SpA patients but only in 5% of the non-SpA patients ($p \leq 0.0001$). The detection of both IgG and IgA anti-CD74 antibodies for diagnosing SpA revealed a sensitivity of 77% and a specificity of 90%. Remarkably, IgA autoantibodies against CD74 alone had a sensitivity of 67% and a specificity of 95%, the likelihood ratio (LR) LR+ was 12.7 and LR- was 0.35. IgA anti-CD74 antibodies were even more frequent in SpA patients with short disease duration and significantly correlate with more advanced radiological sacroiliitis and reduced spinal mobility.
Conclusion: Anti-CD74 IgA antibodies were strongly associated with SpA. Antibodies against CD74 could provide an important additional tool for diagnosis of SpA.

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Risk factors for insufficient linezolid blood concentrations after standard dosing in critically ill patients

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Background: Linezolid is an important treatment option against infections caused by Gram-positive bacteria in ICU-patients. However, some recent studies showed that linezolid standard dosing often results in insufficient blood levels in this high-risk patient group. Little is known about potential causes for these sub-therapeutic levels so far.
Objectives: We aimed to identify and to quantify covariates influencing linezolid levels in critically ill patients.
Methods: 52 adult critically ill ICU-patients with infections receiving linezolid intravenously 2 x 600 mg daily were included in a prospective observational study. Multiple serum samples (median 33) were taken from each patient over 4 days and linezolid was quantified by LC-MS/MS. Evaluation for independent covariates on pharmacokinetics was performed by studying effects of different clinical and laboratory parameters on linezolid concentrations in an explorative multivariate analysis and by development and use of a population pharmacokinetic model (NONMEM).
Results: Independent covariates showing significant effects ($p < 0.01$) on pharmacokinetics as determined by NONMEM (a) or by multivariate analysis (b) included body weight (a,b), the liver parameters fibrinogen (a,b) and antithrombin (b), lactate (a,b), creatinine-clearance (b) and presence of acute respiratory distress syndrome (ARDS) (a,b). ARDS patients showed an elimination clearance augmented by 81% resulting in 9-fold lower trough values within the study patients. These results revealed that ICU patients presenting some of the following characteristics might be especially at risk for sub-therapeutic levels: ARDS, elevated body weight, normal lactate levels, normal liver or renal function.

P133

Celiac disease serology in naive Rheumatoid Arthritis patients

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Objective: Symptoms associated with the inflammatory processes in Rheumatoid Arthritis (RA) are often indistinguishable from symptoms of other chronic diseases and conditions. RA shares many aspects with celiac disease (CD). The aim of this study was to evaluate the CD associated serological profile in newly diagnosed RA patients.
Methods: Sera of 135 adult patients with confirmed RA diagnosis were compared to 79 adult blood donors using the following ELISAs: *AESKULISA®* CeliCheck New Generation (IgA and IgG against anti-tTg neo-epitope antibodies), *AESKULISA®* tTg Check (for in house research use only, IgA and IgG against anti-tTg antibodies), and *AESKULISA®* CCP IgG.
Results: 3/135 (2.2%) RA patients tested at their first visit showed elevated anti-tTg neo-epitope antibodies levels ($p<0.0001$). However, 15/135 (11.1%) of these RA patients were positive for tTg autoantibodies ($p<0.0001$). 60% of these were seronegative for the RA marker CCP and remained negative until the 4th visit. In the follow-up 2/15 were still tested positive for anti-tTg autoantibodies and negative for CCP.
Conclusion: Incidences of RA and CD are similar. CD positive serology is significantly higher in RA patients, much more for anti-tTg than for anti-tTg neo-epitope. RA and CD share clinical, epidemiological, pathophysiological, environmental and genetic aspects. Since both are mediated by enzymatic posttranslational protein modifications, common environmental factors, increased intestinal permeability and dysbiotic diversity, the significance of CD autoantibodies in RA is interesting. Is it an epiphenomenon or a result of pathophysiological mechanisms? Further studies are needed to unravel this dilemma.

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Midkine inhibition can reverse the MPA modulated TJ permeability in Caco-2 cell monolayer.

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Background: Immunosuppressant MPA is prescribed to prevent allograft rejection in organ transplanted patients. However, its use is sporadically linked to the leak flux diarrhea and other gastrointestinal disturbances in patients through yet unknown mechanism. Recently,

we described an increased tight junction permeability through MLCK pathway in MPA treated Caco-2 monolayer. Here we studied a possible involvement of midkine dependent PI3K pathway in alteration of TJs under MPA treatment.
Method: Caco-2 cells were grown in monolayer to develop the TJs and treated for 72 hours with MPA or iMDK + MPA and or DMSO. Caco-2 monolayer integrity was assessed by TEER and FITC-dextran value and chromatin complexes that contain active and repressive promoter regions, along with their binding partners, were precipitated with specific antibodies.
Results: Mass Spectrometry results show 2.5 fold increase of midkine protein in MPA treated cells as compared to the control. Our functional assays showed that iMDK (an inhibitor of midkine) significantly recovers compromise integrity of MPA-treated Caco-2 cells monolayer. ChIP analyses showed a significant increase of active mark (H3K4me3) and decrease of repressive mark (H3K27me3) in the promoter of midkine, PI3K, Cdx-2 and claudin-2 genes and vice versa in claudin-1 gene, which was further confirmed by mRNA and protein expression assay.
Conclusion: We observed a MPA dependent over expression of midkine protein and midkine-dependent activation of PI3K pathway that alters TJ assembly and increases permeability of Caco-2 monolayer as compared to control cells which was reversible by iMDK. The study indicates a possible use of midkine inhibitors as therapeutic agent to prevent MPA related GI disturbances.

P135

Elevated MMP-3 levels in early RA patients after borrelia infection

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Objective: To investigate the influence of an infectious disease on Rheumatoid Arthritis (RA), patients from the ADAPTHERA study were screened for antibodies to borrelia antigens.
Methods: 204 sera from early RA patients with disease duration < 6 months were screened using the following ELISAs (AESKU.DIAGNOSTICS): *AESKULISA®* Rf-AGM, *AESKULISA®*, CCP-IgG and -IgM, *AESKULISA®* Borrelia-IgG and -IgM and *AESKULISA®* DF MMP-3.
Results: 10.8% of the patients tested at their 1st visit were positive for borrelia-specific antibodies. 18/204 patients were positive for IgG or IgM, or both, and 4/204 patients had equivocal results. Borrelia positive sera showed negative results in the classical RA parameters Rf-AGM and CCP. 5/22 borrelia positive patients were CCP positive but negative for Rf-AGM. Interestingly, 10/13 patients which were negative for classical RA parameters had high MMP-3 levels, while only 1/9 of CCP positive patients showed elevated MMP-3 titres. Interestingly, classical RA parameters remained negative in 3 patients until their 4th follow-up visit. Only one patient was positive for CCP testing throughout the investigated time period.
Conclusion: Not only borrelia inflammatory responses, but also periodontitis, an inflammatory disorder of the mouth, and RA share common pathogenic mechanisms. The co-measurement of MMP-3 may assist in improved differential diagnosis and helps to eliminate unnecessary patient therapies, thus saving time, money and improving overall patient care. Further studies are needed to investigate the role of MMP-3 testing for early RA diagnosis and the role of borrelia infection in the development of RA.

P136

Microparticles cause preeclampsia and embryonic growth restriction by activation of inflammasome in the placenta

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Preeclampsia (PE) is a hypertensive disorder of pregnancy leading to maternal and fetal complications. Its causes remain unknown and delivery is the only remedy for the disease. Procoagulant microparticles (pcMP) of different cellular origins are elevated in PE but their mechanistic relevance is unknown. PE is also associated with a pro-inflammatory condition but there are few studies exploring mechanistic insights. To address these questions pcMP (endothelial or platelet derived) were injected into C57/Bl6 pregnant mice and the pregnancy outcome (embryonic survival and growth, placenta morphology) was studied. Blood pressure, kidney histology (PAS staining and EM) and proteinuria was done to evaluate PE. Inflammasome activation by pcMP in mouse placenta and trophoblast cells was studied using western blotting and immunohistochemistry. Human trophoblast derived cells and placentas from PE patients were also studied for inflammasome activation. NLRP3 and Casp-1 KO mice were used to rescue the mice from disease conditions and establish causality of the mechanism. pcMP caused PE along with fetal loss and embryonic growth restriction in mice. Human and mouse placenta analysis indicated inflammasome activation seen by elevated expression of NLRP3, cleaved casp-1 and IL-1β. Apyrase and purinergic receptor antagonist treatment rescued the pregnancy outcome indicating the involvement of purinergic signaling. The pregnancy outcome and renal function was also rescued in NLRP3 and Casp-1 KO mice. Our results establish that MP are causative of PE, fetal death and embryonic growth restriction. These pathogenic effects of MP are mechanistically linked with purinergic receptor induced inflammasome activation in the placenta.

Molekulare Diagnostik

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Regulation of the phospholipase A2 receptor expression in prostate cancer cells

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Background: There is a growing body of evidence suggesting that the M-type phospholipase A2 receptor (PLA2R1) act as tumor-suppressor and play a crucial role in the process of cellular senescence.
Questions: For this reason we were interested in the expression and regulation of PLA2R1 by DNA methylation and signaling pathways in human prostate cancer cell lines in comparison to normal prostate epithelial cells.
Methods: DNA methylation of the *PLA2R1* gene was analyzed using direct sequencing and methylation specific-high resolution melting analysis of bisulfite-modified genomic DNA. Cellular transcript levels of PLA2R1 and secreted phospholipases A_2 ($sPLA_2$) were determined using RT-qPCR.
Results: Levels of PLA2R1-specific mRNA inversely correlated with the degree of PLA2R1 promoter methylation in normal and prostate cancer cells. LNCaP cells that had down-regulated levels of PLA2R1-specific mRNA showed increased *PLA2R1* promoter methylations whereas in PC-3 cells that up-regulated PLA2R1 expression had a low degree of methylation in comparison to normal prostate cells. In human prostate tissue specimens obtained by prostatectomy in comparison to matched non-malignant tissue specimens a subset of prostate cancer specimens was identified with higher methylation degrees in comparison to non-malignant specimens. By using pharmacological inhibitors, NF-κB and SP1 were identified as positive regulators and the PI_3K/Akt/mTOR signalling pathway as negative regulator of the cellular PLA2R1 expression. Furthermore, an inverse correlation was observed between the expressions of PLA2R1 and group-IIA and -V sPLA2s suggesting that PLA2R1 may act as transcriptional suppressor of these two sPLA2 enzymes.

P138

A New Blood Collection Tube for the Stabilization of circulating cell-free DNA (ccfDNA)

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Background: Circulating cell-free DNA (ccfDNA) has become an emerging tool in non-invasive prenatal testing (NIPT) and in cancer diagnostics. PreAnalytiX has developed the PAXgene® Blood ccfDNA Tube, which prevents the release of genomic DNA (gDNA) from blood cells during transport and storage. The stabilization of ccfDNA is mandatory to maintain a constant fraction of fetal ccfDNA in the maternal plasma, especially for NIPT. Here, we demonstrate the performance of the PAXgene Blood ccfDNA stabilization chemistry in quantitative real-time PCR assays and show first results of prototype tubes used for the next-generation sequencing (NGS)-based downstream application PrenaTest® (LifeCodexx AG).
Question: Evaluation of a new blood collection tube for stabilization and purification of (fetal) ccfDNA for NIPT analysis.
Method: Whole blood was drawn into PAXgene Blood ccfDNA Tubes, Streck Cell-Free DNA BCT® or EDTA tubes and stored for up to 10 days. ccfDNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit and analyzed by qPCR. Specimens from pregnant donors were analyzed with QuantYfeX® assay followed by NGS and PrenaTest® DAP.plus software analysis (LifeCodexx).
Results: The fraction of ccfDNA in blood collected and stored for 6 days in EDTA tubes showed an up to 100-fold increase of 18S rDNA fragments. By contrast, copy numbers of these fragments remained constant when blood was stabilized with PAXgene stabilization chemistry. PrenaTest of ccfDNA from pregnant women revealed comparable values for fetal fractions and identical outcomes of NGS between Streck BCT and PAXgene Blood ccfDNA Tubes. For Research Use Only. Not for use in diagnostic procedures.

P139

Inflammatory Bowel Disease (IBD) Locus 12: Is Glutathione peroxidase-1 (GPX1) the Relevant Gene?

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Background: Genome wide association studies (GWAS) have identified and repeatedly confirmed the association of rs3197999 in *MST1* with inflammatory bowel disease (IBD). However, the underlying pathophysiology remains unclear. rs3197999 is a non-synonymous SNP which modifies the function of macrophage stimulating protein-1 (MST1).
Methods and Results: We show by haplotyping that rs3197999 is in linkage disequilibrium with rs1050450 in *GPX1*, with almost complete cosegregation of the minor alleles. As shown by immunoassay, rs3197999 influences the MST-1 level in serum. But also rs1050450 causes an amino acid exchange in glutathione peroxidase 1 (GPx-1) and reduced activity of this antioxidant enzyme. Furthermore, the association of GPx deficiency and IBD in mice was already shown.
Conclusion: We propose that GPx-1 is a better candidate than MST1 for the pathophysiologic link between IBD locus 12 and IBD.

P140

The isolation of prostate cancer specific circulating tumor cells in the blood of patients with metastatic castration-resistant prostate cancer

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Background: Circulating tumor cells (CTCs) consist of a heterogeneous population of very rare cells of the primary tumor or its own metastasis. The aim of this pilot study is the development of a cancer specific functionalized wire, which detects prostate cancer specific circulating tumor cells (PCTC) in metastatic PCa patients. The CTC detection rate will be compared between an EpCAM functionalized wire and a PCa specific functionalized wire.
Methods: We combined 4 antibodies against PSMA, PSA, PSCA and EpCAM. Evaluations of these antibodies were performed by immunofluorescence analysis of the cell lines PC3 and LNCaP. PCa specific functionalization of the wire was determined with spiking experiments. Next we investigated blood samples of 15 metastatic castration-resistant prostate cancer patients showing metastatic progression documented by prostate-specific antigen or radiologic criteria. The captured cells were identified by immunofluorescence staining using cytokeratin and Hoechst positive as well as CD45 negative criteria.
Results: In summary, PCTC counts ranged from 0-122 (MD 9) per PCTC and CTC counts ranged from 0-22 (MD 3) per CTC. Our data shows that a more sensitive isolation of PCTC's is possible using PCa functionalized wire compared to the EpCAM wire ($p \leq 0{,}001$). The CTC isolation sensitivity was about 86% for the PCa- wire and about 73% for EpCAM functionalized wire.
Conclusions: PCTC can be isolated with the PCa-specific functionalization of the wire. At once CTC's that underwent an EMT can be isolated, too. This proof of concept shows how important it is to optimize the EpCAM-based CTC detection methods.

P141

Characterization of uracil catabolism variability in healthy volunteers and cancer patients

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Background: Pharmacokinetics of 5-fluorouracil (5-FU) is mainly determined by Uracil (U) catabolism pathway. Decreased activity of the first catabolizing enzyme, dihydropyrimidine dehydrogenase (DPD), is a major predictor of 5-FU toxicity with known risk variants in the DPD gene accounting for ~30% of toxicities. Conversely, phenotypic variability in the catabolism downstream of DPD by dihydropyrimidinase (DHP) and β-ureidopropionase (bUP) and its potential contribution to 5-FU toxicity has not been investigated. Here, we aimed to characterize the variability of metabolites and metabolic ratios of uracil catabolism and to evaluate their association with genetic variation in the DHP and bUP genes (*DPYS* and *UPB1*).
Methods: Plasma concentrations of U, 5-FU, and their metabolites were determined by LC-MS/MS and three variants in *DPYS* and *UPB1* previously associated with 5-FU toxicity were genotyped in 320 healthy volunteers and 27 cancer patients.
Results: In healthy volunteers, we observed lower concentrations ($P \leq 0.007$) of all metabolites as well as lower β-ureidopropionic acid/dihydrouracil ratios (UPA/UH_2; $P < 0.001$) in women. Among volunteers, *DPYS* c.265-58T>C carriers had higher UPA/UH_2 ratios ($P = 0.036$). In cancer patients, only the UH_2/U ratio was significantly altered during 5-FU infusion ($P < 0.001$).
Conclusions: Observed changes in endogenous metabolite ratios during 5-FU infusion support the rate-limiting role of DPD in uracil catabolism. Higher UPA/UH_2 ratios in females suggest that reduced 5-Fluoro-UH_2 catabolism contributes to higher 5-FU toxicity rates in women. The association of *DPYS* c.265-58T>C with the UPA/UH_2 ratio is in agreement with reduced 5-FU toxicity previously observed in c.265-58C carriers.

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TREND-seq – a highly multiplexed sequencing approach to interrogate transcriptome 3' end diversity in health and disease

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Post-transcriptional diversification of the genome output is vital for the cell to execute biological programs establishing and maintaining the integrity of complex multicellular organisms. Transcriptome 3' end diversity (TED) is known to play a crucial role in this process: deregulation can cause developmental defects, and aberrant TED is widespread in numerous disease processes including cancer. Here we established a method based on deep-sequencing, which allows interrogating the dynamics of transcriptome 3'ends in a highly multiplexed fashion (TREND-seq). As a proof of concept, we analyzed transcriptome 3' end diversity in response to depletion of more than 170 potential executing regulators by RNAi in human neuroblastoma. We demonstrate that this protocol reliably works with minimal amounts of RNA (100 ng) following a straightforward, fast and scalable workflow. In our experimental model system we observe a massive deregulation of TED during tumorigenesis, which interestingly affects numerous oncogenes and tumor suppressors. Depletion of potential executing regulators reveals components of the mRNA processing machinery as key regulator genes, which confirms functionality of the protocol. Thus we show TREND-seq to be a powerful and straightforward tool to assay the transcriptome 3'end diversity and dynamics in a highly multiplexed format. This technique will likely also help to disentangle complex genome diversification and its deregulation in human disorders.

P143

Methylated free circulating HPP1 DNA is an early response marker in patients with metastatic colorectal cancer treated with a combination of fluoropyrimidine, oxaliplatin and bevacizumab.

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Background: Previously, we demonstrated that detection of methylated free-circulating DNA (mfcDNA) of *HPP1* in blood is correlated with a poor prognosis of patients with metastasized colorectal cancer (mCRC).
Objective: Here, we analyzed the levels of *HPP1* mfcDNA in plasma samples of mCRC patients that have been treated with FOLFOX or XELOX combined with bevacizumab to test whether *HPP1* mfcDNA is a suitable prognostic and response biomarker.
Methods: Pre and post therapeutic plasma samples were collected from 467 patients (clinical study AIO-KRK-0207). Free-circulating DNA (fcDNA) was isolated and Bisulfite-treated fcDNA was quantified using methylation specific PCR.
Results: 337 out of 467 patients were positive for *HPP1* mfcDNA before therapy. *HPP1* mfcDNA negativity of the pre therapeutic sample was significantly correlated with a better overall survival (OS) (33.8 versus 21.1 months). Treatment resulted in a reduction of *HPP1* mfcDNA to non-detectable levels in 167 patients. These patients showed a significantly better OS compared to patients with still detectable *HPP1* mfcDNA levels (27.5 versus 15.9 months). Remarkably, OS of patients with non-detectable *HPP1* mfcDNA levels after treatment was similar to the OS of patients that had been negative for *HPP1* to begin with (27.5 versus 33.8 months). According to these data, *HPP1*mfcDNA levels could be used as a prognostic and a response marker. Multivariate analysis showed that the *HPP1* mfcDNA level in the post therapeutic sample is an independent prognostic factor for OS. In addition, ROC analysis confirmed that the level of *HPP1* mfcDNA in the post therapeutic sample could be used as a response marker to discriminate between patients who (do not) benefit from chemotherapy (AUC=0.704).

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CYP3A5 genetic variation influences everolimus maintenance dose requirement in heart transplant patients

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Background: The immunosuppressive drug everolimus (ERL) has become an alternative to nephrotoxic calcineurin inhibitors (CNI) in transplant patients due to its antiproliferative properties and renal-sparing mode of action. However, ERL therapy is associated with a large variety of adverse events owing to its narrow therapeutic window combined with substantial variability in response. Mechanisms underlying this inter-individual variability are poorly characterized. We aimed to evaluate the effect of clinical factors and genetic variation in ERL pharmacokinetic pathways on ERL maintenance dose requirement in heart transplant (HTx) patients.
Methods: This pilot study comprised of 37 patients recruited at the Bern University Hospital who were treated with CNI-free ERL therapy for at least three months. Variants in *CYP3A5*, *CYP3A4*, *CYP2C8*, *POR*, *NR1I2*, and *ABCB1*were genotyped and clinical data were retrieved from patient charts.
Results: Although ERL trough concentration (C_0) was within the targeted range for most patients, over 30-fold variability in the dose-adjusted ERL C_0 was observed. Regression analysis revealed a significant effect of the common splice-site variant *CYP3A5*3* on the dose-adjusted ERL C_0 ($P = 0.031$). Patients carrying the *CYP3A5*1/*3* genotype required 0.02 mg/kg/day higher ERL dose compared to patients with *CYP3A5*3/*3* genotype to reach the targeted C_0. Additionally, ERL therapy substantially improved estimated glomerular filtration rate (28.6 ± 6.6 ml/min/1.73m^2) in patients starting ERL therapy due to kidney dysfunction.
Conclusion: Our preliminary data indicates that ERL pharmacokinetics in HTx patients is highly variable and that CYP3A5 genetic variation may contribute to this variability.

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Higher active tamoxifen metabolite concentrations associate with CYP2D6 activity (metabolic ratio) and continuing therapeutic drug monitoring (TDM)

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Background: Tamoxifen (TAM) is a selective estrogen receptor modulator used in the treatment of receptor positive breast cancer. CYP2D6 metabolizes the pro-drug TAM to endoxifen (ENDOX), the most important active metabolite. In contrast, CYP3A4/5 leads to formation of inactive N-desmethyl-TAM (DM-TAM). The metabolic ratio (MR) of DM-TAM / ENDOX reciprocally reflects CYP2D6 activity and is subject to significant genetic polymorphism. We analysed data from TAM metabolite TDM and report effects of time under TDM and the MR on ENDOX levels.
Methods: TAM, OH-TAM, ENDOX and DM-TAM were measured using d5-TAM and d5-ENDOX as internal standards after ACN/methanol precipitation and chromatographic separation on a C18 column by mass spectrometry (API 4000). All compounds were identified by their typical mass transitions.
Results: The range of MRs was split in 3 groups (high, medium and low CYP2D6 activity) by judgement from a distribution plot of log(MR). ENDOX concentrations decreased significantly with increasing MR (lower CYP2D6 activity) (n=1007, median 12.8, 7.8, and 3.2 µg/L, p<0.001). ENDOX concentrations increased over time in follow-up TDM samples (median 2.8, 3.6, 4.2, 5.3 µg/L, p=0.002, n=64, 22, 8, 4) but only in the group with the lowest MR. Median intervals between sampling were 61, 55 and 80 days, respectively.
Conclusion: Our findings suggest clinically appropriate TAM dose escalation when low ENDOX levels were detected by TDM. ENDOX levels showed the known correlation with CYP2D6 activity. We recommend TDM of TAM metabolites for treatment individualization.
References: von Ahsen N et al., J Lab Med. 2009;33:293. Mürdter TE et al., Clin Pharm Ther 2011;89:708.

Autorenverzeichnis