Abstracts*)

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Symposiums-Vorträge

Symposium 2: Neue Entwicklungen in der Massenspektrometrie

V01

Standardization and Metrological traceability of MS-based protein tests: challenges and opportunities

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For the sake of patient safety medical test results should be accurate and consistent in time and space. To that end, standardization of test results is key. Standardization of tests can only be reached if internationally recognized reference materials, procedures and systems are available. So far, proteins in body fluids are generally measured using immunoassays. Conceptual drawbacks of immunoassay-based protein tests are the indirect measurement of the compound(s) of interest and the variable specificity of the antibodies across manufacturers. The latter prevents to define the measurand in an unequivocal way, implicating that the precise molecular entities are often unknown. Also, test results may be misleading and can lead to unnecessary treatment or missed opportunities for therapeutic interventions. These cases stem from problems inherent to immunoassays such as lack of reference materials, lack of concordance across platforms and presence of heterophilic or autoantibodies. Finally, notwithstanding the appearance of many promising protein biomarkers in the past decades, only a few made it to the clinic. As most medical labs became fully dependent on protein test development initiated by IVD-manufacturers, medical laboratories have been forced into a position where they wait and see.
Tandem mass spectrometry (MS) has potential as a detection and quantitation method to alleviate most of the drawbacks and flaws inherent to immunoassays. In 2012, targeted proteomics based on LC-MS/MS has been declared method of the year as it allows quantitation of specific subsets of proteins of interest. On top, MS-based targeted proteomics can be used in a highly multiplexed format. Moreover, targeted LC-MS/MS allows precise molecular characterization of the measurands, which is a prerequisite for test standardization. Targeted proteomics is frequently performed using multiple reaction monitoring (MRM) - instrumentation, such as triple quadrupoles. A typical MRM-workflow consists of five steps: defining the protein set of interest based on the clinical or biological question; selecting appropriate peptides which are proteotypic and which have suitable LC and MS properties; selecting precursor-fragment transitions based on their intensity; validating the transitions in order to prevent interferences and quantitating the specific protein(s) by calibration using either value-assigned peptide calibrators respectively matrix-based calibrators.
The most critical step for absolute quantitation of serum proteins is the trypsin digestion step as equimolar conversion from protein to proteotypic peptides should be complete and reproducible for all proteins
MS-based targeted proteomics has great potential as an alternative technology to antibody-based protein assays in hypothesis driven clinical trials in the context of precision diagnostics and in case of flawed immunoassays. From a standardization viewpoint major advantages of MRM coupled to mass spectrometry are its potential to unequivocally define the molecular entities, the direct assessment of the compounds of interest, and the lack of non-selectivity if transitions are well selected. Challenges are related to the fact that internationally recognized reference materials are hardly available. From a value based healthcare viewpoint, this technology enables lab professionals to reestablish their role in developing home brew MS-based protein tests in case of promising biomarkers that have potential to fulfill unmet clinical needs.

V02

Effects of introducing MS in round robins for endocrine markers

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is now becoming a routine method of analysis in many clinical laboratories, especially for the small molecules. LCMS/MS is a highly sensitive and specific method for many hormones and shows low cross reactivity. In addition it offers the possibility of measuring several hormones in a single run, thereby increasing diagnostic value and reducing costs per test. Immunoassays are replaced with increased speed by LCMS/MS methods, a technique considered the gold standard in hormone analysis. If so, it should be reflected in the EQAS results.
We have analysed 8 year external survey data from the endocrine section of the Dutch EQAS (SKML) and looked at the performance of the LCMS/MS methods compared to immunoassays. This endocrine section organizes 6 schemes annually with two frozen serum samples for the hormones in each scheme. All laboratory trimmed means, CV's and mean and CV per method are calculated.
The increasing popularity of the LCMS/MS technique is shown by the yearly exponential increase of results obtained with LCMS/MS. In early spring 2017 there were 23 laboratories (110 in total) that used an LCMS/MS for one or more analytes and 22% of the steroid measurements were from a LCMS/MS. For cortisol in urine and saliva, 17 hydroxyprogesterone and androstenedione in serum 50 to 60% of the results are now from LC-MS/MS.

The better specificity of LCMS/MS is seen in the lower concentration ranges of the steroids e.g. testosterone or 17 hydroxyprogesterone < 1 nmol/L, as compared to the immunoassays, which show often much higher values due to cross reactivity. However, between-laboratory CV, is sometimes higher for the LC-MS/MS group compared to the immunoassay methods (e.g. 10% vs 5%). In addition, not all laboratories perform equally well using this technique showing higher within-laboratory variability. Better method validation and standardization of the method are crucial for a better performance.
Although the LCMS/MS technique shows better accuracy, introduction of this technique is not a guarantee for better performance. Most LCMS/MS methods are in-house developed assays and more attention should be paid to for instance preparation of calibrators, use of a proper internal standard, samples preparation, and optimising chromatography. Traceability to a reference measurement procedure or a reference preparation is necessary for a better accuracy. Then, this technique will become a true gold standard in hormone analysis.

Symposium 3; Instrumentelle Analytik in der Infektionskrankheitserreger-Diagnostik

V03

Culture free Microbiology: facts, future or fiction

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Currently, the field of medical microbiology diagnostics is changing rapidly. New molecular technologies enable new information about micro-organisms during disease but also in health. Detection of uncultivable, new and/or unexpected species result in a more complete picture of the world of microbes in the human body and show that what we know now, based on culture, is only the tip of the iceberg. The other side of the same coin shows spread of antimicrobial resistance and ermerging worldide epidemics of specific microbes that urge us to act rapidly and develop molecular diagnostics to detect these microbes as soon as possible. These two forces of technological possibilities and global events are important drivers behind a basic change in the field of microbiological diagnostics. Classical culture is to slow and not always able to identify all the new bacterial species and/or emerging viruses. In addition, specific culturing techniques for viruses are stopped in routine labs due to the ease of use of molecular techniques, their rapid result and low cost compared to virus culture. Due to all the new molecular developments also new fields for microbiological diagnostics are coming within reach. These include microbiome analysis, metabolome analysis and resistome analysis. This all results in the fact that more and more also the classical cultivable micro-organisms are replaced by real time PCR technologies in routine diagnostics.
This raises the question whether we are moving towards a culture free microbiology. Can we do microbiological diagnostics without culture and what are the risks/benefits for patient care? Are these molecular techniques missing specific results or detecting non-viable microbes? In this presentation the aspects of new molecular developments are addressed as well as their role in future microbiological diagnostics and consequences for the current microbiological laboratory.

DGKL-AG-Symposium 1: Leitlinien - AG „Diagnostische Pfade“

V04

S3 Leitlinie: Epidemiologie, Diagnostik, Therapie, Prophylaxe und Management unkomplizierter bakterieller ambulant erworbener Harnwegsinfektionen bei erwachsenen Patienten, Update 2016

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Background: Uncomplicated, bacterial, outpatient acquired urinary tract infections are among the most common infections in the outpatient area. However, the resistance level of the pathogens of uncomplicated urinary tract infections has significantly increased in recent years. This guideline therefore contains up-to-date evidence for the rational use of antimicrobial substances, to avoid an inappropriate use of certain antibiotic classes and to avoid the development of resistances. For the first time, it also treats the prophylaxis of recurrent uncomplicated urinary tract infections.
Methods: In an interdisciplinary evidence-based and consensus-based AWMF S3 guideline, the current knowledge about epidemiology, diagnostics, therapy, prevention and management of uncomplicated, bacterial, outpatient acquired urinary tract infections in adult patients was combined. The S3 guideline was updated under the leadership of the German Society of Urology (DGU). Evidence level and bias risk were used for quality testing.
Results: Updated information on the eradication rate, sensitivity, collateral damage and the safety of antibiotics of the first and second choice were re-listed. Fosfomycin trometamol, nitrofurantoin, nitroxolin, pivmecillinam or trimethoprim are recommended for the treatment

of uncomplicated cystitis. Fluoroquinolones and cephalosporins should not be used as antibiotics of the first choice. In the case of uncomplicated pyelonephritis with slight-moderate course forms, preferably cefpodoxim, ceftibuten, ciprofloxacin or levofloxacin are to be used as oral antibiotics.
Conclusion: Antimicrobial stewardship aspects have significantly influenced the therapeutic recommendations. A broad implementation in all clinical practice settings is necessary to ensure a predictive antibiotic policy and thus to improve care provision.

V05

S2k Leitline: Präanalytik

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The challenges of the preanalytical phase are multifold with patients' integrity and safety as the highest targets. Preanalytics includes the selection of suited tests, ordering these tests, in some cases conditioning of patient, preparation of sample vessels, the non-invasive or invasive sampling, preparation of the primary sample, in some cases storage of the samples and the transport of the specimens into the laboratory. Most of these steps take place not within the laboratory but in an outpatient setting or a patient ward and many different professions are stakeholders in these processes.
Stability at defined preanalytical conditions for a sample is assumed, when the medical conclusions from the test result are identical to the testing with optimized preanalytics. This issue is highly related to the definition of performance specifications, a rather complex matter in which very recently a number of achievements have been reached by members of the EFLM.
Previously, stability has been assumed if the result differs less than 10% from the "true" value. However, the 10% threshold does not take into account differences in the biological variability and in the permissable uncertainty of the tests. E.g. in serum sodium testing, the difference from the "true" value may only be 0.6%.
The section of Laboratory Management is currently in the process of developing a 2k, consensus-based guideline (registration number 115-002) which covers some aspects of preanalytics. This guideline is developed according to the rules of the AWMF (Association of the Scientific Medical Societies in Germany) using a systematic approach and in collaboration with other scientific societies such as Deutsche Gesellschaft für Humangenetik e.V. (GfH), Deutsche Gesellschaft für Transfusionsmedizin und Immunhämatologie (DGTI) and Deutsche Gesellschaft für Liquordiagnostik und Klinische Neurochemie e.V.(DGLN). Our focus is on the stability of the sample (which can be effected by certain additives to the sampling vessels, preparation of the primary sample (such as delayed centrifugation), certain transport conditions (such as different temperatures) and the time in transit of the primary tubes) for the most frequent tests in a clinical laboratory. Other tests will be selected because of the irretrievability of the sample (such as in bone marrow testing or cerobrospinal fluid testing) or particular instability of certain tests.
In a first step, >100 tests with similar stability and handling conditions will be grouped. Because of the high number of tests, 2 members of the section of Laboratory Management of DGKL will suggest stability conditions based on publications and own experiences for these groups of tests. In a second step, a formal Delphi process will be employed in which all participants will comment on the guideline. Final results of this project are expected to be published in 2018.

Stiftung für Pathobiochemie und Molekulare Diagnostik - ausgewählte, aktuelle Projekte

V06

The cell surface receptor Toso links regulatory B cell function to the control of T cell immunity and self-tolerance

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The immune system has developed tightly controlled regulatory mechanisms that allow for the elimination of invading pathogens, while avoiding immunopathological damage due to excessive inflammation. The immune cell-specific surface molecule Toso (also known as Faim3 or FcμR) is highly expressed on B cells and, as an IgM-binding molecule, has been suggested to interact with the B cell receptor. While Toso has been implicated in the regulation of inflammatory responses in vivo, the mechanism underlying this regulatory function is still largely unclear. Utilizing conditional gene deletion in mice, we demonstrate a B cell-inherent function of Toso, that unexpectedly provides protective T cell immunity against viral infection. Our study specifically links the immunoregulatory function of Toso to a set of IL-10-producing regulatory B cells. We identify Toso as a critical regulator for the differentiation/maintenance of regulatory B cell subsets in vivo. We further demonstrate that B cell-specific deletion of Toso affects normal immune homeostasis with consequences for both T cell immunity and B cell tolerance and

show how this is interrelated with regulatory B cell function. Mechanistically, Toso fine-tunes B cell activation thresholds, thereby affecting regulatory B cell differentiation. Finally, we unveiled a novel therapeutic strategy by inhibiting Toso function using antibody-blockade, which led to regulatory B cell induction at local sites of inflammation.

V07

Unraveling disordered RNP-complexes in health and disease: sCLIP for illuminating RNA-protein interactomes with single nucleotide resolution

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RNA-binding proteins (RBPs) are central for gene expression by controlling the RNA fate from birth to decay. Various disorders arising from perturbations of RNA-protein interactions document their critical function. However, deciphering their function is complex, limiting the general functional elucidation of this growing class of proteins and their contribution to (patho)physiology. Here, we present sCLIP, a simple and robust platform for genome-wide interrogation of RNA-protein interactomes based on crosslinking-immunoprecipitation and high-throughput sequencing. sCLIP exploits linear amplification of the immunoprecipitated RNA improving the complexity of the sequencing-library despite significantly reducing the amount of input material and omitting several purification steps. In addition, it permits a radiolabel-free visualization of the immunoprecipitated RNA as quality control, making this protocol widely applicable for many research institutions.
In a proof of concept, we identify that CSTF2tau binds many previously not recognized RNAs including histone, snoRNA and snRNAs. CSTF2tau-binding is associated with internal oligoadenylation resulting in shortened snRNA isoforms subjected to rapid degradation. We provide evidence for a new mechanism whereby CSTF2tau controls the abundance of snRNAs resulting in alternative splicing of several RNAs including ANK2 with critical roles in tumorigenesis and cardiac function. Combined with a bioinformatic pipeline sCLIP thus uncovers new functions for established RBPs and fosters the illumination of RBP-protein interaction landscapes in health and disease.

Symposium 4: „Liquid profiling", eine interdisziplinäre Herausforderung

V08

„Liquid Biopsy" in der tumorgenetischen Diagnostik: Aktueller Stand und Erfahrungsberichte

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Liquid biopsy is a method which detects cell-free nucleic acids also called circulating free DNA (cfDNA) from blood. In the frame of tumor diagnostic liquid biopsies have a lower sensitivity as compared to conventional tissue biopsies and negative results without detection of genetic aberrations are difficult to interprete since they can be caused by the absence of genetic alterations or an ineffective technical sensitivity. However liquid biopsy is well established for follow-up therapies, specificially for the detection of the EGFR resistance mutation T790M in lung cancer. Since its diagnostic meaningfulness is clearly increased by the knowledge of the genetic aberrations detected from conventional biopsies, liquid biopsies and tissue biopsies can supplement each other and the combination of both methods will improve the accuracy of tumor diagnostic.

Symposium 5: Hämostaseologie und Inflammation

V09

Factor XI in arterial hypertension and vascular inflammation

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Multicellular interactions of platelets, leukocytes, and the blood vessel wall support coagulation and precipitate arterial and venous thrombosis. High levels of angiotensin II cause arterial hypertension by a complex vascular inflammatory pathway that requires leukocyte recruitment

and reactive oxygen species production and is followed by vascular dysfunction. We delineate a previously undescribed, proinflammatory coagulation-vascular circuit that is a major regulator of vascular tone, blood pressure, and endothelial function. In mice with angiotensin II–induced hypertension, tissue factor was up-regulated, as was thrombin-dependent endothelial cell vascular cellular adhesion molecule 1 expression and integrin aMb2– and platelet-dependent leukocyte adhesion to arterial vessels. Theresulting vascular inflammation and dysfunction was mediated by activation of thrombin-driven factor XI (FXI) feedback, independent of factor XII.The FXI receptor glycoprotein Iba on platelets was required for this thrombin feedback activation in angiotensin II–infused mice. Inhibition of FXI synthesis with an antisense oligonucleotidewas sufficient to prevent thrombin propagation on platelets, vascular leukocyte infiltration, angiotensin II–induced endothelial dysfunction, and arterial hypertension in mice and rats. Antisense oligonucleotide against FXI also reduced the increased blood pressure and attenuated vascular and kidney dysfunction in rats with established arterial hypertension. Further, platelet-localized thrombin generation was amplified in an FXI-dependent manner in patients with uncontrolled arterial hypertension, suggesting that platelet-localized thrombin generation may serve as an inflammatory marker of high blood pressure. Our results outline a coagulation-inflammation circuit that promotes vascular dysfunction, and highlight the possible utility of FXI-targeted anticoagulants in treating hypertension, beyond their application as antithrombotic agents in cardiovascular disease.

V10

IL-22 ein Schlüsselzytokin intestinaler Homöostase und Entzündung

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The instestinal surface is closely exposed to a plethora of potential harmful microorganisms. The mucosal immune system has evolved mechanism to enable close interaction between host and microorganisms on the epithelial surface while dampening its pathogenicity. Inflammatory bowel disease (IBD) is an archetypical disease in which the orchestration of the closely intertwined host-microbe interaction is misdirected. IBD is characterized by chronic relapsing inflammation of the gastrointestinal tract.
IL-22 belongs to the IL-10 family and is a master cytokine in the regulation of intestinal homeostasis. IL-22 is secreted from residing immune cells (T-Cells, innate lymphoid cells, neutrophil granulocytes) and exerts its protective function on epithelial cells via activation of the STAT3 signal transduction pathway.
Thus, understanding the mechanistic pathways which are employed by IL-22 to confer epithelial protection will help to understand a cornerstone of the pathophysiology of IBD.

V11

Novel insights into platelet activation via the pattern recognition receptors CD36 and FcgammaRIIA

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Beside their role in hemostasis and thrombosis platelets modulate immune responses during inflammation and infection. In response to injury activated platelets secret hemostatic but also inflammatory and immunomodulatory factors by granule exocytosis. Platelets sense danger molecules or pathogens via pattern recognition receptors such as CD36 and FCγRIIA. CD36 is abundantly expressed on platelets and represents a prominent thrombospondin-1 and danger-associated molecular pattern receptor thereby promoting platelet reactivity and a pro-thrombotic state. Using CD36 blocking antibodies and CD36-deficient human platelets we addressed the role of platelet CD36 in thrombin generation. We showed that CD36 amplifies coagulation factor XIa-driven thrombin generation in concert with GPIbα and αIIbβ3 integrin on human platelets. This process requires fibrin which mediates the binding of distinct coagulation factors to the platelet surface. Thus, CD36 induces a hyper-reactive platelet phenotype by triggering platelet-dependent thrombin generation. In a second study quantitative LC-MS based proteomics was used to analyze novel protein alterations of platelets from patients with hereditary deficiency of the fibrinogen receptor αIIbβ3 integrin (type 1 Glanzmann thrombasthenia). Interestingly, levels of the classical pathogen recognition receptor FCγRIIA which exhibits high avidity for IgG immune complexes and IgG-opsonized cells or bacteria, were up to 2.5-fold higher in thrombasthenic platelets than in platelets from controls. Crosslinking of FCγRIIA via anti- FCγRIIA IgG-complexes resulted in increased surface expression of the dense and lysosomal granule marker CD63 but not of the selective lysosome marker LAMP-1 or the α-granule marker CD62P on thrombasthenic platelets. These data indicate that elevated FCγRIIA levels on patients' platelets trigger increased dense granule exocytosis in response to IgG-complexes and therefore might represent a compensatory mechanism of platelet activation in Glanzmann thrombasthenia.

Symposium 6: „MassCyto“ - Kombination von Durchflusszytometrie und Massenspektrometrie

V12

Immune profiling of chronic inflammation by mass cytometry

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The development of mass cytometry (CyTOF® technology) has pioneered a new era of multiparametric single-cell analysis. Combining time-of-flight mass spectrometry with single-cell cytometry at a speed of several hundreds of events per second allows the study of cells and cellular networks at an unprecedented depth and complexity. In contrast to conventional fluorochrome-based flow cytometry, mass cytometry uses antibodies coupled to highly-pure stable rare earth metal isotopes for the detection of cellular properties. Thereby, it is comparatively easy to perform single-cell cytometric experiments with currently ~45 parameters without the typical obstacles inherent to fluorescence-based cytometry such as spectral overlap and autofluorescence.
We have employed mass cytometry to investigate rheumatoid arthritis (RA) patients receiving an experimental therapeutic treatment with helminthic worms. For this, we developed a mass cytometric antibody panel of 44 markers for complex immune profiling of patient’s peripheral blood mononuclear cells (PBMC). The panel includes also a novel beta-2-microglobulin-based live cell barcode that allows for joint processing of 10 pooled cell surface barcoded samples, establishing identical conditions during sample preparation and data acquisition and greatly diminishing data variability. We here report first data of the mass cytometric immune phenotyping of RA patients before and after helminth or placebo treatment and the comparison of their profiles to healthy age and gender-matched controls.

BNLD-Symposium: Arzneimittelsicherheit - TDM von Antiinfektiva

V13

Arzneimittelsicherheit

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There are many synonyms and quasi-synonyms for the term “drug safety”. Internationally recognized and comprehensible is the generic term „pharmacovigilance”. The WHO defines “Pharmacovigilance (PV) is … the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem”. In the overview lecture the areas of pharmacovigilance in clinical routine, studies and environmental safety will be described with a special focus on drug therapy safety.

V14

Grundprinzipien und Praxis der personalisierten Therapie mit Beta-Lactamen

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Bacterial infections are potentially life-threatening disorders. Prognosis depends on effective antibiotic treatment right from the outset. Therapeutic efficacy is determined by the susceptibility of the bacterial pathogen, expressed as minimum inhibitory concentration (MIC), and the concentration of the antibiotic achieved at the focus of infection, which is in turn influenced by drug metabolism and pharmacokinetic factors. The effect mechanism of beta-lactams is time-dependent, correlating with the duration of drug concentrations above the MIC of the pathogen.
Critical illness is associated with major pharmacokinetic changes which may lead to unexpected drug concentrations and unpredictable dose requirements differing significantly from standard dosages. Emerging dosing strategies are therefore based on pharmacokinetic/pharmacodynamic (PK/PD) principles. In the past, therapeutic drug monitoring (TDM) was mainly used in antibiotics with concentration-dependent

adverse side effects. Increasingly, TDM plays a key role in antibiotic treatment optimisation in general and in particular in beta-lactam therapy - notably in severely ill patients. Furthermore, evidence of the superiority of continuous beta-lactam infusions over shorter administration regimens is growing. Target drug concentrations have to be defined, considering MIC values especially in pathogens with limited susceptibility. For reliable TDM results, correct pre-analytical sample handling is indispensable.
Presently, personalised, TDM-guided therapy offers the most promising approach for an efficacious beta-lactam treatment especially in critically ill patients.

Symposium 7: The Human Glycome in Health and Disease

V15

Serum glycome alterations in malignancy: a novel class of tumor biomarkers

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Background: Epithelial ovarian cancer (EOC) is the most frequent cause of death from all gynecological malignancies because of its late diagnosis. As N-glycosylation is modified in the course of ovarian cancer, it is a promising source of tumor biomarkers. In this work, we investigated the glycome of total serum of primary serous ovarian cancer patients, patients suffering from benign ovarian tumors and healthy controls. We also investigated for the first time the N-glycome profiles of ascitic fluid from primary serous EOC patients and compared them with the serum N-glycome of the same patients as well as healthy controls.
Methods: Serum N-glycans were released from total serum proteins, permethylated and measured by MALDI-TOF-MS. The areas of the glycan structures that were significantly up- or downregulated were combined as a score named GLYCOV developed in our laboratory. The diagnostic performance of the GLYCOV value was compared with CA125 using Receiver Operating characteristics curves. Sensitivity and specificity were calculated using binary logistic regression.
Results: GLYCOV was able to diagnose early-stage as well as late-stage serous EOC better than CA125 and even allowed the discrimination between malignant and benign ovarian tumors. Ascites showed qualitatively as well as quantitatively different N-glycosylation pattern compared to healthy serum. Overall, increased antennarity, branching, sialylation and LewisX motives were observed in ascites samples. Indeed, different intensities of N-glycans were detected especially for the highly branched N-glycans. In addition, a correlation was established between ascites volume and degree of sialylation.
Conclusion: Our data suggests the power of the glycan marker GLYCOV to diagnose early-stage EOC. In addition, we reported for the first time the N-glycome of ascitic fluid and showed that the glycome modulations detected in EOC serum were also present in ascites. Both serum and ascitic fluid from EOC patients exhibited typical features of inflammatory conditions, when compared with healthy serum.

Symposium 8: Autoimmunerkrankungen/ Immundiagnostik

V16

Neue Autoantikörper zur Diagnose von Autoimmunerkrankungen

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Studies and reports of new autoantibodies and their clinical associations remain a very dynamic field of study. Novel diagnostic and/or pathogenic autoantibodies (AABs) in patients with systemic autoimmune rheumatic diseases (e.g., rheumatoid arthritis, idiopathic inflammatory myopathies, systemic sclerosis), autoimmune neurologic diseases (e.g., neuromyelitis optica, autoimmune encephalopathies, paraneoplastic neurologic syndromes, autoimmune peripheral neuropathies), autoimmune nephropathies (e.g., idiopathic membranous nephropathy) and chronic inflammatory bowel diseases have been integrated in routine diagnostics in the last years. In addition, novel assays for autoantibody determinations have been developed and transferred into routine diagnostics. Furthermore, novel results of evaluation studies and meta-analyses have led to a reassessment of some of the clinical relevances of some autoantibody specificities. All these aspects have led to the improvement of the serological diagnostics and prognostics of systemic and organ specific autoimmune diseases.
More and more novel AABs are described every year. However, not all of these AABs are relevant in routine diagnostics. In the following cases the determination of novel AABs is recommended: 1. Closing of diagnostic gaps by increasing the diagnostic sensitivity of AAB profiles that include novel AABs (e.g., myositis profiles including MDA5, NXP2, TIF1y, HMGCR and Mup44 antibodies, among other AABs); 2. Improvement of the diagnostic sensitivity and/or specificity compared to established parameters (as has been shown for anti-CCP antibodies compared to

rheumatoid factors); 3. Improvement of diagnostic possibilities of early, atypical and/or mono/oligosymptomatic („sine syndroms“, forme fruste disease) manifestations of autoimmune diseases (e.g., interstitial lung disease within the scope of the antisynthetase syndrome, sclerosis sine scleroderma); 4. Further differentiation of clinically and pathologically heterogeneous diseases (e.g., idiopathic inflammatory myopathies); 5. Definition of novel disease entities (e.g., neuromyelitis optica can be clearly differentiated form early stages of multiple sclerosis by anti-aquaporin 4 antibodies); 6. Differentiation between autoimmune and non-autoimmune causes of a defined disease (e.g., anti-phospholipase A2 receptor antibody as a serological marker of an autoimmune form of membranous nephritis); 7. Additional information regarding disease activity, prognosis, organ manifestations and/or response to therapy (e.g., anti-PAD4 antibodies in rheumatoid arthritis); 8. Exclusion of an autoimmune disease (e.g., anti-DFS70 antibodies in the absence of disease specific AABs may exclude a connective tissue disease); 9. Insights in the pathophysiology of the disease (e.g., anti-GP2 antibodies in Crohn´s disease); and 10. Hints of autoimmune side effects of biological therapies (e.g., TNF and checkpoint inhibitors).
Important to note that the use of AABs for the above proposed fields of application requires carefully designed studies that assess the cost effectiveness and rational approach of AAB testing. Hopefully, the integration of new AABs into routine diagnostics will save patients from needless, expensive and invasive tests, and will lead to earlier diagnosis and better differentiation from other diseases to lower morbidity and mortality significantly.

V17

Autoimmunität als potenzieller Stratifizierungsfaktor bei chronisch entzündlichen Darmerkrankungen

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The recent “re-discovery” of brown adipose tissue (BAT) in humans is one of the most intriguing findings in the research area of metabolic diseases as it raised hope for the treatment of obesity. In addition to brown adipocytes present in BAT depots, inducible brown-like adipocytes so called beige adipocytes can be found in specific WAT depots under various catabolic conditions such as cold exposure in wintertime. Cold-activated beige and brown adipocytes trigger an energy-demanding process known as adaptive thermogenesis, which requires increased uptake of dietary carbohydrates and lipids for maintaining caloric balance. The relevance of BAT is exemplified by the fact that in rodents, BAT and liver take up equal amount of energy from the bloodstream, a process which is able to normalize glucose and lipid values in insulin resistant and hyperlipidemic mice. Notably, also in humans most of the standard metabolic parameters routinely determined by physicians such as glucose and blood lipids are influenced by brown and beige adipocyte activity.
Recently, we investigated in more detail the regulation as well as the molecular processes of lipid disposal into activated BAT, using pharmacological and genetic interventions in mice. We found that short-term BAT activation by cold exposure or beta-3-adrenergic receptor agonism triggers insulin secretion, a process depending on fatty acid release by white adipose tissue. Furthermore, we showed that both insulin release and brown adipocytes insulin sensitivity is essential for the replenishment of endogenous energy stores and efficient adaptive thermogenesis. These data demonstrate that both catabolic and anabolic processes are important for energy balance and function of BAT. In addition to increased fatty acid disposal, we found enhanced uptake of dietary cholesterol into activated BAT as consequence of lipoprotein internalization. Following the fate of cholesterol, we observed the induction of hepatic bile acid synthesis, interestingly via the alternative but not the classical pathway. This process, depending on hepatic CYP7B1 induction, results in elevated plasma levels and pronounced fecal excretion of conjugated bile acids, accompanied by distinct changes in gut microbiota. Pharmacological intervention using ezetimibe, a drug blocking dietary cholesterol uptake, prevented both the rise in bile acid excretion and compositional changes in gut bacteria in response to cold. These results identify bile acids generated in the liver as the determinant of cold-induced gut microbiota, highlighting the relevance of cholesterol metabolism by the host for diet-induced changes on gut microbiota and energy metabolism.
Altogether, our results demonstrate the functional relevance of thermogenic adipocytes for systemic energy and lipoprotein metabolism. In this light, increasing adaptive thermogenesis may represent a promising therapeutic approach for obesity-associated metabolic diseases.

Symposium 9: Pädiatrische Laboratoriumsmedizin

V18

Referenzwertermittlung bei Kindern, wo stehen wir?

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Reference intervals are an essential tool for the evaluation of laboratory test results. Whether a test result is “within” or “outside” the accompanying reference limits often has major diagnostic and therapeutic consequences, making reliable reference a critical part of laboratory medicine.

Establishing pediatric reference intervals is a particular challenge. Physiological development from birth to 18 years results in extensive age- and sex-specific dynamics in most biomarkers and their reference intervals, and adequate representation of these dynamics requires a sophisticated approach. Most commonly, reference intervals are divided into discrete age groups; however, this strategy can only approximate the continuous changes in many analytes with age and leads to misclassification of test results at age groups limits. Alternatively, continuous reference intervals and percentile charts as used in anthropometric quantities (e.g. pediatric weight- and height-for-age charts) allow adequate appreciation of the continuous age-dependent dynamics in biomarkers and furthermore enable exact assessment of the smooth transition of test results between values considered healthy and values considered pathologic.
In addition to complex age-dependent dynamics, unique ethical and practical challenges have to be considered when establishing pediatric reference intervals. Population-based methods use blood samples from a homogenous group of ≥ 120 healthy volunteers to create reference intervals and are still being regarded as the gold standard. However, blood sampling of healthy children for the purpose of reference interval creation is only possible in the setting of comprehensive studies, and the age-dependency of most analytes enormously increases the number of samples required to cover the complete spectrum of pediatrics. These difficulties limit the availability of high-quality pediatric reference intervals, with most pronounced restrictions in neonates and infants, and ultimately negatively impact pediatric decision-making.
In recent years, international initiatives such as CALIPER, KiGGS, and the LIFE project have greatly improved the availability of pediatric reference intervals. However, despite these advances, percentile charts from birth to adulthood are still not available for most biomarkers.
Data mining of laboratory information systems is an emerging alternative to population-based approaches to pediatric reference intervals, and the high number of samples available when using this strategy dramatically improves age-related accuracy. In the multi-center PEDREF study (www.pedref.org) we analyzed laboratory data collected during patient care from 12 German centers and created percentile charts for hematology and biochemistry analytes in girls and boys from birth to 18 years. This approach complements conventional reference interval initiatives and demonstrates the potential of digital approaches for medical research and to support clinical decision-making.

V19

Immundefizienz erkennen - verstehen - behandeln

Manfred Hönig[1]
[1]*Universitätsklinikum Ulm, Ulm, Germany*

More than 290 diseases are summarized with the term „Primary Immunodeficiency, PID“ and represent a phenotypically and genetically heterogeneous group of rare diseases.
The phenotype of PIDs comprises more than the classical presentation with frequent, recurrent or severe infections and includes signs and symptoms of autoimmunity and autoinflammation. Accordingly, diagnostic tools are extremely diverse but nevertheless some basic methods as a full blood count, plasma levels for immunoglobulins and specific antibodies allow a first assessment.
Therapeutic options are equally include a broad and heterogeneous spectrum from merely supportive measures, prophylactic or preemptive antimicrobial agents, substitution of immunoglobulins, immunosuppressive/ anti-inflammatory drugs, specific pathway inhibitors or cellular therapies as hematopoietic stem cell transplantation or gene therapy.
The awareness for this group of diseases is important as the prognosis often depends on the early diagnosis. For this reason a national screening approach is about to be established to identify patients with severe T-cell deficiency shortly after birth (“SCID-screening”).

DGKL-AG-Symposium 3: Bioinformatik in der Laboratoriumsmedizin: IT-Unterstützung bei der Befunddarstellung - AG „Bioinformatik“

V20

Einführung: Visualisierungsverfahren in der Laboratoriumsmedizin und Bioinformatik

Georg-Erich Hoffmann[1]
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The lab report is a critical link between the medical laboratory and the physician: It provides to the requester the essence of the laboratory’s work and triggers a considerable amount of diagnostic and therapeutic actions. Given this great importance, it is surprising how little attention has been paid so far to the record format. It still looks very much the same as half a century ago: a long list of current and preceding measuring values, flanked with units and reference limits, cryptic flags and a few graphical elements. This traditional table format makes laboratory results hard to read and difficult to assess, especially when complex diagnostic situations or time trends are concerned.

With the ever increasing number of available laboratory tests and especially with the advent of high-throughput techniques such as MS/MS and NGS in the routine laboratory, this negligence must no longer be tolerated. Fortunately enough, the young science of bioinformatics provides a cornucopia of algorithms and presentation formats that will help capturing large amounts of data at a glance, detect critical changes over time, and make complex biomarker relationships transparent.
The talk will present some basic ideas of multivariate analysis and result visualisation, e. g. data normalisation, reduction of dimensionality, and hierarchical clustering. The focus will be on the graphical support of routine laboratory reports; some specific formats for the new "omics era" will also be shown.

V21

Standardisierung und Farbkodierung von Labordaten

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The most common way to interpret laboratory data is a comparison of measured values with reference intervals. Their limits determine whether a result is to be classified as "normal" or as "pathological". However, reference intervals depend on many variables such as gender and age, analytical methods and measuring units. This variability makes the interpretation difficult, especially for physicians and other healthcare providers, who are not familiar with the specific area of medical or methodological expertise.
Moreover, decision limits may vary with the underlying medical question. An essential information is therefore whether the value is clearly within the reference interval, deviates slightly or strongly, or exceeds certain alarm limits, so that an immediate action is needed.
A simple standardised representation of laboratory values that takes into account many – if not all – of these aspects, is therefore desirable. Ideally, a standardised result should be independent of the specific type of laboratory value, and based on a reliable and robust estimation of reference intervals and maybe other decision points, derived from sufficiently large data sets.
Several working groups have dealt with this issue. Their recommendations usually include some centring and scaling with so-called z values, which fall into a uniform range around the centre of the reference interval. To achieve this uniformity, one may incorporate a suitable transformation before computing the z value (i.e. zlog values based on a logarithmic transformation or fitted Box-Cox transformation), and process the result even further, in order to yield only positive values, symbolic representations such as (+), +, and ++, or standardised colours from deep blue through white to deep orange. An alternative to z values could be to transform the values in terms of a "seriousness indicator" which would map all values into a scale reaching from extremely low to extremely high; this could be achieved by suitable spline functions based on clinical decision points.
The talk demonstrates the effects, advantages and disadvantaged of the different ideas and also illustrates how colour coding could support cluster analysis of laboratory data.

a.u.l.a. Symposium: Update „Gynäkologische Endokrinologie"

V22

Gynäkologische endokrinologische Fragestellungen in der niedergelassenen Laborarztpraxis

Jochen Ludwig[1]
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The interpretation of laboratory values represents an important interface between the laboratory physician and gynecologists. However interpretation of the findings requires anamnestic data such as the menstrual cycle day or drug therapy. In addition a clinical question must be obvious otherwise interpretation of the findings is not possible. In addition to the lab order, close cooperation and good communication between laboratory doctors and gynecologists are also necessary. The laboratory physician can provide important help with his knowledge and experience regarding individual laboratory methods. The gynecologists also demand close collaboration with the laboratories for a good patient care.
The lecture will show how the laboratory physician can provide the gynecologists with sufficient individual interpretation favourable to a targeted individual interpretation. It is shown which prerequisites are necessary and how the laboratory doctor acquires the knowledge necessary for the interpretation of the findings. Frequent questions are discussed as well as the elaboration of solutions for specific questions on the basis of case histories. In addition to specific courses, working groups and expert networks can help to discuss complex gynecological endocrinological issues and provide an individual solution for an optimal interpretation of the findings.

V23

Schilddrüse: Labordiagnostik und Therapie bei Kinderwunsch und Schwangerschaft

Nicolas von Ahsen[1]
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Thyroid disease is very common and hypothyroidism caused by Hashimoto's thyroiditis (with positive anti-TPO-autoantibodies) affects as much as 5-10% of women. The female:male sex ratio for the disease is 9:1. Subclinical hypothyroidism is defined by an elevated TSH (>4 mU/L) and fT4 within the reference range. TSH results must be interpreted in the context of age, body weight, comorbidities and medication. It is increasingly understood that subclinical hypothyroidism must not be treated in all instances. However, women diagnosed with subfertility and persistent subclinical hypothyroidism should be treated with L-thyroxine to TSH targets well within the normal range. Some experts initiate treatment even with TSH in the high normal range, which is not generally accepted, especially when anti-TPO is negative. TSH reference ranges are different in pregnancy and generally lowest in the first trimester. Assay manufacturers recommendations should likewise be followed for free hormone (fT3, fT4) reference ranges in pregnancy. L-thyroxine dosing must consider the increased demand during pregnancy. After delivery L-thyroxine demand decreases again and the indication for continued treatment should be critically reviewed.
Pregnancy-associated temporary hyperthyroidism is often observed at the end of the first trimester. It must be distinguished from true hyperthyroidism e.g. due to Graves' disease (M. Basedow). Confirmed hyperthyroidism can successfully be treated but experienced clinicians should be involved.
Expert laboratory staff should be aware of these gender related differences in reference ranges and decision limits.

Symposium 10: Stoffwechselerkrankungen

V24

Brown adipose tissue – new option in the therapy of metabolic disease?

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The recent "re-discovery" of brown adipose tissue (BAT) in humans is one of the most intriguing findings in the research area of metabolic diseases as it raised hope for the treatment of obesity. In addition to brown adipocytes present in BAT depots, inducible brown-like adipocytes so called beige adipocytes can be found in specific WAT depots under various catabolic conditions such as cold exposure in wintertime. Cold-activated beige and brown adipocytes trigger an energy-demanding process known as adaptive thermogenesis, which requires increased uptake of dietary carbohydrates and lipids for maintaining caloric balance. The relevance of BAT is exemplified by the fact that in rodents, BAT and liver take up equal amount of energy from the bloodstream, a process which is able to normalize glucose and lipid values in insulin resistant and hyperlipidemic mice. Notably, also in humans most of the standard metabolic parameters routinely determined by physicians such as glucose and blood lipids are influenced by brown and beige adipocyte activity.
Recently, we investigated in more detail the regulation as well as the molecular processes of lipid disposal into activated BAT, using pharmacological and genetic interventions in mice. We found that short-term BAT activation by cold exposure or beta-3-adrenergic receptor agonism triggers insulin secretion, a process depending on fatty acid release by white adipose tissue. Furthermore, we showed that both insulin release and brown adipocytes insulin sensitivity is essential for the replenishment of endogenous energy stores and efficient adaptive thermogenesis. These data demonstrate that both catabolic and anabolic processes are important for energy balance and function of BAT. In addition to increased fatty acid disposal, we found enhanced uptake of dietary cholesterol into activated BAT as consequence of lipoprotein internalization. Following the fate of cholesterol, we observed the induction of hepatic bile acid synthesis, interestingly via the alternative but not the classical pathway. This process, depending on hepatic CYP7B1 induction, results in elevated plasma levels and pronounced fecal excretion of conjugated bile acids, accompanied by distinct changes in gut microbiota. Pharmacological intervention using ezetimibe, a drug blocking dietary cholesterol uptake, prevented both the rise in bile acid excretion and compositional changes in gut bacteria in response to cold. These results identify bile acids generated in the liver as the determinant of cold-induced gut microbiota, highlighting the relevance of cholesterol metabolism by the host for diet-induced changes on gut microbiota and energy metabolism.
Altogether, our results demonstrate the functional relevance of thermogenic adipocytes for systemic energy and lipoprotein metabolism. In this light, increasing adaptive thermogenesis may represent a promising therapeutic approach for obesity-associated metabolic diseases.

Symposium 11: Kardiale und kardiovaskuläre Erkrankungen

V25

Herzinsuffizienzmarker – Was ändert sich durch Neprilysin-Inhibitoren?

Johannes Mair[1]
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Since the approval of sacubitril-valsartan for the treatment of chronic heart failure with reduced left ventricular ejection fraction a commonly raised suspicion is that a wider clinical use of this new drug may diminish the clinical utility of B-type natriuretic peptide (BNP) testing as sacubitril may interfere with BNP clearance. This hypothesis is critically assessed based on the pathophysiology of the natriuretic peptide (NP) system and the limited published data on the effects of neprilysin inhibition on NP plasma concentrations in humans. As the main clinical application of BNP testing is and will be the rapid rule-out of suspected acute heart failure (HF) there is no significant impairment to be expected for BNP testing in the guideline recommended applications. However, monitoring of chronic HF patients on sacubitril-valsartan treatment with BNP testing may be impaired. In contrast to N-terminal-proBNP (NT-proBNP), the current concept that the lower the BNP result in chronic HF patients, the better the prognosis during treatment monitoring may no longer be true during the first month after start of treatment with sacubitril.

V26

Lipoprotein (a) - Was muss ein Labormediziner wissen?

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Lipoprotein(a) and its role in laboratory medicine
High Lp(a) concentrations are associated with cardiovascular disease (CVD) and aortic stenosis. This relationship is highly likely to be causal as impressively demonstrated by epidemiologic and genetic data and is supported by first interventional data. Currently, the therapeutic options to lower Lp(a) are limited with exceptions but there is reasonable hope for the upcoming years. The awareness is not yet well developed that high Lp(a) concentrations can be the cause for CVD independently of other risk factors. Therefore, the “sleeping beauty” Lp(a) needs to be kissed awake and be integrated in clinical diagnostics. In case of high Lp(a) concentrations a strict management of risk factors and surveillance of the patient is highly recommended. This also includes the consultation and surveillance of family members. Often the nagging question about the reason for CVD might be answered and it might be possible to avoid unnecessary suffering.
This lecture will bring after an introduction to the topic also the issues which are important for laboratory medicine: isoform-sensitivity of Lp(a) assays, measurement of Lp(a) in nmol/L or mg/dL, which cut-off is most appropriate: 30 mg/dL or 50 mg/dL, determination of SNPs or apo(a) isoforms by Western blot.

Symposium 12: Systeme und Werkzeuge der Qualitätssicherung in der Laboratoriumsmedizin (DGKL/RfB)

V27

Der irreguläre analytischer Fehler. Ein neues Konzept zur Bewertung laboranalytischer Qualität

Michael Vogeser[1]*; Christoph Seger*[2]
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Internal quality assessment based on quality control samples and external quality assessment based on proficiency testing are the key elements of quality assurance in laboratory medicine. These process-oriented assessment allows for insight into random analytical variation and systematic calibration error. However, in such a setting, any individual sample is not under individual quality control. The quality control measurements act merely at the level of the analytical batch. Notably, many effects and interferences associated with an individual diagnostic

sample can compromise any quantification. It is obvious that a process-oriented quality-control-sample-based approach of quality assurance is not sensitive to such errors.
To address the potential causes and nature of such analytical interference in individual samples more systematically, we suggest the introduction of a new term: irregular (individual) analytical error. This term can be applied in any analytical assay that is traceable to a reference measurement system. For an individual sample an irregular analytical error is defined as an inaccuracy (which is the deviation from a reference measurement procedure result found for the sample) of a test result that is so high that it cannot be explained by measurement uncertainty of the utilized routine assay operating within the accepted limitations of the associated process quality control measurements.
Based on this terminology, recognized causes of irregular analytical error can be given in the talk as a risk catalogue for clinical chemistry. These issues include reproducible individual analytical errors (e.g., caused by anti-reagent antibodies) and non-reproducible, sporadic errors (e.g., errors due to incorrect pipetting volume due to air bubbles in a sample), which can both lead to inaccurate results and risks for patients.

DGKL-AG-Symposium 4: Messunsicherheit – AG „Entscheidungsgrenzen“

V28

Performance specifications from the point of view of external quality control / proficiency testing

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In its first strategic conference in 2014 the EFLM has defined the rationale for analytical performance specifications. At that time point also several task finish groups (TFGs) were assigned in order to develop definitions and procedures on how to apply the outcome of the conference to the different measurands. As a member of the TFG ‘performance specifications for eqas’ the author was involved in defining how external quality assurance (EQA) programs can be used to establish whether a certain measurand in a certain laboratory indeed meets the performance specifications. As a first step in that process, the TFG has first published a paper that describes a 6 factor terminology to describe and compare different EQA programs. A next step is to apply this terminology to comply with the terms of reference of the TFG to define performance specifications for the most common measurands that should be used by EQAS organisers (for category I EQAS). In the presentation the author will present the current status of the work of the TFG and show examples on how this could be applied.

DGKL-AG-Symposium 2: Biobanking im Zeitalter der personalisierten Medizin – AG „Biobanken“

V29

Biomaterialsammlungen im Kontext klinischer Studien: Rahmenbedingungen für einen „broad consent“

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Background: In the last few years biobanks have been recognized as important resources for the progress in human targeted therapy. Since decades the pharmaceutical industry collects human biological materials in the context of industry-sponsored clinical trials. Generally, the intended use of such bio-specimen has to be described in the study protocol (mostly specific pharmacokinetic and/or -genetic issues) together with a narrow consent of the study-participants including the exemptions of withdrawal regulated according to Medicines Act.
In view of the openness of future medical questions and the increasing demand for novel (targeted) drugs, the vision of an establishment of industry-driven biobanks aiming at a broad usage of biomaterials (and related data) is legitimate considering the aspects (a) opening new vistas for medical research, and (b) optimization of public health care. However, as for academic biobanks, the unpredictability of the future use must be compensated by procedural methods as a pre-condition for the legal validity of the donor’s broad consent.
Methods: The common concept of informed consent to use human biological material in the context of clinical studies assumes that the bio-specimen are collected and used for a specific purpose. From 09/2016 to 06/2017 the biobanking task force of the Working Party of the German Medical Ethics Committees has developed a master template for the broad use of additional human biomaterials (and related data) donated by study-participants on the occasion of an industry-sponsored clinical trial, notably, without being part of the trial. Thus, for the use of such bio-specimen the right of withdrawal at any time without any reprisal must be secured.

Results: To compensate for the unpredictability of the future use of such additionally collected bio-specimen industry-driven biobanks must implement procedural methods. In this regard, independent ethics committees are of particular importance also for the assessment of industry-driven human biobanks (set up and operation) as well as for the assessment of new industry-driven research projects making use of such additional "broad consent" bio-samples. In addition, the donating study participant has to be informed unambiguously on the broad scope of the intended use of these additional bio-samples and related data. Last not least, also industry-driven biobanks are recommended to reciprocate by making processes transparent and intensifying participant's and public involvement.
Conclusion: The nation-wide applicable master template for the broad use of additional human biological materials (and related data) donated on the occasion of industry-sponsored clinical trials fulfils the current WMA-recommendations for human biobanks and furnishes a framework that permits industry-driven biobanks to store and use additional human bio-samples without restrictions regarding the scope and/or field of biomedical research. It appears well-suited to serve as a model for a harmonized European-wide consent facilitating broad usage of human biological material for collaborative academic and industry-driven (cross-border) biomedical research directed towards precision medicine that may extend to hitherto unknown disease entities and/or genetic disorders.

Morphologische Hämatologie 2017 – Sind morphologische Kenntnisse im Routinelabor noch von Bedeutung?

PK01

Morphologische Hämatologie 2017 – Sind morphologische Kenntnisse im Routinelabor noch von Bedeutung?

Peter Schuff-Werner[1]; Katrin Dreißiger[2]
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The importance of conventional hematological morphology is controversial in view of new automated technologies concerning morphological, immunological, genetic and molecular methods and the question arises whether morphological expertise is still necessary in the laboratory.
Particularly outside the regular service times and because of personnel savings, the classical workplace in the haematological laboratory is increasingly being filled with medical and technical assistants from other laboratory areas, which lack morphological experience in blood cell differentiation.
The central routine laboratory is still the first step for clinical-chemical, haemostase and hematological diagnostics, where well trained and experienced technicians may set the course for further specialist diagnostics and transfer to a department suitable for the basic disease.
Examples for this are the distinction of atypical lymphocytes suspected to be of reactive or neoplastic origin, the identification of promyeloic leukemia cells by their granulation and their typical configuration of the nucleus and the reliable classification of fragmentocytes in blood films of patients with HELLP or HUS. The morphologically untrained might misinterpret the heterogeneous "coloured picture" of infant blood smears in the sense of haematological disease.
The morphological particularities of RBCs largely escape from automated machine tools and therefore remain reserved to the morphologically experienced MTA or laboratory medical doctor.
The offered course is intended to convey and deepen knowledge about the morphology of white and red blood cells in the peripheral blood image, using selected blood smears and individual microscopes. The discussion of blood images on the demonstration microscope should supplement the knowledge acquired during the course.

Liquordiagnostik, Schwerpunkt Proteinanalytik

PK02

Cerebrospinal fluid diagnostics

Peter Lange[1]; Manfred Uhr[2]
[1]UMGL Göttingen, Göttingen, Germany; [2]Max-Planck Institut für Psychiatrie, München, Germany

Basic CSF knowledge:
• Cerebrospinal fluid rooms, cerebrospinal fluid production and cerebrospinal fluid flow
• Reiber quotient diagram

Präanalytic challenges:
• Cerebrospinal fluid & serum administration
• Difficulties with sample tubs
• Artificial blood contamination
Analytics:
• Cell counting
• Methods of the immune analytics (albumen, IgG, IgA, IgM.)
• Oligoclonal bands (Isoelectric focusing (IEF)).
• Specific antibodies (AI)
Result reporting:
• Integral report
• Results examples interactive training (TED system)

Praktischer Kurs: Referenzintervall-Schätzung aus großen Datenpools mit dem Reference Limit Estimator

PK03

Referenzintervalle aus großen Datenpools mit dem Reference Limit Estimator

Bernd Wolters[1]; Farhad Arzideh[2]; Rainer Klauke[3]; Georg-Erich Hoffmann[4]
[1]St. Joseph-Stift Bremen, Bremen, Germany; [2]Universität Bremen, Bremen, Germany; [3]Medizinische Hochschue Hannover, Hannover, Germany; [4]Trillium GmbH Medizinischer Fachverlag, Grafrath, Germany

The software tool ‚Reference Limit Estimator' (RLE) has been developed by members of the working group ‚Guide Limits' of the 'German Society of Clinical Chemistry and Laboratory Medicine' (DGKL). It uses large data pools collected from routine laboratory information systems - consisting of pathological and non-pathological results- for the indirect estimation of reference intervals. The standard is the direct method where the estimation is calculated from so called ‚healthy' individuals only. The motivation behind our approach is that in most medical laboratory information systems sufficiently large data pools are available to apply statistical (here the RLE tool) methods to estimate the distribution of the non-pathological results from the whole data set. The tool has been verified for many measurands of clinical chemistry and hematology.
The RLE is based on the statistical programming language 'R' using Microsoft Excel as user interface for data preparation and transmission. In this workshop, the participants will be instructed on how to install the RLE on a PC or an USB flash drive. For this, it is advisable that the participants bring their own windows laptops with Microsoft Excel and 'R' (www.r-project.org) already installed. A link with the newest version of the tool can be found on the website of the working group (www.dgkl.de [English] -> Working groups -> Guide Limits -> Excel Tools).
The workshop is divided into an introductory section in which the applied statistical methods and software tools RLE, Excel, and VBA are explained and an extensive practical part, including discussion of results. All attendees will analyse authentic datasets with their own computer systems and will learn how to interpret the results. The required data set can be derived from the available data with an additional data preparation step. The participants are encouraged to bring their own data sets (xls or csv) containing at least three columns with analytical results, gender and age of patients. Other recommended fields are date of analysis, information about the ward and patient identification code.
The estimation of the reference limits is carried out in three steps:
1. Evaluation of the distribution of the raw data, suggested by the system.
2. Check for analytical stability over the time or if a possible drift effect existed.
3. Finishing with the estimation of the reference limits, the results will be discussed.
At the end of the workshop the participants should be able to use the 'Reference Limit Estimator' in their medical laboratory with their own data.

Freie Vorträge / Free Talks

Symposium 1: Nucleic acid based biomarkers in body fluids

FV01

Novel biomarkers on the horizon? Transcriptome 3'end organization by PCF11 drives neurodifferentiation and determines neuroblastoma outcome

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Anton Ogorodnikov[1,2,3], Michal Levin[1,2,3], Surendra Tattikota[1,2,3], Sergey Tokalov[1,2,3], Mainul Hoque[4], Ansgar Poetsch[5,6,7], Bin Tian[4], Michael Schaefer[8], Frank Westermann[9], Karl Lackner[2] and Sven Danckwardt[1,2,3,10,11]

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4 Rutgers New Jersey Medical School, USA
5 Max-Planck-Institute for Heart and Lung Research, Germany
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11 Lead contact

Diversification at the transcriptome 3'end is essential for functional complexity by as yet largely unknown mechanisms. Perturbations of this process may become pathogenetically relevant and, for example, account for the unexpected low number of disease-eliciting driver mutations. Variations at the transcriptome 3'end are prevalent. Yet, typically they escape standard detection techniques (such as conventional RNAseq), with important implications for the identification and elucidation of disease-eliciting perturbations.
By applying a newly developed tailored high throughput sequencing approach (TRENDseq), we identify massive alterations at the transcriptome 3'end in childhood neuroblastoma. This tumor entity is characterized by a general paucity of recurrent somatic mutations and an unusually high frequency of spontaneous tumor regression. In a massively parallel RNAi screening we illuminate the landscape and identify drivers of transcriptome 3'end diversification (TREND). We discover a component involved in RNA processing (PCF11) as a key regulator of TREND, which controls a differentiation RNA-operon in this tumor entity. It shapes various inputs converging on WNT-signaling, and thereby governs cell cycle, proliferation, apoptosis and neurodifferentiation. Low PCF11 is associated with favorable outcome and spontaneous tumor regression, while high level PCF11 arrests neuronal precursors in a fatal, highly proliferative embryonic state. Our data document an unexpected critical role for TREND perturbations in tumor development and describe a novel mechanism for cell fate reprogramming in neuroblastoma with far-reaching implications. From these studies, TREND signatures arise as surprisingly potent novel prognostic biomarkers.

Symposium 2: Neue Entwicklungen in der Massenspektrometrie

FV02

Development of a LC-MS/MS Method for the Quantification of 16 Steroids in Plasma from Children

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Steroids are biological compounds playing an essential role in a multitude of processes ranging from water and salt homeostasis, stress response, development and maintenance of male and female secondary sex characteristics as well as maintenance of pregnancy. To date, many medical conditions are known to alter the steroid homeostasis. Such disorders can be often diagnosed and monitored by measuring the steroid concentrations in either blood or urine. From the clinical point of view, it is more informative to determine their concentrations in blood. However, the analysis of steroids in blood is very challenging due to (a) their structural similarity, (b) their broad dynamic range (from few pmol/L to several umol/L) and (c) their relatively low concentrations especially in newborns and children. Several methods exist for the quantification of steroids in blood, which are mainly based on immunological assays. While these methods are usually quick, they are often suffering from low specificity due to the structural similarity of the recognized epitopes. Furthermore, such methods can only detect one

single steroid at a time and usually require large amounts of sample. It is therefore crucial to develop analytical methods, which enable the simultaneous measurement of many different steroids in a small amount of blood with good precision, accuracy and sensitivity.
The present method is based on solid-phase extraction and subsequent LC-MS/MS analysis in positive polarity and enables the measurement of 16 steroids in just 100 µL of plasma. No chemical derivatization is required. The reported method is linear (r2 > 0.99), sensitive, precise (intra-assay CV = 0.3% – 7.0%; inter-assay CV = 1.5% – 12.4%) and has a broad dynamic range. Analysing external quality control samples with established consensus concentrations revealed that >95% of the measurements were within ±2σ and none of them was outside the ±3σ. The recovery ranged between 83% and 120%. A comparison between the results obtained with this method and those obtained with the immunological methods revealed good correlations for all tested analytes. However, the concentrations measured by LC-MS/MS were in general slightly lower, probably due to lack of cross-reactivity intrinsic to the immunological assays. In conclusion, we present a sensitive, precise and robust LC-MS/MS method to measure 16 steroids in plasma of children.

Symposium 3; Instrumentelle Analytik in der Infektionskrankheitserreger-Diagnostik

FV03

Rapid bacteria identification in CSF through MALDI-TOF MS ID

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Introduction:
Bacterial meningitis is a neurological emergency associated with high mortality. During the last decade optimized diagnostics, emergency casualty workflows and improved therapy led to a reduced mortality (1). Rapid identification of the causative pathogen and the consecutive adjustment of antimicrobial therapy is the cornerstone of a successful therapy. It is undeniable that Matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized and accelerated the identification process. Many groups could show that a direct identification from positive blood culture (BC) flasks is possible without the time consuming step of subcultivation.
Materials, methods and results:
The inhouse extraction assay for the processing of BC bottles has been validated for CSF culture bottles. Analogous to the BC bottles workflow CSF cultures were processed. Blood culture bottles that turned positive outside the routine working hours were analyzed by MALDI-TOF MS and direct bacterial identification protocol without subcultivation was performed batch-wise twice daily.
After the implementation of an inhouse extraction assay to process BC flasks, we could show that optimized workflows in combination with this new method can accelerate the identification process considerably, saving up to 53 hours (2). Analysis of turn-around times showed that identification results were available after about 7 hours after the culture was flagged positive. Comparison with routine microbiological workflow showed a significantly earlier availability of identification results, thus enabling the clinician to adjust the antimicrobial treatment much sooner.
Conclusion:
Identification of meningitis causing bacteria through MALDI-TOF MS is an important technology. Direct identification in CSF culture can provide the clinician with valuable information and therefore facilitate the adjustment of antimicrobial therapy. We could show that the implementation of this technology into routine workflow was able to accelerate the identification process significantly.
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2. Schneiderhan, W, Grundt, A, Worner, S, Findeisen, P, Neumaier, M: Work flow analysis of around-the-clock processing of blood culture samples and integrated MALDI-TOF mass spectrometry analysis for the diagnosis of bloodstream infections. Clin Chem, 59: 1649-1656, 2013.

Symposium 4: „Liquid profiling“, eine interdisziplinäre Herausforderung

FV04

High-Coverage NGS as Diagnostic Tool for Mitochondrial Disease – two Years of Experience

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Objective: Using next generation sequencing (NGS) to improve diagnostic sensitivity for mitochondrial disease.
Method: We established a high-coverage NGS procedure for mtDNA. The entire mtDNA was enriched by a single amplicon long-range PCR and analyzed by massive parallel sequencing. Sequence alignment to NC_012920 sequence with subsequent variant calling was performed. Technical sensitivity was 1% heteroplasmy for SNVs and allowed determination of heteroplasmy with high precision. For 297 samples, we achieved an average coverage of 6801 reads/sample. 27.6 variant per sample were detected.
For diagnostic purposes, we set a heteroplasmy threshold of 10% (blood samples) and 20% (urine or muscle samples) for sequence variants to be evaluated, in order to exclude likely insignificant variants. mtDNA deletions were detected by both long-range PCR and the coverage analysis, the latter providing breakpoint information.
Result: In 2015 and 2016 we performed diagnostic high-coverage NGS of mtDNA for 189 index patients. Most of the patients were analyzed because of suspected mitochondrial disease (146/189, 17 among them MELAS), 22/189 for LHON and 21/189 for other indications. For most patients with suspected LHON or MELAS, the common specific mutations had been excluded by other methods before using high-coverage NGS. In one family we were able to identify the most common MELAS mutation m.3243A > G which had been missed by Sanger sequencing.
Overall we obtained 72 clinically relevant results. Half of them were deletions (36), which frequently showed association with suspected CPEO (12/18) or other suspected mtDNA-deletion syndromes (3/10). In 16 cases, we found confirmed pathogenic point mutations, in 20 cases yet unreported variants of unknown significance (VUS) likely to be of relevance. Deletions were found in a higher proportion in adult than in pediatric samples as opposed to point mutations.
In 29 cases with negative result of mtDNA high-coverage NGS we performed additional whole exome sequencing (WES). We found pathogenic relevant nuclear sequence variants in 14 cases (48.2%).
Conclusion: High coverage mtDNA sequencing is an efficient tool for diagnosing mtDNA mutations in mitochondriopathies, especially in adult samples.

Symposium 5: Hämostaseologie und Inflammation

FV05

Procoagulant extracellular vesicles impair trophoblast function by a thrombo-inflammatory pathway in preeclampsia

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Objectives: Procoagulant extracellular vesicles (EV) and platelet activation have been associated with pregnancy complications. We have recently identified that EV cause preeclampsia by platelet mediated placental inflammasome activation. Whether this thrombo-inflammatory pathway alters trophoblast differentiation and function remains unknown. We therefore aim to identify whether the EV induced thrombo-inflammatory pathway modulates trophoblast proliferation, differentiation, and invasion in PE.
Methods: We injected C57BL/6 mice with endothelial or platelet-derived EVs to study the role of EVs in vivo. Blood pressure, kidney histology (PAS staining and EM), proteinuria, sFlt-1 were assessed to evaluate PE. We exposed human and mouse trophoblast cells to EV and platelets to study their role in vitro. Trophoblast proliferation and cell death was studied using Ki-67 immunostaining, BrdU incorporation and TUNEL staining. RT-PCR for marker genes (PL-II, Tpbpa, Gcm1) was done to study trophoblast differentiation. Matrigel based tube formation assays were used to assess trophoblast invasion. Translational relevance was studied in human PE placenta.
Results: EV injection into pregnant mice results in platelet activation and PE and activated platelets accumulate in the placenta. EV treatment enhanced cell death and impaired trophoblast differentiation and proliferation both in vitro and in vivo. EV treatment resulted in impaired trophoblast tube formation indicating impaired trophoblast invasion. Platelet depletion and genetic (NFE2-/-, Gαq-/-) or pharmaceutical (Apyrase, Aspirin) platelet inhibition abolished these effects. Furthermore, EV induced inflammasome activation in trophoblast cells, and NLRP3 or Casp-1 deficiency or IL-1 receptor antagonist (Anakinra) abolished the effects of EV. Inflammasome activation, platelet activation and cell death were positively correlated in human PE placenta.
Conclusion: These results demonstrate that EV mediated platelet activation impairs trophoblast function by reducing trophoblast proliferation, differentiation and invasion by platelet mediated inflammasome activation. These results support the pathophysiological relevance of enhanced maternal platelet activation at the feto-maternal interface. Monitoring platelets activation and / or EVs in maternal blood may provide insights into placental failure.

Symposium 6: „MassCyto“ - Kombination von Durchflusszytometrie und Massenspektrometrie

FV06

Elicitation of within-laboratory reference values for flow cytometric analysis of thrombocytic glycoproteins

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Background: The flow cytometric analysis of platelets is becoming more and more important because it offers a wide variety of possibilities for platelet function analysis. Using flow cytometry enables the investigation of activation status and reactivity of platelets via evaluation of secretion, degranulation and glycoprotein conformation. Platelet dysfunctions resulting from glycoprotein defects or deficiencies can also be identified. The evaluation and classification of measurement results require elicitation of within-laboratory reference values of all the glycoproteins tested.
Methods: A platelet number of 1x106 was adjusted for each sample. Every selected antibody was titrated to identify the right antibody concentration. In order to determine activation status, platelets were activated with TRAP (10 µM) for 15 minutes before antibodies were added. The basal activation was determined by inhibition of platelet activation with 2 µM of PGI2, also for 15 minutes. Samples were incubated for 15 minutes in the dark after antibody addition and were then fixed with 0.5 % paraformaldehyde. Platelets were identified by size, granularity and the expression of abundant CD41a. Samples from up to 51 healthy blood donors were analyzed for the elicitation of reference values.
Results: Reference values for the percentage of antigen positive platelets and the fluorescence intensity (MFI) of these platelets were established. The data generated were normally distributed and so normal ranges were defined as 2σ. Normal ranges for the percentage of antigen positive platelets for the selected glycoproteins are as follows: CD36 80.3-100 %, CD42a 91.9-100 %, CD42b 92.8-100 %, CD29 71.1-100 %, CD61 93.8-100 %, GPVI 89.6-99.1 %, CD62P inhibited 0-15.5 %, CD62P TRAP activated 75.8-96.9 %, CD63 inhibited 0-9.6 %, CD63 TRAP activated 60.1-87.4 %, PAC-1 inhibited 8.6-68.3 % and PAC-1 TRAP activated 72.4-99.5 %. Reference values for MFI are as follows: CD36 9919-27955, CD42a 32045-50317, CD42b 31401-43898, CD29 5511-7852, CD61 37508-53123, GPVI 7087-10400, CD62P inhibited 1941-5220, CD62P TRAP activated 7262-14921, CD63 inhibited 1438-4606, CD63 TRAP activated 5036-8856, PAC-1 inhibited 1076-3487 and PAC-1 TRAP activated 4849-10408.
Conclusion: The elicitation of reference values is a laborious but significant step for the establishment of in-house validation of flow cytometric analysis. With the calculated reference values we are able to detect different reasons for platelet dysfunctions such as Glanzmann thrombasthenia, Bernard-Soulier syndrome or receptor shedding resulting from a left ventricular assist device or stenosis.

Symposium 7: The Human Glycome in Health and Disease

FV07

Mutational screening of the ENPP1 gene in pseudoxanthoma elasticum (PXE) patients lacking ABCC6 mutations

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Objectives: Mutations in the ATP-binding cassette subfamily c member 6 (ABCC6) gene are the basic cause for pseudoxanthoma elasticum (PXE), a rare autosomal-recessive disease. Progressive mineralization and fragmentation of elastic fibers in soft connective tissues is a hallmark of PXE. Furthermore, ABCC6 mutations were detected in sporadic cases of the genetic disorder generalized arterial calcification of the infancy (GACI) which is characterized by severe calcification of the lamina elastica interna in arteries. The molecular etiology of PXE and GACI has become increasingly challenging as mutations in both ABCC6 and the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene were shown to cause overlapping phenotypes and modifier genes are beginning to be identified. Here, we evaluated the causal role of ENPP1 mutations in PXE patients with an incomplete ABCC6 genotype.
Methods: Using direct Sanger sequencing and high resolution melting analysis (HRM), we analyzed the coding sequence as well as the 5’- and 3’-UTR regions of ENPP1 in 36 patients suffering from PXE but lacking ABCC6 mutations. Furthermore, we developed high resolution melting (HRM) analysis for selected exons of ENPP1 and compared the efficiency of this mutational screening method to the results of direct Sanger sequencing.
Results: We detected 15 different sequence variants in the non-coding regions of ENPP1, therein the variant c.2101-11delT; and a synonymous mutation in exon 25: c.2661A > G (p.Ala887 =). We identified the missense variant c.517A > C (p.Lys173Gln) in exon 4 in seven PXE patients which was associated with diabetes mellitus type 2 and insulin resistance in other cohorts but not as causative for either GACI or PXE or phenotype modifying. The missense mutation c.2320C > T (p.Arg774Cys) previously described as causative for GACI was identified in one PXE

patient carrying only one heterozygous ABCC6 mutation in exon 22: c.2252T > C (p.Met751Lys). With the use of HRM analysis, we identified no sequence variants in six exons of ENPP1 and detected all variants identified by direct sequencing in ENPP1 exons 4, 8 and 23. Results of HRM analysis of exon 6 amplicons proved to be false positive.
Conclusion: HRM analysis turned out to be an efficient cost-effective tool for prescreening genomic DNA for mutations with a recovery rate of about 90%. We further identified merely one case of di-genic PXE in our cohort of more than 200 genotyped PXE patients. However, larger deletions/insertions may be found in ENPP1, which will not be detected by direct Sanger sequencing. In conclusion, although similar pathways underlie the genetic disorders PXE and GACI, ENPP1 mutations are rarely in PXE patients lacking ABCC6 mutations.

Symposium 8: Autoimmunerkrankungen/Immundiagnostik

FV08

TrkA signaling, activated by NGF or DHEA, is a regulator of microglia-mediated inflammation

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Dehydroepiandrosterone (DHEA) is the most abundant circulating steroid hormone in humans, produced by the adrenals, the gonads and the brain. DHEA was previously shown to bind to the Nerve Growth Factor (NGF) receptor, Tropomyosin-related kinase A (TrkA), and to thereby exert neuroprotective effects. Here we show that DHEA reduces microglia-mediated inflammation in an acute LPS-induced neuroinflammation model in mice and in cultured microglia in vitro. DHEA regulates microglial inflammatory responses through phosphorylation of TrkA and subsequent activation of a pathway involving Akt1/Akt2 and cAMP response element-binding protein (CREB). The latter induces the expression of the histone 3 lysine 27 (H3K27) demethylase Jumonji d3 (Jmjd3), which thereby controls the expression of inflammation-related genes and microglial polarization.
As the prototype ligand NGF also exerts anti-inflammatory effects in microglial cells in a TrkA-dependent manner. This is mediated by down-regulation of phosphorylation of JNK1/2 and activation of the NFkB pathway. Consistently, NGF attenuates the LPS-induced glycolytic shift in microglia and alters the expression of key metabolic enzymes.
Together, our data indicate that TrkA signaling, activated by NGF or DHEA, is a potent regulator of microglia-mediated inflammation, thereby providing the platform for potential future therapeutic interventions in neuro-inflammatory pathologies.

Symposium 9: Pädiatrische Laboratoriumsmedizin

FV09

The PEDREF data-mining study for the establishment of comprehensive next-generation pediatric reference intervals

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Background: Interpretation of pediatric laboratory test results is challenging due to physiological changes with age resulting in extensive age- and sex-specific dynamics in most biomarkers. Continuous percentile charts from birth to adulthood allow accurate consideration of these dynamics and enable appropriate quantification of test results in relation to a reference distribution. However, the ethical and practical challenges associated with blood sampling thousands of healthy children have restricted the creation of such percentile charts using population-based methods and warrant alternative approaches. Likewise, consideration of influences due to genetic and analytical diversity requires a collaborative and global approach to pediatric reference intervals.
Methods: We established the multi-center data mining initiative PEDREF to create pediatric percentile charts for laboratory analytes using clinical laboratory data collected during patient care. In an initial analysis, we examined a data set from 10 German tertiary care centers and 2 German laboratory service providers containing > 15,000,000 samples from > 350,000 individuals. The proportion of non-pathologic samples was identified from the input data set using an established statistical approach and used to construct percentile charts.
Results: A database of percentile charts for 25 biochemistry analytes and 9 hematology analytes enables precise assessment of laboratory test results in girls and boys from birth to 18 years. These results from a mainly Caucasian population measured on Roche Cobas, SYSMEX, and Beckman-Coulter analyzers are the starting point for the establishment of reference intervals for more specialized analytes and the investigation of influences due to genetic and analytical heterogeneity.

Conclusions: We initiated the PEDREF multi-center data mining study to complement conventional reference interval initiatives when the setup of comprehensive population-based studies is not feasible and when age-related accuracy in infants and neonates is restricted due to ethical objections. The participatory design allows examination of reference interval variation caused by differences in genetic background and analytical procedures, and we invite healthcare providers and clinical laboratories worldwide to participate at www.pedref.org.

Symposium 10: Stoffwechselerkrankungen

FV10

Vitamin D and health care costs: results from the population-based Study of Health in Pomerania

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Objective: Observational studies suggest that vitamin D deficiency is related to a multitude of pathologic changes and may be related to increased health care costs. However, the association between vitamin D deficiency and health care costs is hardly examined; especially data from the general population is sparse. We therefore analysed the cross-sectional and longitudinal associations between the serum 25-hydroxy vitamin D concentration (25OHD) and direct health care costs and hospitalization in Northeast Germany.
Methods: Data were obtained from the first and second follow-up of the population-based Study of Health in Pomerania (SHIP-1 and SHIP-2). Total, out- and inpatient annual costs were estimated in 3225 SHIP-1 and 2210 SHIP-2 participants, based on survey data. Vitamin D deficiency was defined as 25OHD concentrations 10-<20 ng/ml, severe vitamin D deficiency as 25OHD concentrations <10 ng/ml and vitamin D sufficiency as 25OHD concentrations ≥20 ng/ml. The cross-sectional (SHIP-1) and longitudinal (SHIP-1 to SHIP-2) associations between baseline 25OHD concentrations and baseline or follow-up average annual health care costs were examined using a generalized linear model with gamma distribution and a log link. Multivariable fractional polynomials were used to determine the best-fitting dose-response relationship. Poisson regression models were used to model relative risks of hospitalization.
Results: More than 60% of our study population was vitamin D deficient (46.9%) or severely vitamin D deficient (13.6%) at baseline. Vitamin D deficient or severely deficient men and women had about 500€ less income than vitamin D sufficient subjects. In cross-sectional models, participants with 25OHD concentrations of 5, 10 and 15 ng/ml had 107%, 19% and 0.6% higher inpatient health care costs than those with a 25OHD concentration of 20 ng/ml (overall p-value = 0.013) after full adjustment for age, sex, season, years of schooling, unemployment, equivalent household income, type of insurance and waist circumference. In contrast, 25OHD concentrations were not associated with outpatient (overall p-value = 0.601) or total annual costs (overall p-value = 0.096) or hospitalization (overall p-value = 0.908). In longitudinal models no significant associations between the 25OHD concentration and total, out- or inpatient costs or hospitalization were detected.
Conclusions: In our large sample with a high prevalence of vitamin D deficiency, there was no relevant association of 25OHD concentrations to total or outpatient costs but to inpatient costs that are associated with rather serious disease. Our results thus indicate a limited influence of vitamin D deficiency on health care costs in the general population.

FV11

Fr1dolin: Pediatric population screening for type 1 diabetes and familial hypercholesterolemia in Lower Saxony, Germany

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Ziele: Pilotstudie zur Durchführbarkeit eines kombinierten Screenings auf präsymptomatischen Typ 1-Diabetes (T1D) und familiäre Hypercholesterinämie (FH) bei Kindern in Niedersachsen. Das T1D-Screening wurde bereits in Bayern mit dem Fr1da-Projekt erfolgreich begonnen, das für die Fr1dolin-Studie eine Vorreiterrolle spielt.
Studiendesign: Populationsbasiertes Screening bei Kindern im Alter von 2 bis 6 Jahren (ab 2. und bis zum letzten Tag vor dem 7. Geburtstag) im Rahmen üblicher (kinder-) ärztlicher Vorstellungen im Vorschulalter.

Methoden: Kapilläre (oder venöse) Blutentnahme (200 µl) im Rahmen der Untersuchungen U7a bis U9 oder bei einem (Kinder-) Arztbesuch zur pseudonymisierten Bestimmung von T1D-assoziierten Antikörpern (IAA, GADA, IA-2A, ZnT8A, Helmholtz-Diabetes-Zentrum, München) und LDL-Cholesterin (LDL-C)-Messung (Kinder- und Jugendkrankenhaus AUF DER BULT, Hannover) sowie Erfassen der krankheitsspezifischen Familienanamnese mit einem Fragebogen.
Die LDL-C-Messung erfolgt aus 3 µl Probenvolumen auf einem Siemens Dimension EXL 200 (30 µl Totvolumen, ggf. 5 µl für die Messung des freien Hämoglobins nach Drabkin). Positive Ergebnisse werden in einer zweiten Blutprobe kontrolliert.
Wird die Diagnose bestätigt, werden krankheitsspezifische Beratung und Unterstützung der Familie organisiert. Bei LDL-C-Werten zwischen 135-160 mg/dl erfolgt das Angebot an die Familie eine genetische Untersuchung durchzuführen.
Bei unauffälligem genetischem Befund erfolgen jährliche Kontrollen beim Kinderarzt. Sollte sich ein LDL-C ≥ 160 mg/dl zeigen, erfolgt die Vorstellung in einem Lipidzentrum zur weiteren Diagnostik und ggf. Therapieinitiierung. Nachuntersuchungen sind bis zum Alter von 12 Jahren oder bis zu 6 Monate nach klinischem Beginn des T1D geplant.
Zwischenergebnisse: Derzeit (26. Juni 2017) wurden Proben von 2.253 Kindern (52,8 % Jungen, Alter: 4,0 ± 1,2 Jahre, Alter der Mutter 34,6 ± 5,4 Jahre, Alter des Vaters: 37,8 ± 6,7 Jahre, Mittelwert ± Standardabweichung) untersucht.
Bei 95 / 2253 (4,2 %) der Teilnehmer konnten Laboruntersuchungen wegen unzureichender Probenmenge oder totaler Hämolyse (48; 2,1 %) nicht durchgeführt werden.
91 / 2159 (4,2 %) Kinder hatten erhöhte LDL-C-Werte, 22 LDL-Resultate (0,98 %) waren ≥ 160 mg/dl. 9 / 1629 (0,55 %) zeigten mehrere T1D-Antikörper.
Eine Zweitanalyse konnte bisher bei 44 / 91 durchgeführt werden. Dabei blieben 6 / 7 der Erstanalysen mit Resultaten ≥ 160 mg/dl in diesem Bereich, hingegen stiegen 2 / 32 in diesen an (159 -> 179, 140 -> 163 mg/dl). 11 / 24 der Erstresultate sanken aus dem Bereich 135 – 159 mg/dl darunter ab.
Schlussfolgerung: Unter Berücksichtigung der bisherigen Zweitanalysen zeigen die ersten Ergebnisse des Fr1dolin-Screenings eine geschätzte Prävalenz für präsymptomatischen T1D (ca. 1: 200), und eine signifikant höhere Prävalenz für FH (> 1: 200) als erwartet in der Gesamtbevölkerung.

Symposium 11: Kardiale und kardiovaskuläre Erkrankungen

FV12

Comprehensive molecular profiling in acute aortic dissection: seeking diagnostic biomarkers

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Aim: Acute aortic dissection (AAD) is associated with extremely high lethality also because of a high rate of initial misdiagnosis. As yet, easily accessible and cost-effective blood tests only play a minor role in the diagnostics of patients with suspected AAD. Such biomarkers may also aid in the monitoring of patients at increased risk for AAD, for example patients with Marfan syndrome or with thoracic aneurysm. To discover biomarker candidates for the clinical management of AAD, we performed a comprehensive molecular profiling study as well as initial validation of biomarker candidates.
Methods: First, we compared protein abundances in secretomes, which were produced by tissue culture of aortic samples from patients with AAD, aortic valve replacement (AVR), or thoracic aortic aneurysm (TAA). Second, we used RNA-Seq to quantify gene expression in aortic tissues themselves. Third, we measured 360 inflammation- or cardiovascular disease related proteins in plasma by using Proseek multiplex immunoassays.
Results: Almost 1500 proteins were identified in aortic secretomes. First standard classification of proteins with fold change greater than two revealed 149 proteins differentiating AAD from AVR or TAA. Further computational analysis via regularized classification based on fold change identified platelet factor 4 (PF4), intelectin-1 and platelet basic protein as the best discriminators of AAD from either control group. In a first validation study, PF4 plasma levels were significantly decreased in AAD patients when compared to patients with AVR. The transcriptomic approach and Proseek immunoassays produced 20 and 8 top biomarker candidates, respectively, which are currently validated in plasma samples of patients with either AAD, TAA, AVR or the two most relevant AAD differential diagnoses, namely pulmonary embolism and myocardial infarction.
Conclusion: In a proof of principle study, we demonstrated the feasibility of discovering biomarkers for AAD by comprehensive molecular profiling of aortic tissues and plasmas and identified several promising candidates.

Symposium 12: Systeme und Werkzeuge der Qualitätssicherung in der Laboratoriumsmedizin (DGKL/RfB)

FV13

Datengetriebene Qualitätssicherung mit aggregierten Z-Werten aus Qualitätskontrollen und Patientenmedianen zur Detektion eines Bias

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Zielsetzung
Die interne Qualitätskontrolle verhindert fehlerhafte Messungen und ist daher ein wesentlicher Teil der Laboratoriumsmedizin. Die RiliBÄK gibt für die wichtigsten Analyte eine zulässige relative Abweichung der Kontrollprobeneinzelmessung vor. International ist die interne Qualitätssicherung heterogener, zumeist werden aber die Regeln nach Westgard eingesetzt. Alle Regeln basieren auf der Annahme, dass sich Qualitätskontrollen genauso wie Patientenproben verhalten. Häufig ist diese Annahme aber aufgrund fehlender Kommutabilität nicht zulässig. Die Mediane der Patientenmessungen leiden nicht unter Kommutabilitätproblemen und können daher die Kontrollprobenmessungen ergänzen. Im Folgenden wird mit den „Aggregierten Z-Werten" ein mathematisches Verfahren vorgestellt und evaluiert, mit dem sich Qualitätskontrollen und Patientenmediane gemeinsam auswerten lassen um einen Bias zu erkennen.
Methode
Kontrollprobeneinzelmessungen und die Mediane der Patientenmessungen wurden in Z-Werte umgerechnet Z = (x-mean)/sd. Diese Z-Werte wiederum wurden mit folgender einfachen Formel aggregiert: sum(Z)/sqrt(n).
Um die Aussagekraft der aggregierten Z-Werte zu evaluieren und mit etablierten Verfahren zu vergleichen, wurden Analyte und Messungen simuliert. Dazu wurde das Softwarepaket „rSimLab" in der Programmiersprache R geschrieben und veröffentlicht (https://github.com/acnb/rSimLab). Simuliert wurden Albumin, Hämoglobin A1c, Testosteron, Troponin I und Vitamin D3. Die Verteilung der Werte orientierte sich dabei an den entsprechenden Werten im Klinikum rechts der Isar der TU München. Die Impräzision wurde mit der sogenannten „Charakteristischen Funktion" simuliert, um eine absolute Impräzision in der Nähe der Nachweisgrenze und eine Impräzision relativ zum Messwert für höhere Wertelagen zu erhalten. Für jeden Analyten wurden 720 Tage mit unterschiedlichen Messabweichungen (Bias) simuliert. Jeder dieser Läufe wurde 200 Mal wiederholt.
Ergebnis
In den Simulationen konnten die Kontrollregeln Tage mit zu hohem Bias besser als Kontrollprobeneinzelmessungen oder Mediane detektieren. Mediane waren insbesondere bei einer hohen Probenanzahl und einem niedrigen Verhältnis zwischen Streuung und Mittelpunkt der Werte aussagekräftig. Die Aggregation von Z-Werten war traditionellen Westgard-Regeln vor allem dann überlegen, wenn Kontrollprobeneinzelmessungen oder Mediane von dem Bias unterschiedlich betroffen waren.
Zusammenfassung
Mediane von Patientenmessungen können Kontrollprobenmessungen ergänzen um einen Bias zu erkennen. Mit der Aggregation von Z-Werten lassen sich Kontrollprobeneinzelmessungen und Mediane gemeinsam auswerten. Dieses neue Verfahren ist traditionellen Westgard-Regeln häufig überlegen.
Literatur
Bietenbeck, A., Thaler, M. A., Luppa, P. B., & Klawonn, F. (2017). Stronger Together: Aggregated Z-values of Traditional Quality Control Measurements and Patient Medians Improve Detection of Biases. Clinical Chemistry, (https://doi.org/10.1373/clinchem.2016.269845).

FV14

DAkkS and IQsmart: Accreditation faces process orientated quality management

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Objectives:
The latest DIN EN ISO 9001 (2015) does not know the term "document" anymore. It rather refers to "documented information". This opens the door for more user-friendly IT-tools such as process oriented Quality Management Systems which reach far beyond modern document management systems. Instead of reading long texts, users are guided by graphical workflows. Many QM documents in these process oriented QM systems are resolved and can no longer be turned in. Instead the auditor may visit the system online to view the processes.
Regular DAkkS audits, which cover the ISO 15189 take place in part of our laboratory for years. Since high process quality and a living QM system are our aim, we decided to replace the traditional path of QM systems and underwent as the first laboratory an ISO audit with the process orientated QM system IQsmart for acccreditation.

Methods:
In 2002 Lufthansa Technik, a world wide operation company developed an integrated process management system (IQ MOVE). It offers a comprehensive method for the development and supervision of processes, enabling a high process quality and acceptance of the system within the company. Furthermore it is the basis for the maintenance of its existing aviation law licenses. As a Lufthansa Technik spin off, IQsmart was founded, aiming at transferring the processed based QMS software to the medical field. In cooperation with the University Medicine Greifswald and the medical information system provider c.a.r.u.s. the software was developed and adapted.
Results:
The process management system IQSmart was successfully introduced within the newborn screening laboratory, which is part of the Institute for Clinical Chemistry and Laboratory Medicine in Greifswald. Besides the process orientated presentations a unification and consolidation of existing SOP's was realized. Additionally, a deep cross-interlocking with the existing legal framework of the German newborn screening and the laboratory processes were achieved. All processes are displayed in a role-based way. Thereby, the employee is able to find his/her relevant process steps quite easily, all resulting and deriving process steps and process changes and modifications in a timesaving and effective way. Furthermore, IQsmart focuses on an active process management, allowing all employees to contribute to the systems enhancements and actualization for example with a feedback function.
We provided the auditor with an external web based access to our QM system, in order to prepare his audit in advance. Furthermore, in preparation of the audit, adaptions in how to present demanded documents were arranged with the DAkks.
The ISO 15189 audit was successfully performed with IQsmart.
Conclusion:
IQsmart proves to be not only capable of passing an ISO audit but more important, it is a QM tool which is being used by employees and supports and improves every day's laboratory work.

Poster

Endokrinologie

P001

Plausibility of thyroid function test results – subject to objective evaluation

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Objective: Individual laboratories yearly analyze thousands of samples for thyrotropin (TSH) and free thyroxine (fT4). As applied immunoassays are prone to interference it is essential to recover discordant results before reporting them to clinicians. This plausibility check is still performed manually, and by individuals with varying degrees of expertise. To increase the accuracy of this procedure we developed a unique, objective algorithm to test the plausibility of TSH/fT4 combinations.
Method: Historical TSH/fT4 data, obtained by immunoassays on random access analyzers were extracted from laboratory information systems and used to construct a plausibility algorithm.
Values were consolidated by multiplying TSH with its corresponding fT4, and this product was then plotted against the corresponding TSH to yield the overall TSH/TSH_fT4 relation. This was performed with 13021 data points. The 95th upper and lower limits of this relationship were used to identify discordant samples as outliers. Results of this algorithm were compared to validated test samples with confirmed discordance and to the subjective evaluation of TSH/fT4 combinations by clinicians and laboratory specialists.
Result: The plausibility algorithm correctly classified 100% of 54 published and confirmed discordant TSH/fT4 combinations, as well as 100% of our own collected discordant samples. Professionals correctly classified 78% of concordant and only 56% of discordant TSH/fT4 combinations. Routine application of the algorithm in a large (887 bed) teaching hospital for 6 months identified 12 new cases.
Conclusion: The objective plausibility check offers a robust, automatable method to identify discordant samples and is superior to the current subjective approach.

P002

Association of serum chemerin with subclinical parameters of atherosclerosis – results of a population-based study

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Background and objectives:
Chemerin is an adipokine that has been shown to be associated with markers of inflammation and components of the metabolic syndrome, which are in turn known as leading risk factors for cardiovascular diseases (CVD). Recent research demonstrated an increasing interest in the role of chemerin in CVD. However, existing studies revealed very divergent findings and thus it is still not clear if chemerin acts as predictor for CVD in humans. Therefore, we aimed to investigate the association of serum chemerin with different subclinical parameters of atherosclerosis in a general population.
Study design and methods:
Linear and logistic regression models with the carotid intima-media thickness (IMT) and the ankle-brachial index (ABI) as subclinical outcomes for atherosclerosis were applied to analyze data from 4143 subjects of the German Study of Health in Pomerania (SHIP). All regression models were adjusted for age, sex, waist circumference, systolic blood pressure, glycated hemoglobin, total triglycerides, and estimated glomerular filtration rate.
Results:
Linear regression adjusted for age and sex revealed that high serum chemerin levels are significantly associated with a higher burden of subclinical atherosclerosis, represented by lower ABI and higher IMT values. After additional adjustment for waist circumference and other metabolic as well as inflammatory parameters only the inverse association between chemerin and ABI remained statistically significant. Logistic regression models confirmed the relation of serum chemerin with ABI and revealed that each increase in chemerin per 25ng/ml was associated with a 25% higher odds of having a low ABI value, defined as values under the 25th age and sex-specific quartile [odds ratio 1.25 (95% confidence interval 1.12-1.40), $p < 0.01$].
Conclusion:
Our analyses revealed a modest, but significant inverse association between chemerin and ABI that remained consistent after adjustment for confounding factors. However, the association of chemerin with IMT got lost after adjustment for the same set of metabolic and inflammatory confounders. ABI and IMT are known to measure the burden of subclinical atherosclerosis in different arterial regions. Thus, our results suggest that chemerin might have different associations on these arterial regions. Further longitudinal analyses are necessary to validate the role of chemerin in the vascular system.

P003

Aldosterone and lipid metabolism in the general population

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Background: Aldosterone and high-density lipoprotein cholesterol (HDL-C) are involved in many pathophysiological processes that contribute to the development of cardiovascular diseases. Previously, inverse associations between circulating plasma aldosterone (PAC) and HDL-C concentrations were described in obese patients with insulin resistance. Further, a deterioration of lipid metabolism with decreasing HDL-C and increasing triglyceride concentrations after treatment of primary aldosteronism (PA) was reported. We aimed to assess whether similar associations between PAC and five major components of lipid metabolism exist in a large healthy adult population without PA.
Methods: Data from 739 men and 938 women aged 25-85 years who participated in the first follow-up of the Study of Health in Pomerania were obtained. These participants were free of PA or renal failure and did not take drugs affecting the renin-angiotensin-aldosterone system, such as diuretics, beta blockers or other antiadrenergic agents, calcium channel blockers, ACE inhibitors, or angiotensin II receptor blockers. PAC, total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C) and triglyceride concentrations were measured and the nonHDL-C concentration was calculated. Multivariable analysis of variance (ANOVA) and linear regression models adjusted for sex, age, body mass index, estimated glomerular filtration rate and HbA1c were calculated. PAC was categorized in sex-specific quartiles (ANOVA) or used as continuous predictor (linear regression). All lipid concentrations were log-transformed before being entered in the models.
Results & Discussion: HDL-C concentrations significantly decreased over the PAC quartiles (median, 1st - 3rd quartile in the lowest PAC quartile: 1.22, 0.95 - 1.55 mmol/l; in the highest quartile: 1.13, 0.90 - 1.45 mmol/l), whereas the concentrations of the other lipids remained unchanged. The ANOVA revealed no statistically significant results. The linear regression models showed statistically significant positive associations of PAC with LDL-C (ß-coefficient 0.022, standard error 0.010, p 0.03) and nonHDL-C concentrations (ß-coefficient 0.023, standard error 0.009, p 0.01) and an inverse association of PAC with HDL-C concentrations (ß-coefficient -0.022, standard error 0.011, p 0.04). Our findings confirm the previously described association between PAC and HDL-C and demonstrate that this association is not restricted to selected patient groups, e.g. with PA. Further, our data point towards associations between PAC and LDL-C or nonHDL-C, but do not confirm the previously described associations with triglycerides.
Conclusion: Associations between PAC and lipid concentrations are not restricted to patient populations but also exist within the healthy population.

P004

Density-enhanced phosphatase 1 (DEP-1) modulates obesity induced insulin resistance by targeting the insulin receptor

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Objective: Insulin signaling and glucose uptake is closely regulated by the phosphorylation status of the insulin receptor, which is negatively controlled by protein-tyrosine-phosphatases (PTPs). Since the PTP density-enhanced phosphatase-1 (DEP-1, Ptprj) dephosphorylates a variety of receptor tyrosine kinases, we hypothesized an impact on insulin signaling caused by DEP-1 association to the insulin receptor, thus possibly also modulating insulin resistance in vivo.
Method: We applied a high-fat diet (HFD) induced obesity-associated insulin resistance model to evaluate the relevance of DEP-1 in insulin signaling using conventional DEP-1 knockout (Ptprj-/-) as well as C57BL/6 wildtype mice with or without additional treatment with DEP-1 or control antisense oligonucleotides (ASOs).
Result: HFD-fed-Ptprj-/- mice were characterized by both improved glucose tolerance and enhanced insulin sensitivity, determined by glucose and insulin tolerance tests (GTT, ITT), along with reduced blood pressure and leptin serum levels compared to wild-type animals. DEP-1 deficiency was followed by increased phosphorylation of components of the insulin signaling cascade in the insulin-sensitive tissues liver, skeletal muscle and adipose tissue after insulin challenge. Further, increased glucose uptake was detected in DEP-1 downregulated skeletal muscle cells as well as in skeletal muscle derived from Ptprj-/- mice. To evaluate a potential therapeutic impact by targeting DEP-1, we thus administered after 10 weeks of HFD specific DEP-1 or control ASOs for 6 weeks to wildtype mice. DEP-1-ASOs led to improved insulin sensitivity, reduced basal glucose level, and to a significant reduction of body weight, which was accompanied by both lower leptin and insulin levels in serum. Moreover, DEP-1 physically associated with the insulin receptor in situ, which was evidenced by a proximity ligation assay (PLA) in liver tissue, and recombinant DEP-1 dephosphorylated the insulin receptor in vitro. PLA and immunoblotting also demonstrated that downregulation of DEP-1 through ASOs was followed by hyperphosphorylation of the insulin receptor in liver tissue, underlining the findings in Ptprj-/- mice.
Conclusion: Our results demonstrate that DEP-1 is acting as a previously unrecognized endogenous antagonist of insulin signaling evidenced by physical association with the insulin receptor determined by PLA. Further, DEP-1 knockout and downregulation of DEP-1 by ASOs leads to improvement of insulin sensitivity. Therefore, DEP-1 may represent a novel target in insulin resistance.

P005

Effects of acute hypoxia on hormonal regulations in apnea divers

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Effects of acute hypoxia on hormonal regulations in apnea divers
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Introduction
Intermitted hypoxia and hypercapnia are regularly seen phenomena in patients with obstructive sleep apnea syndrome (OSAS). OSAS is associated with a higher prevalence of stroke, myocardial infarct, atherosclerosis, coronary artery disease and essential hypertension. But the underlying pathomechanisms are not totally understood, because of the high number of comorbidities in these patients and the lack of an adequate clinical model to simulate hypoxia and hypercapnia in humans. Hypoxia results in changes in cerebral blood flow due to increased peripheral vasoconstriction and systemic compensatory mechanisms. Furthermore, elevated sympathetic nerve activity releases catecholamines. Interestingly, the same systemic effects can be simulated due to maximal voluntary apnea. In acute stress situations such as hypoxia hormones play an important role. We therefore investigated hormonal regulations after a single maximal breath hold.
Materials and Methods
Venous blood was obtained before, immediately after, 30 min and 4 h after a single maximal breath-hold. TSH, fT3, fT4, cortisol, prolactin, LH, FSH, hGH, testosterone and SHBG were analyzed by Vista1500™ and Immulite1000™ (Siemens Healthineers, Eschborn, Germany) analyzers. Changes in values were investigated using Friedman's test with Bonferroni correction. Each post-apnea time point was compared to the corresponding baseline value using Dunn's multiple comparison post-hoc test.
Results
17 divers (15 male, 2 female) with a mean age of 39.8 years (SD ± 11.1) performed a mean breath-hold time of 299.5 sec (SD ± 54.2).

Friedman's test was significantly different for TSH ($p < 0.0001$), fT3 ($p = 0.0009$), fT4 ($p < 0.0001$), testosterone ($p = 0.0002$), PTH ($p = 0.0002$), cortisol ($p < 0.0001$), and prolactin ($p < 0.0001$) values. Changes in hGH, LH, FSH, and SHBG were not significant.
Dunn's multiple comparison showed significant differences for TSH after 30 min and 4h ($p < 0.05$; $p < 0.001$); for fT3 after 4 h post ($p < 0.001$), for fT4 post-apnea and after 30 min ($p < 0.001$; $p < 0.05$); for cortisol after 30 min an 4h ($p < 0.05$; $p < 0.05$); for PTH after 30 min and 4h ($p < 0.01$; $p < 0.01$), and for prolactin post-apnea and after 30 min ($p < 0.001$; $p < 0.001$).
Conclusion
We found that even a single maximal apnea results in significant changes in certain hormones. The observed hormonal up- and down regulations in healthy apneic divers might help to understand the underlying pathomechanisms in patients with OSAS. Therefore future studies should further investigate the role of hormonal regulations caused by intermittent hypoxic states.

P006

Integrin-dependent adhesive interactions between inflammatory macrophages and adipocytes inhibit beige adipogenesis in obese adipose tissue

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Zielsetzung: Beige adipogenesis and UCP-1–dependent thermogenesis in white adipose tissue (AT) is diminished in obesity. Although type 2 immunity, including the anti-inflammatory M2 macrophages, regulates beige adipogenesis in the lean AT, very little evidence exists that inflammation could inhibit thermogenic beige adipogenesis in obesity. More specifically, it is entirely unclear whether the M1 inflammatory macrophages present in the obese AT contribute to the obesity-related disruption of beige adipogenesis and thermogenesis in white AT and whether this is happening through direct interaction with adipocytes, likely via integrin molecules.
Methoden: Mice with hematopoietic-specific ablation of VLA4-integrin and littermate control mice, as well as wild type mice administered with a VLA4-integrin inhibitor (and control/vehicle treated mice) were fed a high fat diet to induce obesity. An analysis of the metabolic phenotype of the mice was performed by measuring several metabolic parameters (e.g. glucose, cholesterol, insulin as well as by performing an insulin tolerance test), while beige adipogenesis was evaluated by measuring the mRNA and protein levels of UCP1 in the AT under normal temperature conditions as well as in cold. The accumulation of macrophages (inflammatory and anti-inflammatory) in the AT was determined by flow cytometry, while the retention of the macrophages in the AT of the mice was additionally evaluated by performing in vivo monocyte/macrophage trafficking assays. Co-cultures of macrophages with primary adipocytes or adipocyte progenitors were performed in order to reveal whether the duo VLA4-integrin/VCAM-1 (expressed on macrophages and adipocytes respectively) is involved in inflammatory-macrophage/adipocyte interactions, leading to reduction of beige adipogenesis under obese conditions.
Ergebnis: Inflammatory macrophage retention within the obese AT was mediated by direct adhesive interactions between the VLA-4-integrin on macrophages and its counter-receptor VCAM-1 on adipocytes, which were induced to express VCAM-1 in obesity. Importantly, this VLA-4/VCAM-1–dependent interaction between macrophages and adipocytes, as well as adipocyte progenitors, resulted in reduced beige adipogenesis and thermogenesis in the obese subcutaneous AT. Hematopoietic-specific ablation of VLA4-integrin or pharmacologic VLA-4-integrin antagonism resulted not only in improved insulin sensitivity but also in elevated beige adipogenesis, UCP-1 expression and thermogenesis of the subcutaneous AT, in both diet-induced and genetic obesity.
Zusammenfassung: The pharmacologic targeting of the VLA-4-integrin in obesity may be an effective novel therapeutic approach to promote beige adipogenesis and thermogenesis in obesity, thereby reversing obesity-associated metabolic dysregulation.

P007

ARCHITECT HbA1c: Influence of pre-analytical factors and sample collection tube type

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Background: Measurement of glycated hemoglobin (HbA1c) is recommended for the diagnosis and monitoring of diabetes as it reflects glycemic control over the last 3 months. Anti-coagulated whole blood is recommended as sample matrix. Aim of this study was to compare results of the enzymatic ARCHITECT HbA1c assay (List number 4P52) in different sample collection tubes 2 h after blood draw and after storage for 48 h at room temperature (RT).
Methods: Blood samples were collected from healthy volunteers. EDTA tubes and Fluoride/EDTA/Citrate- tubes from Greiner or Sarstedt were used. The first set of samples was tested within 1-2 h after collection using ARCHITECT HbA1c enzymatic assay, the second set of samples was analyzed after storage for 48 h at RT.

Results: HbA1c Median levels in mmol/mol (Interquartile range (IQR)) in Greiner EDTA and GLUCOMEDICS (GM) tubes tested 2 hours after blood draw are 31.7 (30.0- 34.4) and 31.7 (30.1 – 34.7), with an R2 of 0.99 and linear regression equation HbA1c GM = -0.03 + 1.01x. Respective results for Sarstedt EDTA and GlucoEXACT (GE) tubes are 31.2 (30.1-34.4) and 31.2 (30.1-34.4), with R2 = 0.92 and HbA1c GE = 1.73 + 0.95x. In the second set of samples with 48 h storage at RT, average difference to initial HbA1c value was – 1.19 mmol/mol in Greiner EDTA and -0.90 mmol/mol in GM tubes, for the Sarstedt EDTA and GE difference was -0.50 and -0.40 mmol/mol, respectively.
Conclusion: HbA1c can be measured with the enzymatic ARCHITECT HbA1c assay with high precision and excellent correlation in bothwhole blood EDTA and in Fluoride/EDTA/Citrate. Whole blood EDTA and Fluoride/EDTA/Citrate (GM or GE) samples stored for 48 hours at RT give comparable results to those tested immediately after blood draw.

P008

Urinary excretion of the main metabolite of melatonin relates to all-cause and cardiovascular mortality in renal transplant recipients

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Objective: 6-sulfatoxymelatonin (6-SM) is the major metabolite of melatonin, a multifaceted hormone that rises during the onset of darkness. Melatonin is rapidly hydroxylated in the liver and conjugated to 6-SM prior to excretion in urine. 24-hour urinary 6-SM (U6-SM) excretion is an integrated measurement of total melatonin production over a day.
In patients with chronic kidney disease sleep is often disturbed. This could represent a risk factor for poor long-term outcome. In line with this, we hypothesized that low U6-SM is associated with excess mortality in renal transplant recipients (RTR).
Methods: U6-SM was measured using a newly developed isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) method. The study population consisted of 702 RTR with a functioning graft for at least one year, who visited the outpatient clinic between 2008 and 2010. All participants collected a 24-hour urine sample prior to their visit to the clinic. Baseline associations we explored by linear regression analyses. Kaplan-Meier and Cox regression analyses were employed to investigate the associations of 6-SM with all-cause mortality, cardiovascular mortality and graft-failure.
Results: Mean (±SD) age of the RTR was 53 (±13) years, with 57% males and a mean (±SD) eGFR of 45 (±19) ml/min/1.73m2. U6-SM was associated among others with age, waist-to-hip ratio, eGFR and the use of antihypertensives (standardized ß respectively -0.30, -0.18, 0.14 and -0.18, all with p = 0.001). After median 5.4 [4.8-6.1] years follow-up 133 RTR died (19%), of whom 54 (41%) due to a cardiovascular cause, and 75 (11%) developed graft failure.
U6-SM was significantly associated with all-cause mortality (HR [95%CI] = 0.82 [0.71-0.95], p = 0.008) and with cardiovascular mortality (HR [95%CI] = 0.78 [0.62-0.98], p = 0.03), independent of conventional risk factors and kidney function. There was no significant independent association with graft failure (HR [95%CI] = 1.07 [0.84-1.37], p = 0.57).
Conclusion: In this study, we found that U6-SM, as a measure of total melatonin production, is inversely associated with all-cause mortality and cardiovascular mortality in RTR. Based on these results, evaluation and management of melatonin metabolism could be considered for improvement of long-term outcomes in RTR.

P009

Biological variation of testosterone, dihydrotestosterone and their ratio determined in 30 healthy individuals with LC-MS/MS

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Objective: For correct interpretation of laboratory data, information on the biological variation is essential. This includes the within-person biological variation (CVi), between-person biological variation (CVg) and analytical variation (CVa). When monitoring disease, serial measurements usually reflects clinical improvement or disease progression. Interpretation of data on changes over time in an individual patient can be complicated and therefore the reference change value (RCV) can be determined. With the development of a new LC-MS/MS method, and the ability to measure testosterone (T) and dihydrotestosterone (DHT) in low concentrations, the biological variation was established in order to accurately interpret the measurements of T, DHT and their ratio.
Methods: Plasma total T and DHT were measured as part of the androgen profile in 30 apparently healthy subjects (15 males/15 females) with online-SPE coupled to LC-MS/MS. The blood samples were collected at a standardized time over a period of 4 months, with four-week intervals from January to May. The CVa, CVi, CVg and RCV were assessed according to the method of Fraser and Harris (1989) and Fokkema et al (2006).
Results: CVi's for T, DHT and the DHT/T-ratio were comparable in men and women, varying from 10-16%. However, CVg's were higher in women, T-35%, DHT-45% and the DHT/T-ratio 41%, compared to 20%, 31% and 19% in men respectively. The CVa's in both men and women

were below 7.5% at concentrations ranging from 0.7-15.6nmol/L for T and DHT. And finally, the RCVs were similar between men and women (men; T-41%, DHT-40%, DHT/T-ratio-30% and women; T-42%, DHT-37%, DHT/T-ratio-36%).
Discussion and Conclusion: In general, these novel data on biological variation shows a high degree of inter-individual variation, illustrating its importance and the need for careful interpretation of single measurements of T and DHT. For the DHT/T ratio, as compared to single T and DHT concentrations, less influence from the circadian rhythm was expected, as it reflects 5-alpha-reductase activity. However, this did not became apparent from our data. For clinical conditions associated with T and DHT, the calculated RCVs can be used for assessment of significant changes over time in the patient-status. The RCV data demonstrates that a change of approximately 40% represents a significant shift.
In conclusion, the biological variation determined with LC-MS/MS is valuable for the interpretation of T, DHT and DHT/T-ratio, and shows that these parameters can be useful in patient follow-up.

P010

HbA1c-Bestimmung – Evaluation des Tosoh G11 und Vergleich mit Roche Integra 800 und Cobas c513

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1. Titel
HbA1c-Bestimmung – Evaluation des Tosoh G11 und Vergleich mit Roche Integra 800 und Cobas c513
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3. Zielsetzung
Für glykiertes Hämoglobin (HbA1c) müssen Methoden hohen Anforderungen genügen, um standortübergreifend eine adäquate Führung der Patienten zu gewährleisten. Im internationalen Schrifttum werden für die Bestimmungsmethoden Gesamt-Präzisionen von <2% bis hinunter zu <1% gefordert. Dies ergibt sich aus den Entscheidungsgrenzen für therapeutische Interventionen. Für den Interlabor-VK im Ringversuch erlaubt die RiliBÄK noch 18%, aber international üblich sind wesentlich geringere Toleranzen, z.B. 6% (USA).
Es soll untersucht werden, bis zu welchem Grad aktuell verfügbare Methoden diesen Anforderungen genügen.
4. Methode
Eine neues (Tosoh G11) und 2 etablierte Verfahren (Roche Cobas c513 und Roche Integra 800) werden direkt mittels Patientenproben sowie der jeweiligen herstellerabhängigen Kontrollmaterialien miteinander verglichen. Insbesondere erfolgte eine Evaluation des Tosoh G11 HPLC-Systems.
100 Patientenproben aus der laufenden Routine wurden an allen 3 Systemen taggleich in Doppelbestimmung gemessen. Für jede Probe wurde der Intraassay-VK ermittelt und die Messwerte wurden in Bezug auf die Wertelage miteinander verglichen. Aus Kontrollprobenmessungen (8 x 2) wurden Intra- und Interassay-Präzision bestimmt. Ebenso wurde die Richtigkeit in Bezug auf die Kontrollproben-Zielwerte ermittelt.
Mit Patientenproben wurden ebenfalls die Intra- und Interassay-Präzision (8 x 2 und 10 x 2) ermittelt. Die Lagerung von Tag zu Tag erfolgte bei 4°C. Darüber hinaus wurden Stabilitätsbetrachtungen für die Messung auf Tosoh G11 angestellt.
5. Ergebniss
Es konnten 98/100 Patientenproben im direkten Vergleich ausgewertet werden (2 Proben mit Hb-Varianten wurden ausgeschlossen). Der mittlere Intraassay-VK betrug 1.85% (Roche Integra 800), 0.98% (Roche Cobas c513) und 0.35% (Tosoh G11).
Der Vergleich der Messwerte (Cobas c513 vs. Tosoh G11) ergab mit der Deming-Regression [Cobas c513] = 1.07 * [Tosoh G11] - 0.1% mit einem Vertrauensband von 3.16%.
In 3 verschiedenen Patientenproben (HbA1c 5,01, 5,73 und 10.15) fanden sich auf Tosoh G11 Intraassay-VKs (10 x 2) von 1.24%, 0,47% und 0,36%. Die Interassay-VKs betrugen 0,92%, 0,62% und 0,66%.
Die Kontrollproben-Messungen (8 × 2) ergaben für Tosoh G11 im Intraassay-VK 0,85% und 0,38%, im Interassay-VK 0,72% und 0,59%.
Für Roche Integra 800 fanden sich für den Intraassay-VK 0,33% und 0,75%, für den Interassay-VK 1,61% und 1,75%.
Für Cobas c513 fanden sich im Intraassay-VK 0,55% und 0,75% sowie für den Interassay-VK 2,08% und 1,41%.
6. Zusammenfassung
Im direkten Vergleich der 3 Assays mit 98 tagfrischen Patientenproben ergaben sich deutliche Unterschiede in der Intraassay-Präzision. Es zeigte sich, dass das chromatographische Messverfahren (Kationenaustauscher-HPLC, Tosoh G11) den mit Abstand niedrigsten Intraassay-VK (0.35%) lieferte. Das immunturbidimetrische Verfahren auf Cobas c513 lag um den Faktor 2.8 höher, die Messung auf Integra 800 sogar um den Faktor 5.3, bezogen auf Tosoh G11. Bei der Untersuchung von identischen Patientenproben ergeben sich also deutliche Performance-Unterschiede. Dies ist insofern relevant, da das verwendete Kontrollmaterial artifiziell ist, in diesem Fall herstellerabhängig, gelagert wird und sich damit von Patientenproben unterscheidet.
Unsere Daten bestätigen, dass erhebliche Qualitätsunterschiede zwischen den zur Verfügung stehenden Methoden bestehen, die bei der Auswahl berücksichtigt werden sollten. Da inzwischen Präzisionen von <1-2% für ausreichend aussagekräftige Ergebnisse gefordert werden, schränkt dies die Auswahl ein.

P011

Reference intervals for sodium in seniors: results from the prospective SENIORLAB study

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Background and aim: A majority of resources in clinical medicine is spent on seniors. The correct interpretation of laboratory results plays an important role for accurate diagnosis and therapy. At the moment, there is a lack of accurate decision limits for seniors. The SENIORLAB study set out to define reference intervals in seniors for a variety of laboratory parameters. The present analysis aims to estimate reference interval for sodium, a parameter frequently requested in seniors, for Caucasian individuals aged 60 years and more.
Methods: From a total of 1467 study participants reporting subjective wellbeing at baseline examination in the SENIORLAB study, the following individuals were excluded: death at first follow-up for participants <80 years of age (mean follow-up time 3.7 +/- 0.7 years), survival of less than 3 years between age 80-84, survival of less than 2 years between age 85-89, and survival of less than 1 year for age 90 and older. Further exclusion criteria known to affect sodium concentrations were: medication with NSAID, diuretics, laxatives, steroids, clonidine, lithium, sulfonylurea, fluoexetin, paroxetin, amytryptilin, imipramine, lamotrigine, clofibrate; participants with hypothyreosis (TSH > 4.5 U/L), heart failure (BNP >150 pg/mL), abnormal morning cortisol levels (<101 or >536 nmol/L), abnormal aldosterone (<100 or >900 nmol/L), and evidence of renal (eGFR < 50 ml/min/1.73m2) or hepatic dysfunction (cholinesterase activity <5320 U/L). Sodium was determined by indirect ion selective electrode on a Cobas Integra 800 (Roche, Rotkreuz, Switzerland). Double sided 95% reference intervals together with the 90% confidence intervals (CI) were evaluated according to CLSI guideline EP28-3c.
Results: A total of 468 individuals (207 males, 261 females, mean age 70 years, range 60-99 years) were available for calculation of reference intervals. There was no significant correlation between age and sodium concentrations. Further, no sex difference in sodium concentrations could be observed. Therefore an age- and gender independent reference interval was calculated. With the non-parametric method, the lower limit of the reference interval was 137 mmol/L, 90% CI [137,137], whereas the upper limit was 144 mmol/L 90% CI [143,144]. The upper and lower limits of the reference interval did not change when using the robust method or a method based on normal distribution.
Conclusion: In this carefully selected reference population reference intervals for serum sodium could be established. There is no need for gender and age-specific stratification of serum sodium reference intervals in seniors of Caucasian origin.

P012

Reference intervals for chloride in seniors: results from the prospective SENIORLAB study

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Background and aim: A majority of resources in clinical medicine is spent on seniors. The correct interpretation of laboratory results plays an important role for accurate diagnosis and therapy. At the moment, there is a lack of accurate decision limits for seniors. The SENIORLAB study set out to define reference intervals in seniors for a variety of laboratory parameters. The present analysis aims to estimate reference interval for chloride for Caucasian individuals aged 60 years and more.
Methods: From a total of 1467 study participants reporting subjective wellbeing at baseline examination in the SENIORLAB study, the following individuals were excluded: death at first follow-up for participants <80 years of age (mean follow-up time 3.7 +/- 0.7 years), survival of less than 3 years between age 80-84, survival of less than 2 years between age 85-89, and survival of less than 1 year for age 90 and older. Further exclusion criteria known to affect chloride concentrations were: medication with diuretics, laxatives, corticosteroids, androgens; participants with hyperparathyroidism (PTH more than 6.8 pmol/L), abnormal morning cortisol levels (less than 101 or more than 536 nmol/L), abnormal aldosterone (less than 100 or more than 900 nmol/L), and evidence of renal dysfunction (eGFR less than 50 ml/min/1.73m2). Chloride was determined by indirect ion selective electrode on a Cobas Integra 800 (Roche, Rotkreuz, Switzerland). Double sided 95% reference intervals together with the 90% confidence intervals (CI) were evaluated according to CLSI guideline EP28-3c.
Results: A total of 699 individuals (326 males, 373 females, mean age 70.6 years, range 60-99 years) were available for calculation of reference intervals. There was no significant correlation between age and chloride concentrations. Further, no sex dependent difference in chloride concentrations could be observed. Therefore an age- and gender independent reference interval was calculated. With the non-parametric method, the lower limit of the reference interval was 99 mmol/L, 90% CI [98,100], whereas the upper limit was 107 mmol/L 90% CI [107,108]. The upper and lower limits of the reference interval did not change when using the robust method or a method based on normal distribution.
Conclusion: In this carefully selected reference population reference intervals for serum chloride could be established. There is no need for gender and age-specific stratification of serum chloride reference intervals in seniors of Caucasian origin.

Pädiatrische Labormedizin / POCT

P013

Evaluation der fäkalen Calprotectin-Konzentration in einer Kohorte von Kindern im Alter von 0-5 Jahren

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Evaluation der fäkalen Calprotectin-Konzentration in einer Kohorte von Kindern im Alter von 0-5 Jahren
Einleitung und Zielstellung:
Fäkales Calprotectin ist ein bewährter Biomarker für entzündliche Darmerkrankungen. Da altersspezifische Referenzbereiche in der Altersgruppe von 0-5 Jahren bisher nicht etabliert sind, war das Ziel dieser Arbeit, die Evaluation der fäkalen Calprotectin-Konzentration in einer regionalen Berliner Kohorte von Kindern in dieser Altersgruppe.
Methodik der Labordatenerhebung:
Das MVZ Labor 28 versorgt in der Region Berlin eine große Zahl an Arztpraxen und weitere Gesundheitseinrichtungen im ambulanten Bereich sowie Krankenhäuser mit Leistungen aus den Bereichen der Laboratoriumsmedizin, Mikrobiologie, Virologie und Infektionsepidemiologie, Krankenhaushygiene und Transfusionsmedizin. Das Labor ist nach DIN EN ISO 15189 sowie DIN EN ISO/IEC 17025 akkreditiert. Die vorliegende Arbeit beruht auf einer Stichprobe, die alle Kinder im Alter von 0-5 Jahren (n = 352) umfasst, die vom 01.01.2016 bis zum 20.12.2016 labormedizinisch diagnoseunabhängig durch das MVZ Labor 28 in Berlin untersucht wurden. Doppelbestimmungen konnten ausgeschlossen werden. Die fäkale Calprotectin-Konzentration wurde mit dem Diasorin LIAISON® Assay bestimmt.
Ergebnisse:
Der Median der Calprotectin-Konzentration der Gesamtkohorte der 352 Kinder im Alter von 0–5 Jahren betrug 24,50 mg/kg Stuhl (95 % Perzentile 282,2 mg/kg), wobei der Median der Calprotectin-Konzentration in der Altersgruppe der 0-1 jährigen bei 53,55 mg/kg, der 1-2 jährigen bei 32,50 mg/kg, der 2-3 jährigen bei 20 mg/kg, der 3-4 jährigen bei 18,45 mg/kg sowie der 4-5 jährigen bei 10,95 mg/kg lag.
Die 95 %Perzentile der 1-4 jährigen lag bei 187,7 mg/kg. Bei dem Vergleich der einzelnen Altersgruppen zeigte sich die höchste statistische Signifikanz der medianen Calprotectin-Konzentration zwischen den 0-1 und den 4-5 jährigen (53,55 mg/kg vs. 10,95 mg/kg, $p < 0{,}001$). Die Korrelationsanalyse zwischen der Calprotectin-Konzentration und dem Alter ergab eine signifikante negative Korrelation ($r = -0{,}28$, $p < 0{,}001$).
Diskussion:
Die mediane Calprotectin-Konzentration von Kindern im Alter von 1-4 Jahren ist niedriger verglichen mit Kindern <1 Jahr, aber höher, als die in der Literatur beschriebenen medianen Calprotectin-Konzentration für Kinder im Alter von 4-17 Jahren. (1) Als erste Orientierungshilfe für die Interpretation der Calprotectin-Konzentration wird im Labor 28 die 95% Perzentile (187,7 mg/kg Stuhl) im Alter von 1-4 Jahren und die 90% Perzentile (358 mg/kg Stuhl) im Alter von 0-1 Jahr auf dem Endbefund mitangegeben. Dieses ermöglicht im Einzelfall, die Calprotectin-Konzentration zusammen mit dem Alter des Patienten als nützliches Hilfsmittel für die Vorhersage einer CED zu verwenden. Weitere Studien zu altersspezifischen Referenzbereichen sind erforderlich.
(1) O. R. Herrera, M.L. Christensen, R.A. Helms Calprotectin: Clinical Applications in Pediatrics J Pediatr Pharmacol Ther 2016;21(4):308-321.

P014

Biochemical sample testing in new MiniCollect® Blood Collection Tubes

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Background: Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows highest flexibility and accuracy by collecting blood in unprecedented simplicity. The MiniCollect Serum Separator and Lithium Heparin Separator tubes are intended to collect, transport, separate and process capillary blood for testing serum and plasma, respectively in the clinical laboratory.
Methods: Studies were done at Steyr Hospital (Austria) and Laboratory Rainbach (Austria) using MiniCollect tubes with old design vs. new design. Altogether, 80 hospitalized and 70 healthy subjects were recruited. Informed consent was given by all donors and the studies were approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. After centrifugation for 10 min at 3000g, 28 common biochemical analytes (venous) and 10 analytes (capillary) were tested using an AU680 and DxI800 (Beckman Coulter, precision within-run <3%, total <3%). Comparison testing to Microtainer (BD) was included. Analysis was done with the instrument's accompanying reagents. Statistical evaluation was done by STATISTICA 13.
Results: Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design for performance testing did not reveal any clinically nor statistically significant deviations ($p < 0.05$). The values in both serum tubes

of venous collection resulted in an initial highest deviation of 3.2%, and in plasma tubes of 4.4%. Comparable highest deviations for initial values in relation to 48h values were obtained for serum (5.3%) and plasma (6.4%). Capillary collection led to a highest deviation for LDH of 6.9% in serum, and in plasma tubes of 8.7%.
Conclusion: From a clinical perspective, the MiniCollect Serum Separator and Plasma Separator tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

P015

New MiniCollect® 9NC Coagulation Blood Collection Tubes for pediatric sample testing

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Background: Drawing blood from infants or children is mostly critical, particularly when the amount needed to fill a standard coagulation tube by ensuring the correct ratio of blood to additive can't be guaranteed. The MiniCollect Coagulation Tube is intended for collection of citrate anticoagulated whole blood samples for coagulation assays and allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity.
Methods: Two clinical studies were carried out to compare the performance of the new pediatric tube to a standard VACUETTE Coagulation tube by taking venous blood. Altogether, 20 healthy and 75 hospitalized subjects (Laboratory Rainbach and Hospital Steyr, Upper Austria) were recruited. Informed consent was given by all donors and the study was approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. After centrifugation for 10 min at 3000g, common coagulation parameters were tested using an ACL Top 500 (Laboratory Instruments). Analysis was done with the instrument's accompanying reagents (precision aPTT ≤2.5%; PT ≤ 3%, Fibrinogen ≤8%). Statistical evaluation was done by STATISTICA 13.
Results: Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of pediatric tubes with the new design did not reveal any clinically nor statistically significant deviations ($p < 0.05$). The values in both tubes resulted in maximum deviations of 7.1% for aPTT.
Conclusion: From a clinical perspective, the MiniCollect Coagulation tube with the new design is substantially equivalent to a VACUETTE Coagulation tube. The newly designed tube provides an essentially enhanced blood collection device for pediatric sample testing.

P016

Glucose testing in new MiniCollect® Blood Collection Tubes

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Background: Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity. MiniCollect FX Sodium Fluoride/Potassium Oxalate Tubes are used for the determination of glucose and lactate in capillary blood.
Methods: Studies considering venous and capillary blood collection were done at Steyr Hospital (Austria) and Laboratory Rainbach (Austria) using MiniCollect tubes with the old design vs. new design. Altogether, 80 hospitalized and 20 healthy subjects were recruited. Informed consent was given by all donors and the studies were approved by EC Upper Austria. Directly after venous (hospitalized subjects) or capillary (healthy subjects) blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. After centrifugation for 10 min at 3000g, glucose and lactate were tested using an AU680 (Beckman Coulter, within run precision <3%, total precision <3%). Analysis was done with the instrument's accompanying reagents. Statistical evaluation was done by STATISTICA 13.
Results: Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design for performance testing did not reveal any clinically nor statistically significant deviations ($p < 0.05$). The values of glucose concentration resulted in a highest deviation of 1.7% (for both venous and capillary collection), and the lactate values indicated deviations of 2.5% (venous) and 2.2% (capillary) between both tubes.
Conclusion: From a clinical perspective, the MiniCollect FX NaF/KOx tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the

fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

P017

Pediatric reference intervals of 9 steroid hormones in serum by liquid chromatography-tandem mass spectrometry: from Mini-Puberty to Puberty

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Background: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides high-throughput analyses combined with high specificity and sensitivity. The purpose of the study was the establishment of reference intervals from birth to adulthood for a robust and reliable LC-MS/MS method for measuring the steroid hormones, cortisol, 17-hydroxy progesterone, testosterone, androstenedione, progesterone, estradiol, aldosterone, DHEAS, and cortisone.
Methods: Serum samples from 1095 subjects (age range: 0.3 – 20 years) were analyzed simultaneously using LC-MS/MS. Subjects with concomitant endocrine diseases and or body mass index > |3| standard deviation score (BMI-SDS) were excluded. Reference intervals were established for age-, sex- and pubertal stage-specific cohorts. In addition, the use of oral contraceptives in females was considered as a confounder. Nonparametric or robust method was used to calculate the reference intervals. 90% confidence intervals for upper and lower limits of the reference intervals were calculated.
Results: Increased levels of steroid hormones, especially in adrenal androgens and cortisone, during mini-puberty showed a drastic drop after 1 year from the birth and started to increase again continuously during the pubertal development. Reference intervals of sexual hormones for pre-pubertal ages differentiated themselves between gender by LC-MS/MS. Cortisol and aldosterone showed a relatively constant course over the age range, except for higher cortisol levels in females who took oral contraceptives. In females (age range: 13.8 – 17.9 years) who took oral contraceptives, cortisol levels were significantly higher than age-matched females who did not take oral contraceptives ($p < 0.0001$). Separate reference interval for oral contraceptive cohort was established.
Conclusion: Sex-, age-, and pubertal stage-specific ranges for basal morning serum levels of 9 steroid hormones in 1095 healthy neonates, infants, children, and adolescents are established. LC-MS/MS can facilitate meaningful interpretation of biological data with better differentiation of steroid hormones especially in the low concentration range in children and adolescents.

P018

Verification of the Samsung LABGEO Analyser

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Verification of the Samsung LABGEOPT10 Analyser
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Background.
The Samsung LABGEOPT10 Analyser is a compact POC chemistry analyser that uses different cartridges. In our laboratory, we compared the results of this analyser with our reference system the Abbott Chemistry Architect ci8200 analyser. A mandatory requirement is that the analyser is simple to use near the patient (outside the laboratory) in a carousel for cardiac screening.
Methods.
We evaluated the Biochemistry Test 9 cartridge with the following assays: Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), Gamma-glutamyl transpeptidase (GGT), Glucose (GLU), Creatinine (CREA), Total cholesterol (CHOL), Triglyceride (TRIG), High density lipoprotein cholesterol (HDL) and Low density lipoprotein cholesterol (LDL, calculation).
The verification procedure consists of within-day-variation, between-day-variation and patient comparison.
Results.
The within-day-variation and the between-day-variation of the tested assays varies from 1,8 to 5,9 % VC. The correlation of patient results between Architect and LABGEO is very well (GLU, GGT, CHOL, TRIG, ASAT. Only HDL ($a = 1{,}0195$, $b = -6{,}7306$), CREA ($a = 0{,}8352$, $b = -0{,}0666$) and ALAT ($a = 1{,}25$; $b = 0$) needs correction factors, which could be applied by the local product specialist.

Conclusion.
The LABGEOPT10 is an easy to handle POCT analyser which correlates well with the reference analyser because the correction factors can be applied in this analyser. This analyser fulfils the criteria and can be placed outside the laboratory in the cardiac carousel.

P019

Rule-out of non-ST-elevation myocardial infarction by five point of care cardiac troponin assays according to the 0 h/3 h algorithm of the European Society of Cardiology

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Background: Point of care (POC) assays for cardiac troponins I or T (cTnI or cTnT) may accelerate the diagnosis of patients with acute coronary syndrome (ACS). However, their clinical utility according to the 0h/3h algorithm recommended by the European Society of Cardiology (ESC) for the management of Non-ST Elevation Myocardial Infraction (NSTEMI) is unknown.
Materials and methods: Blood samples from 90 patients with suspected ACS were obtained at admission and 3h later. Concentrations of cTn were determined using five POC assays (AQT90 FLEX cTnI and cTnT; PATHFAST™ cTnI; Stratus CS 200 cTnI; Triage MeterPro cTnI) and two guideline-acceptable high-sensitivity (hs-) immunoassays.
Results: For the diagnosis of NSTEMI (n = 15), areas under the receiver operator characteristic curve (AUCs) for hs-cTnI and hs-cTnT were 0.86 (95% CI 0.75-0.96) and 0.88 (95% CI 0.80-0.95), respectively, at admission, and 0.96 and 0.94, respectively, 3h later. With the 99th percentile cut-off, their sensitivities were 62% and 92%, respectively, at admission, and 77% and 100%, respectively, 3h later. The PATHFAST™ cTnI assay showed AUCs of 0.90 (95% CI 0.82-0.97) and 0.94 (95% CI 0.89-1.00), respectively, and sensitivities of 67% and 75%, respectively. The other cTn POC assays had AUCs of 0.71 (95% CI 0.53-0.89) to -0.84 (95% CI 0.71-0.96) and 0.86 (95% CI 0.72-0.99) to- 0.87 (95% CI 0.75-0.99) and sensitivities of 39%-50% and 62%-77% at admission and 3h later, respectively.
Conclusion: Only PATHFAST™ cTnI assay proved itself equivalent to ESC-guideline acceptable hs-cTn assays. The lower sensitivity of the other POC assays limits their clinical utility and would requires longer follow-up monitoring of patients for the safe NSTEMI rule-out.

P020

Alle Anwender von Point-of-Care Tests bedarfsgerecht und effizient schulen

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Zielsetzung
Viele internationale Studien belegen, dass ungeschulte Anwender eine der Hauptursachen für fehlerhaft durchgeführte Point-of-Care Tests (POCT) sind. Die große Anzahl potentieller Anwender von POCT in vielen Gesundheitseinrichtungen, deren unterschiedliche Vorbildung und die oft hohe Fluktuation erschweren die Qualifikation aller Anwender. Deshalb wurde unter dem Dach des VDEs (Verband der Elektrotechnik Elektronik und Informationstechnik) eine Anwendungsregel erstellt, in der unter anderem Best Practice Beispiele zur Schulung professioneller Anwender zusammengestellt wurden.
Methode
Eine interdisziplinäre Expertenrunde - bestehend aus Anwender aus großen und kleinen Krankenhäusern, Hersteller von POCT-Tests, Juristen und IT-Experten - erarbeitete rechtliche, inhaltliche und organisatorische Handlungsempfehlungen für Schulungen von POCT. Die hohen Ansprüche des VDE Vorschriftenwerks boten hierfür den formalen Rahmen und garantierten eine hohe Qualität. Größtmögliche Partizipation wurde unter anderem mit einer öffentlichen Kommentierungsphase angestrebt.
Ergebnis
Um alle Anwender von POCT zu schulen, bieten sich vor allem zwei Konzepte an. Bei „train-the-trainer“ werden ausgewählte Mitarbeiter (z.B. POCT-Beauftragte) aus den Arbeitsbereichen weitergebildet, so dass diese dann wiederum als Multiplikatoren andere Mitarbeiter schulen können. Diese Experten stehen in ihrem Arbeitsbereich unmittelbar zur Verfügung, dienen als Vorbild und können so für eine größere Akzeptanz sorgen. Die geforderten Schulungsinhalte müssen den Multiplikatoren klar vermitteln werden, damit alle Anwender vollständig und korrekt geschult werden können.
Bei Schulungen durch E-Learning sollten die Inhalte vom Hersteller des POCT-Tests geliefert und durch den Betreiber auf die örtlichen Gegebenheiten angepasst werden. Dafür müssen Schulungsinhalte modular angelegt sein. Als elektronisches Format bietet sich SCORM (Sharable Content Object Reference Model) an. Innerhalb dieses Formats können wiederum verschiedene Techniken zum Einsatz kommen. Da IT-Systeme in medizinischen Einrichtungen häufig nicht alle Techniken unterstützen, sollte frühzeitig auf eine breite Akzeptanz geachtet werden (z.B.

HTML5 anstelle von Flash). Bei E-Learning in einer POCT-spezifischen Software können nach erfolgreicher Schulungsteilnahme die entsprechenden Geräte sofort freigeschaltet werden. Wenn die POCT-Schulungen auf einer Schulungsplattform zusammen mit anderen Schulungen angeboten werden, ist der Wartungsaufwand für die einzelne Schulung geringer, der Datenaustausch mit der POCT-Middleware wird aber komplexer.
Zusammenfassung
Die VDE-Anwendungsregel 2411-2-101 „Schulung professioneller Anwender von patientennahen Tests" stellt eine Handlungsempfehlung zur bedarfsgerechten und effizienten Durchführung von Schulungen dar. Die vollständige Anwendungsregel gibt weitere rechtliche, inhaltliche und organisatorische Hinweise und kann über den VDE-Verlag bezogen werden.

P021

Glucosemessungen in Ringversuchen in Deutschland

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Zielsetzung
Die Glucosekonzentration im Plasma ist bei der Diagnose und Therapie von Diabetes mellitus von entscheidender Bedeutung. Diese Messung kann im Zentrallabor oder als Point-of-Care Test (POCT) durchgeführt werden. Eine Analyse der entsprechenden Ringversuche liefert Rückschlüsse auf die Qualität der Glucose-Messungen in Deutschland.
Methode
Es wurden Daten der Ringversuche „Klinisch-chemische Analyte im Serum Nasschemie - KS" und „Glucose Trockenchemie - GL" vom RfB sowie „100: Klinische Chemie – Nass-chemie" und „800: Trockenchemie 01 - POCT: Glucose" von INSTAND der Jahre 2011 bis 2015 analysiert. Um die Berechnung gruppenspezifischer Konsenswerte zu vereinheitlichen, wurden diese Zielwerte mit einem robusten Huber M-Schätzer, eng verwandt mit dem „Algorithmus A" aus ISO 13528, neu bestimmt. Ringversuchsergebnisse wurden in Übereinstimmung mit Buvke (1) als „gut", „akzeptable", „schlecht" oder nach Rili-BÄK als „durchgefallen" klassifiziert. Für verschiedene Faktoren wurden Odds-Ratios für die Teilnahme mit einem „guten" Ergebnis berechnet.
Ergebnis
In den beiden Ringversuchen für Großgeräte RfB KS und INSTAND 100 wurden Referenzmethodenwerte verwendet. Im Gegensatz dazu mussten bei fehlender Kommutabilität des eingesetzten Materials in den POCT-Ringversuchen RfB GL und INSTAND 800 Konsenswerte als Zielwerte eingesetzt werden. Insgesamt wurden über 130.000 Proben in 4.437 unterschiedlichen Laboren vermessen.
Die entsprechenden Ringversuche beider Organisationen zeigten große Übereinstimmung. Die zentralen 95% aller Ergebnisse wichen weniger als 9,3% (Großgeräte) bzw. 16,7% (POCT) vom Zielwert ab. Die mittleren Variationskoeffizienten (mVK) der einzelnen Großgeräte lagen zwischen 0,020 und 0,032, der mVK der POCT-Geräten reichte von 0,022 bis 0,084. Faktoren, die die Wahrscheinlichkeit ein „gutes" Ergebnis im Ringversuch zu erzielen, erhöhten, waren Anzahl vorangegangener Ringversuche und ein „gutes" Ergebnis im vorherigen Ringversuch. Teilnehmer, die sowohl am POCT - als auch am Großgeräte - Ringversuch teilnahmen, hatten ein höheres Odds-Ratio ein „gutes" POCT-Ergebnis zu erzielen. Unterschiede zwischen POCT-Chargen waren in 13,7% größer als 5% des Zielwerts.
Zusammenfassung
In Ringversuchen messen Großgeräte die Glucosekonzentration präziser als POCT-Geräte. Allerdings sind POCT-Geräte heterogen und die Präzision der besten Geräte reicht an die Präzision der Großgeräte heran. Fehlende Kommutabilität erschwert die Auswertung von POCT-Ringversuchen, aber Ergebnisse von RfB und INSTAND stimmen weitgehend überein. Faktoren, die mit mehr Erfahrung assoziiert sind, erhöhen die Wahrscheinlichkeit guter Ergebnisse. Ringversuchen fördern Standardisierung, deren Analyse kann Verbesserungspotentiale aufzeigen.
Literatur
(1) Bukve, T., Stavelin, A., & Sandberg, S. (2016). Effect of participating in a quality improvement system over time for point-of-care C-reactive protein, glucose, and hemoglobin testing. Clinical Chemistry, 62(11), 1474-1481.

P022

Evaluation of discrepancies between sodium values obtained with direct and indirect ion-selective electrodes at the emergency department of the University Hospital Bonn

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Introduction
Sodium is one of the most frequently asked analytes by the emergency department (ED). It can be measured by direct or indirect ion-selective electrodes (ISE). Clinician's knowledge about different methods and the so-called ion exclusion effect is sparse. Former studies

revealed problematic discrepancies regarding sodium values obtained with Point of care testing (POCT) compared with central laboratory (CL) analyzers.
Aim of the study
Comparison of sodium values between POCT and central laboratory analyzers in a wide collective of patients presented at our ED. Are there significant differences in sodium values obtained with direct or indirect ISE and has this an important impact for clinicians regarding diagnosis of hyper-/hyponatremia at the ED?
Materials and Methods
In n = 1942 patients sodium values were established with POCT blood gas analyzers (Rapidlab 1265™, Siemens Healthineers, Eschborn, Germany) and in parallel with central laboratory analyzers (Dimension VISTA1500™, Siemens Healthineers). Differences between values were calculated. Outliers were evaluated according to reference ranges and alarm ranges. Data were statistically analyzed with correlation analysis, Bland-Altman plot and outlier evaluation.
Results
Average age of the patients was 55.7 years (5-102 y.). Male-to-female ratio was 1149 to 792. Mean value of the absolute difference between POCT-sodium and Cl-sodium was 0.9 mmol/l (0-21). POCT-sodium mean value was 137.9 mmol/l (SD 4.7) and CL-sodium mean value was 139.6 mmol/l (SD 4.2). POCT-sodium and CL-sodium correlated significantly ($r = 0.81$, $p < 0.0001$). Bland-Altman´s mean difference of the measurement values was 1.6 (limits of agreement -3.8 to +7.1). N = 433 patients had sodium values outside the reference ranges (135-145 mmol/l). In 197 of these patients both values resulted in the same diagnosis. McNemar´s test revealed significant differences ($p < 0.0001$) when comparing both values. CL-Na resulted significantly more often in hyper- or hyponatremia diagnosis. N = 9 patients had values outside alarm ranges (160 mmol/l) while in 5 of these cases also both values resulted in the same diagnosis. No significant differences were found.
Conclusion
We could show that in our collective >85% of the patients had no clinically relevant discrepancies regarding their sodium values. Today laboratory methods have been improved and laws have been steadily tightened to improve quality of sodium measurements.

P023

Increasing patient safety by introduction of electronic bedside assignment of patient identity to blood gas samples

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Background
Assignment of patient identity to samples in point of care testing (POCT) often differs from the processes used for blood samples send to central laboratories. The circumstances under which POCT blood gas samples are taken may vary due to the setting e.g. in intensive care units (ICU). These POCT results are fundamental for immediate therapeutic interventions. A correct and reliable assignment of patient identity to the POCT sample becomes especially crucial for patient safety.
Methods
On two ICUs at the University Medicine Greifswald, we introduced an electronic system, which enables beside assignment of patient identity to POCT samples.
Each patient bed of the ICUs is equipped with bedside monitors which include an integrated barcode reader. These bedside monitors display the ICU's patient data management system, which provides detailed information for patient care and is the established regular work environment for ICU staff. Within this system the assignment of patient identity to POCT samples was realized using a web application (Radiometer FlexLink) in combination with blood gas tubes pre-labeled with unique barcodes (Radiometer safePICO).
The overall workflow for electronic bedside assignment differs in several steps from the one used beforehand. ICU staff therefore received a comprehensive training for the new process. During the implementation period we monitored the number of samples which were analyzed using the old process vs. those analyzed using the new bedside assignment process.
Usability of the tubes as well as the web application was assessed using a one page questionnaire with numeric ratings and open questions regarding problems, advantages and disadvantages of the new products and possibilities for further improvement.
Results
The introduction on the first ICU showed continuous rise in adoption rate. In week 16 the adoption rate reached 75% for the first time. In the following year the average rate was 73% (min 60%; max 82%). On the second ICU, introduction took place one year later, 75% where reached in week 3 after introduction. Within the following six month the average rate was 78% (min 66%; max 86%).
Usability of bedside assignment was rated as very easy or easy by 83% of the respondents (n = 41). Sample taking with the pre-labeled tubes was rated as very easy or easy by 98% of the respondents.
23 ICU staff members reported sporadic problems with software or hardware within the initial bedside steps of the process. 12 respondents regarded reduction in patient mix ups as an advantage of the new process. The amount of time required was perceived divergent by the respondents with 7 reporting it as an advantage while 9 reported it as a disadvantage. For urgent samples the improved process was viewed as too slow.
Discussion
The introduction of the new workflow required intensive training and several weeks of accustomization. Further improvement of the software could facilitate the bedside assignment and reduce the time needed.

Infektionskrankheitserreger / TDM

P024

Increased biofilm formation of Streptococcus gallolyticus subsp. gallolyticus through lysozyme treatment

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Introduction: Streptococcus gallolyticus subsp. gallolyticus (SGG) is recognized as a pathogen in about 20% of streptococcal-caused infective endocarditis cases. Colonization and biofilm formation at the endocardium is an important virulence factor for this bacterium to survive human defend mechanisms. For a better understanding of the mechanisms of lysozyme resistance and biofilm formation of SGG, the transcriptome in presence of lysozyme was analyzed in this study.
Methods: Adhesion of five SGG strains to polystyrene in presence of lysozyme (10 mg/ml and 20 mg/ml) was verified by crystal violet staining after 5 and 16 hours of incubation. For transcriptome analysis, RNA was extracted from two SGG strains in BHI medium with or without 10 mg/ml lysozyme after five hours of incubation to examine early reaction to lysozyme. The RNA was processed, Cyanine3 labeled and hybridized to microarrays. Based on four SGG genomes, the full genome microarray for SGG analysis was developed (Oaklabs, Hennigsdorf). It consists of 10,607 oligonucleotides which represent 4,382 genes. Analysis was done by Direct Array and log2 values were only considered when higher than +1 or smaller than -1 with a p-value smaller than 0.05. Microarray results were verified with relative quantitative real-time PCR.
Results: The biofilm formation of SGG is strain-dependent. The strain DSM 16831 formed more biofilm at polystyrene compared to the other strains after 16 hours of incubation. It was also shown, that lysozyme leads to significantly more biofilm formation in two (BAA-2069 and LMG 17956) of five strains within five hours and of four strains within 16 hours of incubation. The quantity of the biofilm formation of the strain DSM 16831 decreased with increased lysozyme resistance after five and 16 hours of incubation compared to control without lysozyme. Lysozyme resistance and biofilm formation of the SGG isolates BAA-2069 and UCN 34 was examined by transcriptome analysis with full genome microarrays. It was shown that gene expression of the dlt operon was upregulated in presence of lysozyme as well as genes whose products are involved in transcription and translation, DNA repair and hydrogen peroxide resistance. The gene expression of the microcin immunity protein (mccF) and competence induced protein A (cinA) which belong to bacterial immunity were also increased. Expression of genes which belong to bacterial competence, acid tolerance and antibiotic resistance were decreased.
Conclusion: D-alanylation (dlt operon) is increased in presence of lysozyme which leads to resistance to the cationic microbial peptide activity of this enzyme. Additionally, this study showed for the first time that lysozyme triggers biofilm formation in a bacterial species which could be a benefit for the pathogenesis of SGG. The proteins CinA and MccF in SGG whose expression is induced in presence of lysozyme may lead to this rapid biofilm formation.

P025

Ceftolozane/Tazobactam Suceptibility Testing in Extended-spectrum Betalactamase and Carbapenemase Producing Gram-negative Bacteria of Various Clonal Lineages

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Objectives: The rapid emergence of multidrug-resistant bacteria is occurring worldwide and is endangering the efficacy of different antimicrobial agents. Many decades after the first patients were treated with “older” antimicrobials, bacterial infections have again become a matter of great clinical concern. For this purpose, global surveillance data are crucial for empiric therapy decision. The novel antimicrobial agent ceftolozane/tazobactam was approved as a therapy option for complicated urinary tract and intra-abdominal infections. Therefore, we aimed to study the in-vitro activity of ceftolozane/tazobactam against extended-spectrum beta-lactamase (ESBL) and carbapenemase producing Gram-negative bacteria of different origins.
Methods: We performed susceptibility testing on 146 ESBL (107 Escherichia coli and 39 Klebsiella pneumoniae) and 104 different carbapenemase producing Gram-negative bacteria via Etest according to the EUCAST guidelines.
Results: Of the ESBL producing isolates tested, 91 Escherichia coli isolates (85%) and 22 Klebsiella pneumoniae isolates (56.4%) were susceptible towards ceftolozane/tazobactam. Among the 104 carbapenemase producing isolates 102 (98.1%) were reported resistant towards ceftolozane/tazobactam. Only two isolates among these, Escherichia coli and Klebsiella pneumoniae, were susceptible towards ceftolozane/tazobactam. Surprisingly, both were NDM-1/-6 producing isolates.
Conclusion: Ceftolozane/tazobactam should be included in antimicrobial susceptibility testing in order to administer this antimicrobial combination in infections caused by Gram-negative bacteria. This antimicrobial agent is another therapy option for ESBL-associated infections if susceptibility is confirmed.

P026

Temocillin Suceptibility of Extended-spectrum Betalactamase Producing Gram-negative Bacteria of Different Clonal Lineages

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Objectives: The dissemination of multidrug-resistant Gram-negative bacteria, e.g. Extended-Spectrum Beta-Lactamase (ESBL) and carbapnemase-producing patho-gens, has been reported worldwide. Along this occurrence antimicrobial therapy options are very limited. We studied the in-vitro susceptibility of ESBL Escherichia coli and Klebsiella pneumoniae isolates towards temocillin.
Methods: In total 90 E. coli and 44 K. pneumoniae isolates were investigated by use of agar disk diffusion test. The bacteria were isolated from different clinical specimens, but mainly from urine samples of hospitalized and non-hospitalized patients.
EUCAST breakpoints were used to interpret the activity of temocillin.
Results: Relatively high in-vitro susceptibility towards temocillin was observed for the ESBL-producing E. coli isolates (88%) whereas the temicillin susceptibility rate was 95% for the ESBL producing K. pneumoniae isolates. The virulent E. coli clone ST131 was accounted for the one third of the temicillin-susceptible isolates. Resistance towards fluorochinolones (82%) and gentamycin (33%) was recorded. 35% of the temocillin susceptible K. pneumoniae isolates were determined as clone ST16. Among these the resistances towards fluorochinolones (30%) and gentamycin (65%) were detected.
Conclusion: The data support that temocillin is another therapy option besides the carbapenems in infections caused by predominantly disseminating ESBL clones of E.coli and K. pneumoniae.

P027

Potential role of IgM antibodies in the laboratory assessment of hepatitis C virus infection

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The variability in the course and prognosis of hepatitis C virus (HCV) infection including acute and chronic disease, spontaneous healing, cure, insufficient response or relapse after antiviral therapy, asymptomatic carriage and liver cirrhosis requires a differentiated diagnostic approach. The major diagnostic tools are HCV IgG antibody assays, HCV antigen and molecular detection of HCV RNA followed by genotyping, biochemical parameters of liver function and liver biopsy. Although several studies suggest a potential diagnostic value of HCV IgM antibodies, HCV IgM antibody assays are not commonly used and no standardized test is commercially available. Using the well-established Microgen recomline® HCV IgG line assay with an appropriate conjugate for detection of HCV IgM antibodies, we found 32 out of 126 HCV IgG positive human serum samples being HCV IgM positive as well in our routine diagnostic approach, 30/126 were equivocal. While the IgM-negative samples were positive for HCV RNA PCR only in 24/64 (37.5%), IgM positive samples were PCR positive too in 24/32 (75.0%), IgM equivocals in 22/30 (73.3%). The potential role of HCV IgM antibody detection in the laboratory assessment of different stages of the disease as a marker of immunologic activity of the host against HCV, thus supplementing the direct markers of viral amplification, HCV antigen and RNA, should be examined in further studies.

P028

Evaluation des HB&L Uroquattro im mikrobiologischen Labor der Oberlausitz-Kliniken gGmbH

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Zielstellung: Der HB&L Uroquattro der Greiner Bio-one GmbH ermöglicht eine Beschleunigung der mikrobiologischen Analytik in Urinproben durch eine schnelle unspezifische Untersuchung auf Keimwachstum in Flüssigmedien. Ziel war der Vergleich der Sensitivität des Uroquattro mit dem Standard der mikrobiologischen Kultur.
Methode: Nativurin (0,5ml) bzw. boratstabilisierter Urin (0,2ml) wurden am Uroquattro inkubiert, und parallel auf mikrobiol. Nährböden (Blutagar, CNA, Mac-Conkey) ausgestrichen. Am Uroquattro wurden verschiedene Cut-Offs (zwischen dem Detektionsminimum 50 KBE/ml und 10^5 KBE/ml) getestet und ein Methodenvergleich zum Plattentest (Standard) vorgenommen.
Ergebnis: Bei einer Nachweisgrenze von 10^5 KBE/ml wurde eine Übereinstimmung von 90,8% für Nativurin und 90,4% für Boraturin erreicht. Die Sensitivität lag im Nativurin bei einem Cut-Off von 10^5 KBE/ml bei 97,1%. Bei niedrigeren Cut-Offs stieg die Zahl different negativer

Ergebnisse am Uroquattro, bei einem Cut-Off von 10^3 KBE/ml sank die Sensitivität auf 65,7%. Bei der Keimidentifikation konnten 55,4% der different negativen Ergebnisse auf Kontaminationen bei der Probenahme zurückgeführt werden.
Tabelle 1: Übersicht Nativurin
cut-off >10^3KBE/ml >10^4 KBE/ml >10^5 KBE/ml
richtig positiv 88 [28,85%] 59 [19,34%] 34 [11,15%]
richtig negativ 167 [54,75%] 214 [70,16%] 243 [79,67%]
different positiv 4 [1,31%] 11 [3,61%] 27 [8,85%]
different negativ 46 [15,08%] 21 [6,89%] 1 [0,33%]
Tabelle 2: Übersicht Boraturin
cut-off >10^3 KBE/ml >10^4 KBE/ml >10^5 KBE/ml
richtig positiv 68 [22,59%] 61 [20,27%] 37 [12,29%]
richtig negativ 178 [59,14%] 210 [69,77%] 235 [78,07%]
different positiv 3 [1%] 4 [1,33%] 15 [4,98%]
different negativ 52 [17,28%] 26 [8,64%] 14 [4,65%]
Zusammenfassung: Bei einem hohen Cut-Off von 10^5 KBE/ml ergab sich eine 90%-ige Übereinstimmung mit dem mikrobiologischen Ausstrich, die bei niedrigeren Cut-Offs schlechter ausfiel. Insbesondere die Zahl falsch negativer Resultate stieg bei geringeren Cut-Off-Werten.

P029

Comparison of Multiplex Real-Time PCR and Conventional ELISAs for the Diagnosis of Clostridium difficile Infections

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Clostridium difficile infection (CDI) is the most common cause of nosocomial and antibiotic - associated diarrhea. The incidence of CDI in Germany is 5 to 20 cases per 100 000 inhabitants (Lübbert L. et al., 2014). In recent years, a steady increase in severe CDI has been observed; at the same time, the highly virulent epidemic strain ribotype 027 has spread almost everywhere in Germany. The virulence factors enterotoxin A and cytotoxin B lead to a cytotoxic damage of the intestinal cells and thus to diarrhea and colitis. Pathogenic strains usually produce both toxins. Some strains (e.g. ribotype 027) additionally produce the binary toxin CDT which is associated with a higher mortality rate. Strains that cannot form toxins are considered to be apathogenic (Kyne L. et al., 2000; Stewart D, et al., 2013).
For the study, 100 glutamate dehydrogenase (GDH) negative and 400 GDH positive stool samples (Bristol stool scale 6 and 7, using conventional ELISA, Fa. Biopharm) were collected. Patients who had already been treated for CDI and samples without an exact collection date were excluded. The turnaround time (TAT), the test properties and the costs of the currently used enzyme immunoassay were compared to a multiplex real-time PCR method (Fa. Cepheid). CDI-specific GDH was analyzed for a high test sensitivity. In all positive samples, another ELISA for the detection of CDI toxins A and B is carried out to increase specificity. In all discrepant results, the toxin test was repeated using the cultures of CD strains cultivated from faeces. The multiplex PCR was used for testing for the presence of toxins A and B and a deletion in the tcdc gene associated with higher toxin levels (as typical for ribotype 027).
The sensitivity of the multiplex PCR based assay was 99.1%, the specificity was 98.7%, compared to the conventional procedure. The multiplex PCR recognized some more CDI cases (due to CD strains not growing in culture), particularly in discrepant GDH/toxin results (GDH test positive, toxin test negative). The TAT shortened from approximately 40 hours to 15 hours (with the samples transported to the central laboratory) or to even <5 hours with on-site testing. The estimated cost for the classic procedure, for PCR testing all samples, for PCR testing of discrepant cases or GDH positive cases only was 6,55€, 27,10€, 9,03€ and 10,07€, respectively. Compared to the ELISA procedure multiplex PCR provides additional information on ribotype 027.
In conclusion, the overall quality of the multiplex PCR was comparable to the conventional test procedure, with a slightly increased number of positives, but the TAT shortened considerably. The costs are not significantly higher provided that the multiplex PCR is used for selected samples only (GDH positive cases or all discrepant cases). The optimum results (lowest TAT and presumably best sensitivity) can only be achieved with significantly higher costs and examination of all samples on-site.

P030

Drogentestung im Speichel statt im Urin in der Opiat-Substitutionstherapie: Ergebnisse einer retrospektiven Auswertung

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Methadon und Buprenorphin sind verbreitete Substitutionstherapeutika. Zur Einnahmekontrolle werden diese traditionell qualitativ oder quantitativ im Urin bestimmt. Neuerdings ist auch eine Therapiekontrolle im Speichel möglich.
Es soll überprüft werden, ob ein Drogenscreening im Speichel das Drogenscreening im Urin im Rahmen der Substitutionstherapie ersetzten kann.
Eine Besonderheit von Speichel ist, im Gegensatz zu Urin, dass die Konzentrationen mit den Blutkonzentrationen korrelieren. Daher korrelieren auch Speichel-Konzentrationen mit der pharmakologischen Wirkung. Bei oraler Aufnahme ist zu berücksichtigen, dass u.U. Rückstände im Mundraum zum Ergebnis beitragen, weshalb die Zeitspanne seit der letzten Einnahme ggf. zu berücksichtigen ist.
Bei mit Methadon bzw. Buprenorphin behandelten Patienten wurden die Konzentration des Medikamentes und dessen Hauptmetaboliten quantitativ im Speichel und im Urin bestimmt (Methode: LC-MS/MS). Zu 50 Proben-Paaren waren die Art der Medikation, die verordnete Dosis und die Zeitspanne seit der letzten oralen Einnahme bekannt. Zur Prüfung auf einen möglichen Beigebrauch wurde zusätzlich auf Opiate, Opioide, Kokain, Amphetamine, Benzodiazepine und Z-Drugs untersucht. In einer retrospektiven statistischen Auswertung wurden die gemessenen Konzentrationen auf Abhängigkeiten zu Medikations-Daten untersucht.
Die Urinkonzentrationen sind nur schwach mit der Dosis und nur sehr schwach mit den Speichel-Konzentrationen assoziiert, möglicherweise auch dadurch, dass die Urinproben ohne Sichtkontrolle gewonnen wurden (Probenmanipulationen des Urins möglich). Im Speichel findet sich eine deutliche Dosis-Abhängigkeit der Konzentrationen. Zudem ist das Konzentrations-Verhältnis von Gabeform zu Metabolit im Speichel - wie auch im Urin - eine wichtige diagnostische Information. In mehreren Fällen wurde anhand der Speichel-Ergebnisse ein heimliches Absetzen bzw. Abdosieren der Medikation aufgedeckt. Möglich war dies durch eine sensitive und zugleich spezifische Konzentrations-Bestimmung, wie sie derzeit im Speichel ausschließlich mit chromatographischen Methoden wie der LC-MS/MS möglich ist. Immunoassays können Gabeform und Metabolite nicht unterscheiden und haben zu hohe Cut-Offs, d.h. besitzen eine zu geringe Selektivität und Sensitivität.
Speichel ist ein gut geeignetes Untersuchungsmaterial zur Einnahme-kontrolle bzw. Compliance-Überprüfung bei Methadon- und Buprenorphin-Therapie und kann die Urin-Testung ersetzen. Die Ergebnisse belegen eine (Nicht-)Einnahme und lassen auch Schlüsse auf die Therapietreue zu, insbesondere wenn die verordnete Dosis und die Zeit seit der letzten oralen Einnahme vorliegen. Auch wenn diese Informationen nicht bekannt sind, sind die Ergebnisse aufgrund des Verhältnisses von Gabeform zu Metabolit zur Therapiesteuerung geeignet. Dazu kommt der Vorteil von Speichel bei der Probennahme, die schnell, nicht-invasiv und unter Sicht aber ohne Entblößung wie bei Urin - durchgeführt wird.

P031

Therapeutic Drug Monitoring of Opioids with LC-MS/MS – Capabilities and Benefits

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Background:
Opioids are prescription drugs used for treating severe pain. A huge problem is misuse and abuse, especially in cases of non-cancer pains. In 2000 to 2010 the records of first prescriptions in Germany increased from 3.3% to 4.5% (1). According to an US-American meta-analysis the rate of addiction is between 8-12% (2). There are efforts to train particularly primary care physicians in indication and risks of opioid therapy (3). For attending physicians it is not always easy to detect addiction and providing the optimal therapy because of unspecific symptoms, non-compliant patients and unknowingness of the existing drug use. Besides questionnaires-based screening, screening of the acute drug level in blood for a compliance control is helping to identify addiction. With our method it is possible to check 12 opioids and their metabolites in one screening with LC-MS/MS.
Method:
Samples were analyzed by a LC-MS/MS system consisting of a Shimadzu AC 20 HPLC with a SCIEX Triple Quad 5500 MS-detector. Measurement results were evaluated by the SCIEX software MultiQuant.
Results:
With our method it is possible to check 12 opioids (Morphin, Codein, Dihydrocodein, Tramadol, o-Desmethyltramadol, Oxycodon, Methadon, Buprenorphin, Fentanyl, Tilidin, Naltrexon, 6-β-Naltrexol) and their metabolites in one screening with LC-MS/MS.
Conclusion:
In our laboratory it is possible to analyze 12 opioids and their metabolites with one chromatographic run. For all analytes the limit of quantification is lower than the therapeutic range. In cases of positive results not only qualification also quantification is possible. Especially during a treatment with opioids, we recommend regular check-ups of the through level.
For primary care physicians of patients with a suspicion for addiction analyzing blood samples is a tool to estimate the risk and support the therapy. Furthermore this information can help to reduce mortality because of overdose or combined drug intoxication.

P032

Measurement of apixaban, dabigatran, edoxaban and rivaroxaban in human plasma using automated online solid-phase extraction combined with ultra-performance

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Objective: Measurement of direct oral anticoagulants (DOACs) concentration in patient blood is essential in special clinical circumstances. Therefore, a fast, precise, and direct measurement of DOACs such as apixaban, dabigatran, edoxaban, and rivaroxaban in patients' plasma should be developed.
Method: We developed a fast, selective and sensitive method for simultaneous measurement of apixaban, dabigatran, edoxaban, and rivaroxaban in human plasma consisting of protein precipitation with methanol containing internal standards (IS) followed by an automated online solid-phase extraction method coupled with ultra-performance liquid chromatography electrospray ionization-tandem mass spectrometry (online SPE-UPLC-MS/MS). Run time was 4.0 minutes per injection.
Results: The calibration curves of all DOACs were linear over the working range (apixaban: 0.25 - 760 µg/L, r > 0.99; dabigatran: 0.5 - 1800 µg/L, r > 0.99; edoxaban: 0.6 - 800 µg/L, r > 0.99; rivaroxaban: 0.5 – 920 µg/L, r > 0.99). Limits of detection (LOD) in the plasma matrix were < 0.2 µg/L, whereas the limits of quantification (LLOQ) were < 0.6 µg/L for all DOACs measured in the assay. The intraassay and interassay coefficient of variation (CV) for all DOACs were < 6% for clinically relevant concentration range. Mean recoveries were between 61.4% and 91.6% for all DOACs. There were no significant ion suppression or ion enhancements detected at the elution times of apixaban and rivaroxaban, whereas weak ion suppression at the elution time of dabigatran and appreciably ion suppression at the elution time of edoxaban could not be prevented. However, deuterium- and 13C-labeled IS were used to compensate these matrix effects. Apixaban, dabigatran, as well as rivaroxaban were stable in citrate plasma for at least 1 week at -20°C (after thawing), 4°C, RT, which was ascertained with systematic testing over a period of 1 month. Edoxaban, however, was less stable, showing a mean deterioration of about 10% per day when stored at RT, and about 2% per day when stored at 4°C. Repeated freezing and thawing of all four DOACs was uncritical.
Conclusion: We successfully developed and validated an online SPE-UPLC-MS/MS method for fast, sensitive, and specific measurement of the new generation of oral anticoagulants such as apixaban, dabigatran, edoxaban, and rivaroxaban.

P033

TDM as a tool for individualization of pharmacotherapy in severely ill patients, a clinical pharmacological perspective

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Background
Therapeutic Drug Monitoring (TDM) is a diagnostic standard for the individualization of polypharmacotherapy based on validated analytical methods (in particular LC-MS/MS and HPLC-methods) in order to optimize dosing and drug safety. It serves as a pharmacovigilance issue and is based on drug-level control in biological matrices. Factors influencing drug-levels of drugs (e.g.: anti-infectives, psychotropic and anticonvulsant drugs, in blood and tissue in severely ill patients are diverse. For instance, comorbidity, physiological (and age-dependant-) changes, various concomitant drugs, microcirculation, infusion therapy, the "capillary leak syndrome", augmented renal clearance (ARC) and inflammatory processes in the critically ill patient impact on drug-concentration in the different compartments.
Methods
The role of TDM in general and in an intensive care setting (ICU) will be explained on the basis of own studies. An overview of recent publications along with clinical-pharmacological aspects of polypharmacotherapy in ICU will be presented.
Underlying mechanisms of altered pharmacokinetics of drugs (and anti-infectives in particular) in critically ill patients exemplified by a case series of five different etiologies will be demonstrated.
Results/Conclusions
Several factors should be taken into account for calculation of relevant pharmacokinetic parameters: elimination half-life (T1/2), bioavailability (F), volume of distribution (Vd) and clearance (Cl) to deduce a recommendation for dosage in critically ill patients. Specifically we report on potential alterations in Vd, T1/2 and the role of end-organ-dysfunction and inflammatory processes. In this context TDM is taking on a pivotal role to improve outcome from severe disease in critically ill patients.

P034

Quantification of 31 Antidepressants in human serum by using a High Resolution Orbitrap Mass Spectrometer

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Background: Therapeutic drug monitoring (TDM) of antidepressants, which are widely used, is important to ensure compliance and to rule out pharmacokinetic abnormalities such as rapid metabolizer genotype or drug interactions. Therefore, reliable methods for quantification of antidepressants are important in the clinical routine. While most of the currently used mass spectrometry methods use triple quadrupoles as mass analyser, we developed a method for 31 antidepressants using a high resolution Orbitrap mass spectrometer.
Methods: After manual protein precipitation of serum samples, further sample clean-up was ensured by a Turbo Flow column preconnected to the analytical LC column. Stable isotope-labelled counterparts of the target analytes were used as internal standards to ensure reliable quantification. For detection we used a Q Exactive Focus Orbitrap mass spectrometer operating in full-scan mode. Ionization was performed in positive ESI.
Results: Precision (0.58 % - 7.5 %), accuracy (86.9 % - 109 %), recovery (88.9 % - 92.5 %), and matrix effect (85.8 % - 108 %) were acceptable for all analytes. The selectivity of the method was ensured by chromatographic separation of all isobaric compounds. Close agreement between Orbitrap and triple stage based quantification was observed in large series of leftover diagnostic samples.
Conclusions: We were able to establish a selective and sensitive method for the quantification of 31 antidepressants in human serum using a high resolution Orbitrap mass spectrometer applicable for TDM of antidepressants.

P035

24/7 β-lactam LCMS analysis

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24/7 β-lactam LCMS analysis
Aim:
The aim of the present project was the technical and analytical evaluation of a novel combined preanalytical concept consisting of an automated sample preparation module (CLAM-2000, Shimadzu) and an integrated LCMS system (NexeraX2 LCMS-8050, Shimadzu). Our additional intention was to provide proof of principle with respect to the applicability of the concept for 24-hour supply of LCMS-dependent emergency analyses.
"Hit hard and early", the former principle of HIV therapy, is still true for antibacterial treatment. For that purpose we chose the implementation of piperacillin and meropenem concentration determination to ensure personalized efficacious treatment in critically ill febrile patients. LCMS instruments in comparison with automated clinical chemical analyzers need highly skilled personnel capable of handling preanalytical sample preparation and the sophisticated LCMS systems. For these reasons, LCMS methods are currently not suitable for 24/7 emergency parameters. Only recently there is an easy-to-use automated sample preparation module available connected to an LCMS system with a familiar user interface (Shimadzu) resembling software modules of common clinical chemical analyzers waiving trained person-nel.
Method:
Piperacillin and meropenem, measured by our routinely established manually processed method on the LCMS system Xevo TQS (Waters), were compared with the automated CLAM-2000 system.
Using the CLAM method, a deuterated internal standard was added to the sample, protein precipitation with acetonitrile was performed. Afeter a filtration step the filtrate injected into the LCMS system (NexeraX2 LCMS-8050, Shimadzu). For chromatographic separation a sharp linear gradient on a BEH Shield column (Waters) at 45°C was used.
Results:
Precision data of quality control samples of meropenem as well as piperacillin was below 5%, which fulfills the criteria of EMA, 2011. Method correlation was performed using routine patient samples treated with piperacillin (n = 36) or meropenem (n = 20). The method correlation between CLAM-2000 LCMS-8050 system and our established routine LCMS was excellent (r = 0.973 for piperacillin and r = 0.981 for meropenem).
Linearity of the novel method was given over a working range between 0.5 -150 mg/l. The calibration regression curves for both β-lactam antibiotics were y = 1.0202 x and the correlation coefficient was R2 = 0.9999. Stability of the internal standard on the instrument determined by its area under the curve within one week was below 10% CV. The within run precision of patient samples on the instrument was less than 5% CV. All results demonstrated the robustness and routine capability of this device.

Conclusion:
With respect to the novel, innovative approach, method comparison, precision and stability data for piperacillin and meropenem were fully convincing. The new system allows a 24/7 availability of LCMS emergency parameters.

Molekulare Diagnostik / Kardiologische Erkrankungen

P036

Minimal Difference at the 99th percentile of cardiac troponin assays

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Background:
Cardiac troponin (cTn) is considered the most sensitive biomarker for the detection of ischemia related myocardial necrosis (1). In recent years, analytical sensitivity of cTn assays was considerably improved. In Germany the guideline of the German Medical Association forms the legal basis on quality assurance in medical laboratory examinations (Rili-BAEK) (2). However, improved assay sensitivity is not yet translated into adjusted applicable concentration intervals for the permissive relative deviation values leading to the legal validity of the information provided by the manufacturer. Manufacturers typically report assay specific diagnostic cut-offs combined with the coefficient of variation expressed in percent (%CV). To alleviate the interpretation of the assay result, absolute values may be more appropriate instead of imprecision expressed in percent. The minimal difference (MD) is defined as the smallest interval between a measurement and the cut-off at which a measured value is truly different from the cut-off with 95% confidence.
Objective
Evaluation of the analytical discrimination ability of the currently available cTn assays using the MD.
Methods
Data as provided by the manufacturers (3) were used to calculate the MD at the cut-off for the diagnosis according to the formula $MD = k \times \sqrt{(1 \times SD^2)}$ where k = 2 corresponds to a confidence interval of 95%. Data for 16 assays were included.
Results
The individual MD at the 99th percentile diagnostic cut-offs of cTn assays are presented. E.g. a contemporary sensitive cTnI assay from Abbott with a cut-off of 28 ng/L and a %CV of 14% has a MD of 7.84 ng/L. Thus, a measured cTnI is truly above this cut-off if it is > 35.84 ng/L and it is truly below the cut-off if it is < 20.16 ng/L. Assays for the point-of-care are not necessarily characterized by a low analytical discrimination ability as some assays have a MD as low as 2.08 – 5.66 ng/L.
Discussion
This report provides a comprehensive overview of the analytical discrimination ability of cTn assays. As the MD is expressed as a concentration similar to the diagnostic cut-off, the limitations of the %CV are overcome. Thereby, it constitutes a resource for the save interpretation of cTn results and provides a valuable measure of assay quality.
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P037

ANTIBODY TITER AND NON-INVASIVE PRENATAL BLOOD GROUP TYPING IN 419 PREGNANCIES WITH ALLOANTIBODIES

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Introduction: Fetal red blood cell (RBC) antigens are relevant in the pathologic involvement of maternal alloantibodies causing hemolytic disease of the fetus (HDF) or newborn (HDN). Diagnostic procedures include the determination of the fetal blood group from maternal plasma or amniocentic fluid and maternal antibody screening. Here, we present data from 419 pregnancies with alloantibodies.
Methods: Samples from 419 pregnancies with alloantibodies (<20th week of gestation: n = 230; 21th to 30th: n = 143; ≥ 30th: n = 46) were tested. Screening for antibodies was done according to standard procedures and in cases of positive results titration of the antibody was performed.

DNA from plasma samples was checked for the presences of blood group sequences with real-time PCR (RHD and KEL1) or SSP-PCR with fluorescent primer pairs (RHC, RHc, RHE, MNS, JK, FY) and the GeneScan method.
Results: Detection of anti-D antibodies was positive in 222 samples (53%), followed by anti-K (n = 55), anti-E (n = 51) and anti-c (n = 38). The antibody-specifity of the remaining samples (n = 53) was heterogeneous (anti-C, anti-Fy, anti-Jk, anti-M, anti-s). Titers for anti-D antibodies were up to >65000, while anti-c antibodies showed significantly lower values. Out of the pregnancies with anti-D antibodies, in 93 samples a second or third antibody was present. Additional antibodies were found in 42% of samples with an initial anti-c, developing further antibodies during pregnancy. However, anti-K was detected in 94% as single immunisation event. In about 95% of samples, fetal blood group typing showed the expected polymorphism and was in concordance with the detected maternal antibody.
Conclusion: Identification and monitoring of alloantibodies combined with non-invasive blood group genotyping offers a useful diagnostic tool in pregnancies at risk. The main benefit of non-invasive prenatal diagnosis is to avoid amniocentesis, which can include a subsequent minimal bleeding and/ or the risk to booster an antibody. Besides this, our results demonstrate a relatively high number of pregnancies with a second or third antibody.

P038

ANTI-K ANTIBODIES IN A PREGNANCY AFTER ARTIFICIAL INSEMINATION: A CASE REPORT

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Introduction: Determination of HDN-relevant fetal blood groups in amniocentic fluid is a routinely used method while examination of maternal plasma is increasingly utilized as an additional diagnostic tool. Next to RHD, typing of fetal KEL1 is most common in non-invasive prenatal genotyping. In this report we present a case where interpretation solely based on the diagnostic results can lead to a wrong interpretation.
Case report: External samples of a 43-year old pregnant mother (week of gestation: 18) were sent to our institute. In a first serologic test for irregular antibodies an anti-K antibody (titer 1024) was detected. Clinical signs of HDN were not found at this stage. In addition, serological typing of the putative father was mentioned and showed a K-k+ phenotype. On request we received the following additional data: In the first successful pregnancy the woman gave birth to female twins, one being k+k+ phenotype, the other K+k+. At time of birth an anti-K was not detected. Transfusions had not been applicated in the past, as a means of immunization.
After intense discussion with the obstetricians it came out that the woman had undergone artificial insemination with an egg-donor from abroad in the actual and previous pregnancy. According to the national regulations for egg-donations in that country, typing of KEL1 was not requested. Since cell samples from the egg-donor female were not available, samples from the 3 year old twins were examined for the presence of KEL 1.
Methods: Maternal plasma was prepared according to Lo et al. and extracted DNA was examined for KEL1 specific sequences with real-time PCR. Genomic DNA had been genotyped with SSP-PCR (Ready Gene KDK Kit, InnoTrain, Germany).
Results and Conclusion: KEL1 positive results were obtained in real-time PCR with ffDNA. Parallel KEL1 genotyping with DNA samples from the twins showed a positive results for one child, while the other was KEL1 negative, suggesting that the egg-donor inherits a K+k+ phenotype.
Prenatal genotyping of HDN-relevant fetal blood groups is a useful tool in pregnancies with antibodies already present in maternal serum from a previous pregnancy but can lead to a wrong interpretation when the corresponding gene that leads to immunization, does not come as usually from the father, but from another genomic mother as given in this case.

P039

A RETROSPECTIVE ANALYSIS OF TEN YEARS BLOOD GROUP GENOTYPING IN NORTHERN GERMANY

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Introduction: The selection of methods for molecular typing is influenced by the indications and by the alleles encountered. We collated indications and results for samples sent to our laboratory from January 2005 to December 2015.
Methods: Blood samples were genotyped using multiplex-PCR settings to observe the presence of the questionable antigen and determine frequent blood group variants. Detection of rare / new variants was done by sequencing of the corresponding gene.
Results: From January 2005 to December 2015, 4367 samples were routinely send to our laboratory. 1221 samples were for prenatal blood group genotyping: 1156/1221 DNA samples from amniocentic fluid were investigated for the presence of fetal RHD sequences. 65 samples were send to our laboratory to determine fetal RHC, RHE, KEL1 and FY1 in pregnancies with known antibodies.
3153 samples were from blood donors and patients due to problems in serological testing. Frequently observed problems in phenotyping were weak reactions with certain antisera or inconclusive reaction pattern in samples from polytransfused patients.
Conclusion: A large number of molecular variants were the reason for problems in serological testing of blood groups. About 35% of the results could only obtained by sequencing of the corresponding gene, the remaining were detectable with commercial kits.

P040

QUANTIFICATION OF TRANSFUSED PLATELETS IN RECIPIENTS WITH DROPLET DIGITAL™ PCR COMPARED TO QUANTITATIVE REAL-TIME PCR

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Introduction: Determination of recovery and survival of transfused platelets is possible by using genetic markers located in the mitochondrial genome. Quantitative real-time PCR (qPCR) served as reliable tool for the quantification. Droplet digital PCR (ddPCR) was evaluated as an alternative to qPCR for absolute quantification of transfused platelets.
Methods: Platelet rich plasma was prepared from 5 mL blood sample for automated extraction (MagnaPure Compact®) of mitochondrial DNA (mtDNA). Dilution series were performed to assess the sensitivity of DNA extraction. ddPCR for mitochondrial markers (n = 8 SNP alleles) was tested for linearity, sensitivity and reproducibility. Blood samples from 30 patients with haematological diseases were collected for six days after platelet transfusion. Endogenous and exogenous platelet counts measured by ddPCR were compared to qPCR results from identical samples.
Results: MtDNA levels increased linearly with platelet counts in the range of 1 - 50 plt/nL (r = 0.997). Spiking experiments demonstrated a sensitivity of 0.1 plt/nL in a background of 20 plt/nL, using 20 droplets / assay as cut-off value. The coefficients of variation for ddPCR assays were comparable to qPCR: 0.7-3.2% (intra-assay) and 1.7-9.9% (inter-assay) depending on the SNP-allele. Calculated survival times in samples from 30 patients agreed well. The calculated coefficient of correlation for the comparison of platelet counts measured with ddPCR to qPCR was R2 = 0,96 (all samples) and R2 = 0,86 (samples with platelet counts up to 10/nL), respectively.
Conclusion: Our results demonstrate the reliability of ddPCR for quantitative tracking of transfused platelets against a background of endogenous platelets. The limit of detection for transfused platelets is about tenfold lower than for qPCR. The ddPCR introduces an efficient and cost-effective alternative to qPCR for monitoring platelet transfusions.

P041

Evaluation of RealStar® Bordetella pertussis PCR Kit 1.0 for qualitative detection and differentiation of Bordetella pertussis and Bordetella parapertussis specific DNA in respiratory samples

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Objectives:
The goal of the study is the evaluation of the performance of RealStar® Bordetella pertussis PCR Kit 1.0 (altona Diagnostics, Germany) for the reliable detection of Bordetella pertussis and Bordetella parapertussis specific DNA in respiratory samples after automated extraction with NucliSENS® easyMAG® system and amplification on LC480 (Roche). The evaluation is based on the comparison of performance data of RealStar® Bordetella pertussis PCR Kit 1.0 and RIDA® GENE Bordetella (r-biopharm).
Methods:
An initial comparison in qualitative detection of 10 Bordetella pertussis QCMD panel samples (QAB094132 QCMD 2016 Bordetella pertussis DNA EQA Programme) by RealStar® Bordetella pertussis PCR and RIDA® GENE Bordetella PCR was used to show general comparability in sensitivity and specificity of both assays.
After this 50 human clinical nasopharyngeal swabs were processed by NucliSENS® easyMAG® system using generic protocol for DNA and RNA extraction (BioMeriéux). For this purpose retrospective positive and negative tested Bordetella pertussis routine samples were included to this study. All sample eluates were analyzed in parallel with RealStar® Bordetella pertussis PCR and RIDA® GENE Bordetella PCR on LC480 (Roche).
Results:
8 out of 10, and 10 out of 10 educational QCMD samples were classified correctly by RealStar® Bordetella pertussis and RIDA®GENE Bordetella, respectively. 2 samples (Bordetella bronchiseptica and Bordetella holmesii) were methodically missclassified by RealStar® Bordetella pertussis due to the PCR target (IS481) of this test.
Qualitative testing of clinical respiratory samples with RealStar® Bordetella pertussis and RIDA® GENE Bordetella were generally concordant (88%). 17 out of 50 samples were detected positive by both assays, 27 out of 50 samples were detected negative by both assays.
3 samples were detected positive by RealStar® B. pertussis PCR Kit 1.0 only, whereas also 3 samples were detected positive by RIDA®GENE Bordetella Kit only.
In total 6 samples out of 50 samples stayed discordant. 5 out of these 6 samples show ct values higher than 36.
Conclusions:
Both assays show very high concordance in sensitivity and specificity of Bordetella pertussis DNA detection in respiratory samples. Discordant results can be explained by very low pathogen load, where both assays are close to their respective limit of detection. This assumption is based on the ct values of the discordant samples which are at cycle 36 or higher.

P042

Evaluation of RealStar® Pneumocystis jirovecii PCR Kit 1.0 for qualitative detection of Pneumocystis jirovecii pneumonia (PCP) specific DNA in respiratory samples

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Objectives:
The goal of the study is the evaluation of the performance of RealStar® Pneumocystis jirovecii PCR Kit 1.0 (altona Diagnostics, Germany) for the reliable detection of Pneumocystis jirovecii specific DNA in respiratory samples after automated extraction with NucliSENS® easyMAG® system and amplification on LC480 (Roche). The evaluation is based on the comparison of performance data of RealStar® Pneumocystis jirovecii PCR Kit 1.0 and RIDA® GENE Pneumocystis jirovecii (r-biopharm).
Methods:
An initial comparison in qualitative detection of 10 Pneumocystis jirovecii QCMD panel samples (QAF114144 QCMD 2016 Pneumocystis jirovecii pneumonia (PCP) DNA EQA Programme) by RealStar® Pneumocystis jirovecii PCR and RIDA® GENE Pneumocystis jirovecii real-time PCR was used to show general comparability in sensitivity and specificity of both assays.
After this 50 human clinical nasopharyngeal swabs were processed by NucliSENS® easyMAG® system using generic protocol for DNA and RNA extraction (BioMeriéux). For this purpose retrospective positive and negative tested Pneumocystis jirovecii routine samples were included to this study. All sample eluates were analyzed in parallel with RealStar® Pneumocystis jirovecii PCR and RIDA® GENE Pneumocystis jirovecii PCR on LC480 (Roche).
Results:
1 out of 10, and 3 out of 10 weak positive educational QCMD samples were not detected by RealStar® Pneumocystis jirovecii PCR Kit 1.0 and RIDA®GENE Pneumocystis jirovecii Kit, respectively.
Qualitative testing of clinical respiratory samples with RealStar® Pneumocystis jirovecii and RIDA® GENE Pneumocystis jirovecii were generally concordant (90%). 14 out of 50 samples were detected positive by both assays, 31 out of 50 samples were detected negative by both assays. 3 samples were detected positive by RealStar® Pneumocystis jirovecii PCR Kit 1.0 only, whereas also 2 samples were detected positive by RIDA®GENE Pneumocystis jirovecii Kit only.
In total 5 samples out of 50 samples stayed discordant. All 5 samples show ct values higher than 32.
Conclusions:
Both assays show very high concordance in sensitivity and specificity of Pneumocystis jirovecii pneumonia DNA detection in respiratory samples. Discordant results can be explained by very low pathogen load, where both assays are close to their respective limit of detection. This assumption is based on the ct values of the discordant samples which are at cycle 32 or higher.

P043

Standardisierte und automatisierte Auswertung von digitalen PCR-Daten

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Zielsetzung
Digitale PCR ist eine wichtige Methode für den Nachweis und die Quantifizierung von Nukleinsäuren (beispielsweise zellfreier Tumor-DNA) im Blutplasma. Die Digitalität entsteht durch Aufteilung der Probe in viele einzelne Kompartimente („droplets“) und Klassifikation der einzelnen droplets nach Fluoreszenzintensität einer spezifischen Hybridisierungs-Sonde in negativ oder positiv. Aus dem Verhältnis der Zahl der negativen und positiven Kompartimente lässt sich die Konzentration der gesuchten Nukleinsäure in der Probe errechnen. Häufig wird die Klassifikation nach Inspektion der Daten durch einen subjektiv festgelegten threshold vom Untersucher vorgenommen, wodurch zwischen verschiedenen Untersuchern Unterschiede im Ergebnis auftreten können. Eine standardisierte, objektive und automatisierte Auswertung von digitalen PCR-Daten ist daher sinnvoll. Hier stellen wir ein an unserem Institut entwickeltes bioinformatisches Verfahren vor, das eine solche standardisierte Auswertung umsetzt.
Methode
Unser Verfahren verwendet die von einem ddPCR Gerät (QX200, Bio-Rad) exportierten Fluoreszenzintensitäts-Rohdaten, die für jedes droplet gemessen werden, als Input. Leere droplets werden erkannt und ihre Intensität für jede Platte normalisiert. Überlaufeffekte zwischen den Kanälen werden korrigiert und die droplets robust als negativ, positiv oder unspezifischer „rain“ klassifiziert. Die Methode ist als Paket für die frei erhältliche open source Programmiersprache R implementiert.
Ergebnis
Wir haben unser Verfahren mit Proben bekannter DNA-Menge aus Zelllinien in verschiedenen Verdünnungsstufen validiert. Anschließend haben wir unser Verfahren auf ddPCR-Daten zur rare event detection von zellfreier zirkulierender Tumor-DNA im Blutplasma und zur

Quantifizierung von Splicing-Varianten von Tumor-RNA in Vollblut angewendet. Bisher haben wir rund 20 Millionen droplets klassifiziert. Die automatische Auswertung benötigt pro Patientenprobe nur wenige Sekunden. Dabei konnten wir auch Proben klassifizieren, bei denen andere Algorithmen (Bio-Rad QuantaSoft, R-Paket „ddpcr") kein Ergebnis lieferten.
Zusammenfassung
Wir stellen eine standardisierte, automatisierte, und frei verfügbare Auswertung von digitalen droplet-PCR-Daten vor. Diese ist beim liquid profiling von zellfreier Tumor-DNA und Tumor-RNA eine wichtige Voraussetzung für eine Vergleichbarkeit von Befunden zwischen Laboratorien und damit für eine korrekte Beurteilung des zeitlichen Verlaufs bei Tumorpatienten.

P044

Exome sequencing reveals GATA1 mutation in a patient with partial delta-storage pool deficiency and mild thrombocytopenia

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Objectives.
We report about a 35-year-old male patient of Russian background with severe and frequent epistaxis and hematoma since infancy. He presented with mild thrombocytopenia and increased mean platelet volume. Von Willebrand's disease and subhemophilia had been excluded. Previously, he was diagnosed with immune thrombocytopenic purpura. He never underwent elective surgery. His parents were asymptomatic. However, his 4-year-old daughter also suffers from severe bleeding symptoms (multiple, light red hematoma in consequence of minimal trauma).
Methods.
Whole exome sequencing (WES) was carried out for the patient, his asymptomatic wife, his symptomatic daughter and her asymptomatic 8-year-old brother.
Platelet function was assessed by light transmission-, lumi-aggregometry and flow cytometry. Lysates of gel-filtered platelets were analyzed for total granule P-selectin, CD63 and von Willebrand factor (VWF) content by Western blotting and for serotonin levels by ELISA, respectively.
Results.
Platelet function and characterization of the patients' granula suggested a delta-storage pool disease (SPD). In most cases delta-SPD occurs as part of a syndrome, e.g. combined with albinism, immunodeficiency or a thalassemic-like blood disorder. As the patient and his daughter did not show any conclusive phenotype, their DNA was subjected to WES.
Exome sequencing revealed a not yet described GATA1- mutation close to two zinc finger domains (ZnF1 and ZnF2) in a highly conserved region of the GATA1 gene in the 4-year old daughter (c.886A > C, p.T296P, heterozygous) and her father (c.886A > C, p.T296P, hemizygous). This mutation was absent in 150 wildtype-controls but could also be demonstrated in the index patient's asymptomatic mother. Only a few mutations are known to be located in this C-terminal region to date. Mutations in GATA1 may lead to different clinical presentations, depending on their location within GATA1 (e.g. Diamond-Blakfan anemia (exon2), X-linked thrombocytopenia (ZnF1), transient myeloproliferative disorder (Intron 1, exon 2, exon 3) and acute megakaryoblastic leukemia (intron 1, exon 2, exon 3 in case of Down-Syndrome). Significantly increased HbF-levels (reference level: ≤0.8%) in the affected family members of 13.5% (4-year-old daughter) and 2.8% (35-year-old indexpatient) suggested dyserythropoiesis, although thalassemic features of the blood count were lacking and HBA-MLPA did not reveal any copy number variation (CNV) within the HBA gene. Imbalanced X-chromosome inactivation was shown by pyrosequencing-based allele quantification based on RNA isolated from whole blood and might explain the different phenotypes of the GATA1 mutation carriers.
Conclusion.
We describe a GATA1 mutation as the cause of a delta-storage pool disorder. Allele-quantification by ddPCR based on RNA isolated from whole blood and platelet rich plasma is still pending, aiming to further investigate X-inactivation and quantification of BCAM and SLC4A1 transcripts.

P045

The Russell–Silver Syndrome: A Case Report and Brief Review of the Literature

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Background
The Russell-Silver syndrome is a rare genetic disease classified as an imprinting disorder with epigenetic disorders, characterized by intrauterine growth retardation, body asymmetry and specific facial dysmorphism. It is clinically recognizable but its etiology appears to be heterogeneous and has a higher risk of development delay.

Clinical Case
2 month-old baby who attends for low weight gain from birth. Scarce appetite, 5 shots a day of variable quantity. Mother refers to recurrent respiratory problems. No vomiting or cyanosis.
Personal history: 39 + 5 weeks of gestation, CIR, instrumentalized vaginal delivery. Apgar 7/9. Weight: 2590g and length: 48 cm. Cephalic perimeter: 32 cm. P3 at birth. No family history of interest.
Physical examination: Good condition. Mild facial dysmorphism. Cardiopulmonary auscultation: normal.
Abdomen soft and depressible, not meglia. Coffee-with-milk stains. Not hernias. Tests on bags.
Neurological exploration: partially fixes the gaze, social smile. Marked cervical and dorsal hypotonia.
Complementary explorations.
With the initial diagnosis of failure of medro and hypotonia is referred to the principal hospital to complete relevant studies.
Hemogram: Hemoglobin: 7.1g/dL, Hematocrit 20.2%, Leukocytes 10.54x103mm3, Platelets 318x106.
Biochemistry: Glucose 78mg/dL, Urea 30mg/dL, Creatinine 0.20mg/dL, Ion Sodium 140mmol/L, Potassium Ion 5.5mmol/L, Lactate 4.6mmol/L, IGF-I (Sm-C).

P046

Xyloside- and nucleotide treatment of normal human dermal fibroblasts leads to decreased mRNA expression levels of extracellular matrix components

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Background: A fibrosis is defined as an excessive, abnormal deposition of extracellular matrix (ECM) molecules, which can affect different organs. These molecules are secreted by specific cells, to maintain structural and biochemical support to surrounding cells. Although there are a lot of studies concerning the effect and inhibition of pro-fibrotic mediators, currently no appropriate therapy is available to treat the disease. Xylosyltransferases (XT) catalyze the rate-limiting step in proteoglycan biosynthesis. Previous studies have shown, that an increase in XYLT1 mRNA expression as well as serum XT-activity is associated with diseases being characterized by an abnormal extracellular matrix remodeling as for instance fibrosis. Developing anti-fibrotic therapeutic strategies requires a broad knowledge of XT-specific transcriptional regulation processes. The aim of this study was to find molecules, which specifically inhibit XT-activity in normal human dermal fibroblasts (NHDF). Additionally, we examined the influence of these molecules on gene expression of different ECM molecules. For this purpose, we tested molecules of two different molecular classes (xylosides and nucleotides). 4-Methylumbelliferyl-β-D-Xylosid (MU-Xyl) has been shown to inhibit proteoglycan biosynthesis in different cell types. Furthermore, we tested the impact of the nucleotide uridine diphosphate (UDP).
Methods: NHDF cells (n = 3) were seeded with a density of 50 cells/mm2. 24 h after seeding, cells were treated with either 1 mg/ml UDP or 1 mM MU-Xyl. Cells were harvested after 48 h, to determine relative mRNA expression levels by qPCR. The XT-activity was measured in a radiochemical assay. To determine the extracellular XT-activity, supernatants were collected after 48 h and 72 h. The intracellular XT-activity was measured after lysing the cells.
Results: After treatment with MU-Xyl, reduced mRNA expression levels of the genes XYLT1, ACTA2, ELN, ACAN and B4GalT7 were observed in treated cells. As opposed to this, XYLT2 and Col1A1 mRNA expression levels were not significantly different compared to controls. The UDP-treatment resulted in reduced mRNA expression levels of the genes XYLT2, ACTA2, ELN, ACAN, Col1A1 and B4GalT7. There was no significant change in mRNA expression level of the XYLT1 gene. Compared to controls, the relative intracellular XT-activity was significantly increased 72 h after treatment with both, MU-Xyl and UDP. 48 h after UDP-treatment, the extracellular XT-activity was decreased. As opposed to this, the extracellular XT-activity increased 72 h after treatment with UDP.
Conclusion: Treatment with both molecules, MU-Xyl and UDP, leads to decreased mRNA expression levels of different ECM molecules in NHDF cells. Additionally, the treatments result in a time dependent increasement of the intracellular XT-activity. These findings might contribute to a better understanding of regulation mechanisms during a fibrotic disease.

P047

Association of human FOS promoter variants with the occurrence of knee osteoarthritis in a case control association study

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Objective. Rheumatic diseases are characterized by joint inflammation and destruction. The expression of numerous pro-inflammatory and/or pro-destructive proteins depends on the transcription factor AP-1 consisting of JUN and FOS proteins. Expression of these key factors may be

influenced by functionally relevant single nucleotide polymorphisms (SNPs) in their promoters. Our aim was to analyze: i) the presence of SNPs in the promoters of JUN and FOS family genes in patients with rheumatoid arthritis (RA), knee osteoarthritis (OA), and normal controls (NC); ii) their functional influence on the transcription levels of JUN/FOS; and iii) associations of the SNP variants with the occurrence of RA or knee OA.
Methods. SNPs in the JUN and FOS family gene promoters were identified in an initial screening population consisting of RA, OA, and NC DNA samples using the Non-Isotopic RNase Cleavage Assay (n = 25). The functional influence of each identified SNP on mRNA transcription levels was analysed using reporter gene assays. Subsequently, genotyping was done in RA (n = 298), knee OA (n = 277), and NC (n = 484) DNA samples. For replication, significant associations between genetic variants and OA were assessed in the Finnish Health 2000 study cohort (OA: n = 72, NC: n = 548).
Results. In the initial screening population, 2 SNPs (rs4647001, rs4647009) were detected in the JUN promoter and 2 additional SNPs (rs2239615, rs7101) in perfect linkage disequilibrium were detected in the FOS promoter. After transfection into NIH3T3 cells, JUN promoter SNP rs4647009 caused significant downregulation of reporter gene expression, both with or without stimulation by PMA ($P \leq 0.05$). In contrast, reporter gene expression was significantly upregulated in the presence of both minor alleles of the FOS promoter when compared to the major alleles (both with or without stimulation, $P \leq 0.05$). The SNPs in the FOS promoter showed an association of the minor homozygous genotype with susceptibility for knee OA (OR 2.12, 95% confidence interval 1.2-3.7, $P = 0.0086$). Association of this variant was successfully replicated in the Finnish Health 2000 study cohort (allelic OR 1.72, 95% confidence interval 1.2-2.5, $P = 0.006$).
Conclusion. Variants of FOS may represent relevant susceptibility markers for knee OA, potentially involved in its pathogenesis and/or progression by upregulating the expression level of FOS.

P047A

Development of a Versatile Platform for Routine Flourescence in-situ Hybridization

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Introduction and Aims Fluorescence microscopy is a highly sensitive and valuable method in clinical diagnostics. Genetic aberrations in cancer biopsies are routinely observed with fluorescence in-situhybridization (FISH). The increasing number of tumor patients, the high diversity of probes and the diagnostic effort for the correct interpretation of FISH signals, lead to the need for the development of an automated reading and evaluation platform. In fact, challenging parts are the heterogeneity and complexity of the tumor derived section. Thus, pre-filtering of tumor derived areas of interest, the detailed focus of these parts with subsequent probe detection and evaluation is required.
Methods and Results Simultaneous real-time device control The platform is based on a motorized microscope with changeable magnification and fluorescence filters, a LED illumination with multiple wavelengths, a precise moveable X-Y stage and a high-resolution greyscale camera1. All devices are controlled simultaneously by generating the appropriate driver software developed in house2. Pre-filtering Stitching strategy acquires images of DAPI (4,6-Diamidino-2-penylindole) stained specimens with low magnification (100–200 fold) getting a tissue overview within 30 min. Specific algorithms like the entropy filter remove background and artefacts within 1 min and search dense cell areas that are interesting for the detailed FISH probe analysis. Letter requires higher magnification (400–600 fold). Within these images, single cells are detected and separated with an adjustable watershed transformation algorithm. FISH signal analysis Detection and interpretation algorithms are adapted to the specific probes (ZytoVision GmbH), which focus the break-apart and translocation of genes and further events like the gain or loss of sequences. Further, recording sharp images of relevant nuclei in a five stack z-layer image gallery with 500 nm distances combined with a maximum intensity projection is necessary for diagnostic documentation. End-user evaluation Results are demonstrated within a user friendly graphical interface, performed as a diagnostic learning tool. Further, a long term archiving supports the routine workflow. Conclusions the platform is an early stage of development and will be optimized for image processing and the self-learning program by using adapted algorithms. Further, routine diagnostics requires further optimization of scan time and image processing algorithm for multiple cancer sections. This work was supported by the BMBF (Bundesministerium für Bildung und Forschung) with the code 03PSZZF1A.
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Labor-Management/Qualitätssicherung

P048

Identification of steps necessary for general implementation of moving average for continuous QC in medical laboratories

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Introduction: Moving average (MA) can be used for continuous analytical quality control. Though MA has been described decades ago, general implementation in clinical chemistry laboratories has failed. We addressed several issues that we considered to be important to support a more general implementation of MA as continuous QC instrument in medical laboratories.
Method: A MA optimization method described by our group (1,2) was used to generate optimal MA procedures that were implemented for continuous analytical quality control in daily practice (3). During the various phases of MA implementation, issues that potentially complicated the MA implementation and application were identified. Furthermore a MA-alarm case, describing a temporary sodium ion selective electrode (ISE) failure, was used to demonstrate the value of MA.
Results: The first step to support clinical laboratories to obtain and use optimal and validated MA for continuous QC is to make newly developed MA optimization methods commercially available for clinical laboratories. Secondly, improvements in MA management software are required to allow optimal support of MA management on clinical laboratories. These include continuous generation of MA values, adequate continuous alarming, MA resetting, exclusion of samples and presentation of MA in an accuracy plot. Finally, laboratory management issues were identified that included development of a clear protocol how to handle MA alarms and training of technicians.
Conclusions: The issues we encountered during implementation and application of MA illustrate the need to make newly developed MA optimization methods available for clinical laboratories and for improvements in the available MA management software. This should allow a more general implementation of continuous QC by MA on medical laboratories (4).
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P049

Anwendbarkeit des Reference Limit Estimators unter Verwendung verschiedener Kollektive

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Zielsetzung
Die Ermittlung von Referenzwerten für eine bestimmte Population stellt für medizinische Laboratorien eine große Herausforderung dar, da die Auswahl geeigneter Probanden, die Messwerterstellung und die statistische Auswertung nur mit großem administrativen, personellen, und zeitlichen Aufwand möglich sind. Daher werden Referenzbereiche primär aus Packungsbeilagen der Hersteller übernommen.
Die Referenzpopulationen aus den Studien der Hersteller können jedoch von jenen der Patienten einzelner Laboratorien abweichen. Um dieser Problematik zu entgehen und eine einfache Methode zur laborinternen Bestimmung von Referenzwerten zur Verfügung zu haben, wurde von der Arbeitsgruppe Richtwerte der DGKL der Reference Limit Estimator (RLE) entwickelt. Damit können bereits aus dem Routinebetrieb vorhandene Datensätze zur Ermittlung eigener Referenzwerte herangezogen und so der Aufwand reduziert werden.
Ziel unserer Studie war neben der Berechnung von Referenzwerten der Vorarlberger Bevölkerung auch ein Vergleich der Ergebnisse aus zwei unterschiedlichen Patientenkollektiven (Krankenhauspatienten versus Patienten niedergelassener Ärzte).
Methode
Mithilfe des RLE wurden die Daten von 25 Analyten ausgewertet, die im Zeitraum von 2012 bis 2016 keiner relevanten methodischen Änderung unterzogen wurden. Aus den Kollektiven der hospitalisierten bzw. der externen Patienten wurden jeweils 18 - 65-jährige Patienten beider Geschlechter rekrutiert, die vom RLE primär in Pathologische und Nicht-Pathologische eingeteilt wurden. Aus der Nicht-Pathologischen Gruppe wurden daraufhin die Referenzgrenzen als 2,5- und 97,5- Perzentilen berechnet. Einzelne klinische Stationen, die einen überproportional großen Anteil an stark pathologischen Werten erwarten ließen, wurden bereits im Vorfeld von der Berechnung ausgeschlossen, z.B. Intensivstation, Dialyse und Onkologie.
Ergebnis
Eine gute Übereinstimmung zwischen den berechneten Referenzwerten und den Herstellerangaben zeigte sich bei den Elektrolyten (Na, K und Cl). Große Abweichungen waren bei Hämoglobin, GOT (ASAT) und Folat zu beobachten. Für Ferritin war die Berechnung mittels RLE nicht möglich. Die Ergebnisse von Patienten externer Einsender lagen großteils näher an den Angaben der Packungsbeilagen als jene interner Stationen. Ebenso wurden für einige Parameter alters- und geschlechtsabhängige Referenzwerte ermittelt.
Zusammenfassung
Der RLE stellte sich als benutzerfreundliches Programm dar, dessen Installation und Bedienung einfach und intuitiv möglich ist. Die Interpretation der Ergebnisse gestaltete sich in Abhängigkeit des Kollektivs unterschiedlich aufwendig. Die divergierenden Referenzwerte aus den Daten interner und externer Einsender deuten auf einen wesentlichen Einfluss der Selektion und des allgemeinen Gesundheitszustandes

der Patienten hin. Patienten externer Einsender scheinen ein homogeneres und damit geeigneteres Kollektiv für die Referenzwertermittlung mittels RLE darzustellen als Krankenhauspatienten.

P050

External validation of the medical smartphone app Labtracker+

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INTRODUCTION: When following patients over time, physicians may struggle to distinguish 'true changes' in consecutive blood parameters from so-called 'natural fluctuations'. In practice, they have to do so by relying on their clinical experience and intuition. We developed Labtracker+; a medical smartphone app that calculates the probability that an increase or decrease over time in a specific blood parameter is true, given the time between measurements. This clinical validation study investigates whether there is a difference between inexperienced physicians and experienced physicians when it comes to interpreting a change between consecutive laboratory results.
METHODS: We presented patient cases to 135 participants (medical students (n = 92), medical residents from the department of internal medicine (n = 19) and internists (n = 24) to examine whether there is a difference between medical students, residents and experienced clinicians when it comes to interpreting changes between consecutive laboratory results. Participants were asked to interpret if changes in consecutive laboratory values were likely to be 'real or rather due to natural fluctuations. The answers of the study participants were compared to the calculated probabilities by the app Labtracker + and the concordance rates were assessed. Besides, we tested whether physicians with clinical experience scored better concordance rates with the app Labtracker + than inexperienced clinicians.
Results: Medical residents and internists showed significantly better concordance rates with the calculated probabilities by the app Labtracker + than medical students, regarding their interpretation of differences between consecutive laboratory results ($p = 0.009$ and $p < 0.001$, respectively).
Conclusion: The app Labtracker + could serve as a clinical decision tool in the interpretation of consecutive laboratory test results, and could contribute to rapid recognition of parameter changes by physicians.

P051

Reference limits estimation of endocrinological parameters using an indirect method based on intra-laboratory patient data and their relevance in everyday clinical practice

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Objective: A new, indirect approach was used to determine the reference ranges for adults of calcium, phosphate, TSH, fT3, fT4, 25-OH vitamin D, renin and aldosterone. The program, the Reference Limit Estimator (RLE) developed by Arzideh et al., uses the patient values collected in laboratory information systems and determines reference limits by analyzing the value distribution. The possibility to apply the method to the data of the central laboratory of the University Hospital Schleswig-Holstein (Universitätsklinikum Schleswig-Holstein, UKSH) is discussed and the results are compared with established reference intervals.
Methods: The R-based Excel tool Reference Limit Estimator (RLE) makes use of the constantly occurring intra-laboratory measured values of patients. It is assumed that the physiological values are approximately normally distributed and the pathological values, which are far less frequent, can be separated from them. Thus, the RLE calculates the reference intervals from a mixed population. Due to their high proportion of pathological values, gynecological data and values of intensive care units were excluded. Depending on the range of data, age classes between 18 and 80 years were formed. For calcium and phosphate also a seasonal partition is made. Furthermore, the data was analyzed to determine e.g. population-specific effects or differences in measuring instruments.
Results: For the parameters calcium, phosphate, TSH, fT3 and fT4 reference limits were obtained, which are similar to the values given by device manufacturers and the literature. The limits for the thyroid hormones showed greater deviations, whereat these parameters generally feature broader reference intervals. Not enough values were available to perform adequate calculations for renin, aldosterone, and vitamin D.
Conclusion: The RLE provides the ability to easily apply an indirect method to intra-laboratory data sets. However, the RLE is not suitable for parameters whose physiological values differ too much from a normal Distribution. Too little measured values or a high percentage of pathological values prevent reliable results. This is in particular problematic for rarely requested parameters or for parameters whose requirements are linked to specific conditions. For laboratory measurands frequently requested in everyday clinical practice, the RLE is an alternative to complex direct methods for the determination of reference values, which offers significant advantages regarding time, finances and science.

P052

Long term stability of whole blood glucose – a tube comparison study

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Introduction:
Measuring glucose from whole blood samples is a known critical candidate for accurate results owing to unsatisfactory glycolysis inhibition. Former studies showed that Terumo VENOSAFE™ tubes are superior to the conventional NaFl approach. Terumo tubes were taken off the European market in 2016. Since these tubes were integrated in the German National Cohort (GNC) alternatives were urgently required. This circumstance led to this state of the market evaluation of a total of five existing as well as newly available tubes in terms of sufficient long-term glycolysis inhibition.
Methods:
Non-fasting venous blood samples were collected from 61 healthy volunteers. Each proband provided blood for a total of five different tube: (1) Terumo Venosafe® (distributed in Europe), (2) Terumo Venosafe® (distributed in Japan), (3) Greiner Vacuette® FC-Mix, (4) BD Vacutainer® FX-Mixture and (5) BD Vacutainer® SST™ II Advance. Immediately after sampling, tubes were mixed thoroughly and centrifuged within the following hour. Glucose concentrations were determined in all tubes by using the Glucose Hexokinase method on the Dimension Vista® 1500 System (Siemens Healthcare Diagnostics, Eschborn, Germany). Afterwards, the tubes were re-suspendend except from the BD Vacutainer® SST™ II Advance, which contains a permanent gel barrier. Samples from the first 30 probands were then stored at room temperature (RT), while samples from the following 31 probands were stored at 4°C. After 24, 48, 72 and 96 hours all tubes were (re)centrifuged, with the exception of the BD Vacutainer® SST™ II Advance, and repeated glucose measurements were performed.
Results & Conclusion:
Glucose recovery rates differed significantly between the investigated tube types and to a certain extent between the two storing temperatures. The best glucose recovery was observed under both storage conditions for the BD serum tubes with an equal glucose concentration at 96 hours compared to the initial time point. In contrast, glycolysis was most evident in the BD FX-mixture tubes. Within four days, the median glucose concentration decreased by 29.9% at RT and by 10.8% at 4°C in these tubes. Good glucose stability was observed in Greiner tubes, which were introduced as an alternative for the Terumo tubes. The median glucose concentration decreased by 1.9% at RT and increased by 2.1% at 4°C within four days, which might be caused mainly by analytical deviations. Both Terumo tubes displayed comparable glucose stability to former studies. Although Greiner as well as both Terumo tubes are supposed to contain the same glycolysis inhibitor, glucose stability differs significantly between these three tube types.
We were able to show, that Greiner tubes are an acceptable alternative for the GNC. Furthermore, we were able to demonstrate, that no specific glycolysis inhibitor is ultimately necessary in order to have long-term stable glucose concentration, by using gel barrier tubes.

P053

Validation of a mathematic algorithm for specific calculation of clinical reference limits on the example of coagulation parameters

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Objective: In laboratory diagnostics, new methods to calculate lab-specific reference limits become more important. The DGKL published a program (Reference Limit Estimator, RLE), which allows the calculation of individual reference limits. This represents an alternative to the common, administratively complex method of identifying reference limits based on a healthy reference population. With the RLE, lab-specific reference limits for coagulation parameters and liver values are calculated by means of measured data from the laboratory of the University Hospital Schleswig-Holstein (UKSH). These are compared with currently used reference limits from the UKSH central laboratory. Furthermore, potential relations between coagulation parameters and liver values are explored.
Methods: Reference limits were calculated retrospectively from preprocessed laboratory data of the UKSH with RLE version RL35. The different analytical instruments (BCS, ACL, Cobas) were compared by equivalence tests. Additionally relations in laboratory parameters were analyzed by means of Pearson and Spearman correlation tests.
Results: The analysis of the measuring devices resulted in different equivalence groups between the instruments, but no clinical relevant deviations in the parameters were observed. Thus uniform reference limits were calculated. It was possible to determine age- and gender-specific reference limits for most of the coagulation parameters (PTT, INR, AT, fibrinogen, D-dimer, FIX, FVIII). Usually, these were close to the reference limits from the central laboratory (e.g. PTT, male, 18-40 years: 23,8-36,2 s), whereas some age- and gender-specific deviations were observed. The distribution of measured values of other parameters (e.g. CRP) differ too much from Gaussian distribution, so that the calculated reference limits could not be considered as reasonable. Nevertheless, it was possible to calculate specific reference limits for liver

values (GGT, GPT, AChE), which largely corresponded to those from the central laboratory. Correlation analysis did not show any relevant relation between liver values and coagulation parameters.
Conclusion: Unfortunately, for some parameters (e.g. lipoprotein a) the reference limit calculation with the RLE could not be performed due to their non-normal distribution. But it was possible to calculate age- and gender-specific reference limits for various further parameters. Some reference limits were comparable to those from the central laboratory (e.g. AT, PTT), others showed a stronger deviation (e.g. fibrinogen).

P054

Validation of an indirect method for the determination of population specific reference values and the applicability in clinical practice using selected laboratory parameters

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Objective: In order to assess laboratory measurements and to classify them properly, a reference interval is required for each parameter. Indirect methods are a new approach to retrospectively determine specific reference values, especially when a direct determination from healthy reference groups is not possible. This approach is followed by the team "AG Richtwerte" of the DGKL, which has developed the Reference Limit Estimator (RLE). The RLE is a program that determines population-specific reference values from large laboratory-own data sets. Thus collected measurement data of the central laboratory of the University Hospital Schleswig-Holstein (UKSH) was used to calculate reference limits for a selection of electrolyte, liver, pancreas and renal parameters. The results were compared to established values. Method: The RLE is based on the assumptions that the measured values are nearly normally distributed and consist of far more physiological than pathological values. This is the case with many routine parameters (e.g. potassium). For other measurands, particularly for those that are requested rarely or only for specific issues, it might be problematic. In these cases the proportion of the pathological values can be minimized by exclusion of patient groups (e.g. gynecology, intensive care unit) or the use of only one measured value from each patient. Result: For many of the selected laboratory parameters plausible reference values were calculated using the RLE. Very good accordance with the values established in the UKSH were found especially for electrolytes (e.g. sodium RLE: 135-145mmol/l, UKSH: 136-145mmol/l). Using the cholinesterase as an example, the relevance of the size of the data set and the selection of the patient collective becomes particularly clear. The reference values obtained from all data significantly differed from the values of the central laboratory of the UKSH (RLE: 1.31-14.57kU/l, UKSH: 5.3-12.9kU/l). After specific selection of the data in order to keep the proportion of pathological values low, the calculated reference value is much closer to the comparison value (RLE: 3.74-11.17kU/l). Conclusion: The reference intervals of routine parameters (e.g. electrolytes) calculated with the RLE are comparable to the values published in the literature. However, parameters whose values are not normally distributed (e.g. cholinesterase) greatly differ from the established reference intervals. Here, a more accurate preselection can help or an alternative to the RLE must be considered.

P055

Improved whole blood plasma glucose stability and the risk of misclassification of impaired fasting glucose and diabetes mellitus

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The diagnosis of diabetes mellitus is based on accurate plasma glucose measurements and correct patient classification according to established guidelines. Preanalytical decrease in plasma glucose concentration in whole blood samples may be minimized by different additives in the blood sampling tubes. At the Medical Laboratory Rostock (Rostock, Germany), the effect of switching sampling tubes on the diabetes risk classification was investigated in a primary care setting.
Diabetes risk stratifications by fasting plasma glucose were compared between Becton Dickinson NaF-KOx Vacutainer® (January to April 2015, n = 3214) and Greiner Bio-one NaF-Citrate Glucomedics Vacuettes® (January to April 2016, n = 3789). Only patients were covered with glucose and HbA1c requests from identical ordering physicians.
For NaF-KOx tubes, 21 % of patients were classified as having normal fasting plasma glucose (NFG, <5.6 mmol/L), 28 % as impaired fasting plasma glucose (IFG, 5.6 – 7.0 mmol/L), and 51 % as diabetic patients (≥7 mmol/L). After switching to NaF-Citrate tubes, only 11 % of patients demonstrated normal fasting plasma glucose. Correspondingly, a significant increase of IFG (33 %) and diabetes mellitus (56 %) was observed ($p < 0.0001$). HbA1c analysis in IFG-classified patients demonstrated significantly more normal results (HbA1c < 5.7 %) in the NaF-Citrate tubes group (41 %, n = 522) compared to the NaF-KOx tubes group (35 %, n = 315, $p < 0.0001$).
Effective inhibition of preanalytical glycolysis is of paramount importance to obtain accurate plasma glucose measurements, and purpose-optimized blood sampling tubes are commercially available. However, the clinical impact of switching tubes with the potential of patient misclassification should be considered. The present study demonstrated a rise in IFG and diabetes mellitus prevalence after switching from

NaF-KOx Vacutainer to NaF-Citrate Glucomedics Vacuettes, raising the question of interchangeable use of different tube types. Specifically, more patients were observed having IFG as classified by fasting plasma glucose accompanied by normal HbA1c results. In primary care, replacement of tubes with the goal of optimizing plasma glucose measurements generates a substantial number of additional HbA1c orders with the associated costs. Ultimately, fasting plasma glucose concentrations used for patient classification may need revision in the era of purpose-optimized blood tubes to avoid misclassification and rise in impaired fasting glucose or diabetes prevalence.

P056

Validation of a mathematical algorithm for the specific calculation of clinical reference limits using the example of erythrocyte parameters

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Purpose
Reference values are usually determined directly in laboratory diagnostics by means of healthy populations, which entails enormous administrative effort and high costs. The Reference Limit Estimator (RLE), developed by the DGKL, offers the possibility to calculate reference ranges using an indirect method from laboratory measurements. This method is used on the data acquired by the Central Laboratory of the University Hospital of Schleswig-Holstein (UKSH) in order to calculate gender and age-specific reference ranges for erythrocyte parameters.
Method
The calculation with the RLE is based on the assumption that the majority of the data is normally distributed and physiologically, which makes it possible to separate pathological values by mathematical methods. This method is applied to the erythrocyte parameters (erythrocytes, hemoglobin, hematocrit, MCH, MCV, MCHC) derived from the Sysmex XE5000 and XT1800i analyzers. The data is divided into five age groups for women and men, respectively, to calculate their specific reference ranges. A comparison is made with the previous reference values known from the literature (Thomas 2012). It verifies whether these are within the permissible difference or whether a population-specific adjustment is appropriate.
Results
The calculated reference ranges of the erythrocyte parameters are slightly broader compared to the values published in the literature. The deviations are particularly evident in the lower reference range, which are lower on an average of 6-9% in females as well as males at individual age levels.
For the upper reference range, only small deviations can be seen in individual age groups, which is not clinically relevant. Only for hematocrit, lower values (about 8%) are calculated for all ages in both women and men.
Therefore, it might be advisable in clinical routine to use age-specific reference limits for patients, since a shift of the reference range can decide whether or not therapy is initiated.
Summary
With the RLE, age and gender-specific reference ranges for the erythrocyte parameters can be calculated. Overall, these reference ranges are slightly broader compared to the established ones. Hence, in some cases it would be of benefit for the patient to use more specific reference limits. This allows an individual classification and interpretation especially in the clinical diagnosis of anaemias.

P057

Evaluation of External Quality Assessment for Vancomycin

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Objective: Vancomycin is one of the most important agents against severe infections caused by gram-positive bacteria like methillicin-resistant Staphylococcus aureus (MRSA). Therapeutic drug monitoring (TDM) of vancomycin is crucial to avoid blood levels outside its therapeutic range causing side effects such as nephrotoxicity as well as therapeutic failure and development of bacterial resistance. Several different commercial immunoassays are in use for TDM of vancomycin. The aim of our study was to assess the degree of standardization of routine vancomycin quantification.
Method: We studied data of a vancomycin EQA (external quality assessment) program implemented by the Referenzinstitut für Bioanalytik (RfB). Results of 4 campaigns from 2016, involving each 2 samples were assessed, each with an average number of 254 participants using kits from 10 different manufacturers. We studied median reported values and the 16th and 84th percentiles of the reported results, and the CV of all results reported for the respective samples for a total of 8 samples.

Results: The percentage difference between the 16th percentile of results of a sample to the mean concentration observed for this sample ranged up to to 54%. The Percentage difference between the 84th percentile to the respective mean concentration ranged up to 44%. CVs of all reported results for a respective sample ranged from 10% to 12% in the four different EQA campaigns.
Summary: This evaluation of a large EQA data set shows consistently poor between-method agreement and substantial spread of results in vancomycin measurement. Efforts to achieve standardization of vancomycin quantification and of TDM of antibiotics in general - based on the traceability to reference measurement procedures - seem to be of utmost importance to obtain consistent data from different laboratories and in order to develop reliable clinical decision levels.

P058

Optimizing Prediction of Red Blood Cell Concentrate Usage

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Introduction: Stock management of red blood cell concentrates (RBCs) is nontrivial since a surplus leads to wastage, which is both an ethical as well as a financial issue. On the other hand an undersupply of RBCs leads to an increased risk of critical shortages in case of heavy RBC demand. To date, many blood banks use inventory management systems for blood stock calculation. These systems use non-adaptive, empirical methods or average consumption models, which do not take into account temporal changes in blood demand. Therefore, advanced prediction methods could improve blood stock management efficiency by increasing RBC stock when higher demand is likely as well as reducing RBC wastage.
Methods: Data of RBC usage between 2004 and 2016 in the Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria were analyzed. Algorithms like ETS modelling (exponential smoothing) and ARIMA models (autoregressive, integrated moving-average), which were developed for time series analysis, as well as regression models and neural networks, were evaluated. The algorithms were provided with the amount of RBCs utilized on each day, as well as temporal context information where applicable. Predictions were performed for several time points into the future (3, 5, 7, 10 and 14 days), which were defined as periods in the year 2016. Accuracy was defined as the agreement between the model prediction and the real RBC demand. Safety buffers were defined as both the implementation of confidence intervals (80% and 95%) as well as fixed lower RBC threshold values into the model.
Results: The evaluated models were able to outperform both the empirical blood demand model as well as the average consumption calculation methods. Temporal context information increased prediction accuracy. Without safety buffers, underestimation of RBC demand was common in days with unusually high RBC demand, an effect strongly reduced by the inclusion of safety buffers. The advantage of those prediction models was higher for shorter time periods.
Conclusion: Advanced RBC consumption prediction methods perform better compared to both empirical, non-adaptive methods as well as averaging blood consumption models in estimating RBC usage over time. Using those methods, RBC stock management efficiency could be increased, therefore reducing the risk of RBC shortages as well as RBC wastage. Safety buffers are important for the calculations to additionally increase the safety of RBC stock management systems. These effects could be especially beneficial for hospitals with transfusion departments, small to medium-sized blood banks and institutions with a limited stock capacity.

P059

Der irreguläre analytischer Fehler. Ein neues Konzept zur Bewertung laboranalytischer Qualität

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Internal quality assessment based on quality control samples and external quality assessment based on proficiency testing are the key elements of quality assurance in laboratory medicine. These process-oriented assessment allows for insight into random analytical variation and systematic calibration error. However, in such a setting, any individual sample is not under individual quality control. The quality control measurements act merely at the level of the analytical batch. Notably, many effects and interferences associated with an individual diagnostic sample can compromise any quantification. It is obvious that a process-oriented quality-control-sample-based approach of quality assurance is not sensitive to such errors.
To address the potential causes and nature of such analytical interference in individual samples more systematically, we suggest the introduction of a new term: irregular (individual) analytical error. This term can be applied in any analytical assay that is traceable to a reference measurement system. For an individual sample an irregular analytical error is defined as an inaccuracy (which is the deviation from a reference measurement procedure result found for the sample) of a test result that is so high that it cannot be explained by measurement uncertainty of the utilized routine assay operating within the accepted limitations of the associated process quality control measurements.

Based on this terminology, recognized causes of irregular analytical error can be given in the talk as a risk catalogue for clinical chemistry. These issues include reproducible individual analytical errors (e.g., caused by anti-reagent antibodies) and non-reproducible, sporadic errors (e.g.,errors due to incorrect pipetting volume due to air bubbles in a sample), which can both lead to inaccurate results and risks for patients.

Hämatologie / Hämostaseologie

P060

Hematological sample testing in new MiniCollect® Blood Collection Tubes

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Background: Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity. MiniCollect® K2EDTA and K3EDTA Blood Collection Tubes are used to collect, transport, store and evaluate capillary blood specimens for hematology tests.
Methods: Studies considering venous and capillary collection were done at Steyr Hospital and Laboratory Rainbach (Austria) using MiniCollect tubes with the old design vs. new design. Altogether, 65 hospitalized and 90 healthy subjects were recruited. Informed consent was given by all donors and the studies were approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. Complete blood counts including 15 parameters were tested using a DxH800 (Beckman Coulter, precision WBC ≤ 3%/RBC ≤ 1.5%). Comparison testing to Microtainer (BD) was done. Analysis was done with the instrument's accompanying reagents. Statistical evaluation was done by STATISTICA 13.
Results: Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design did not reveal any clinically nor statistically significant deviations ($p < 0.05$). Comparing the initial values of the old and new design for venous collection, both EDTA tubes resulted in a highest deviation of 3.0% for RBC. Comparable highest deviations for initial values in relation to 48h values were obtained for K2EDTA (WBC 0.4%; RBC 0.1%) and K3EDTA (WBC 2.6%; RBC 0.1%). Capillary collection led to a highest deviation for WBC of 0.7% for K2EDTA and of 2.2%.the K3EDTA tubes.
Conclusion: From a clinical perspective, the MiniCollect K2EDTA and K3EDTA tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

P061

Microscopic investigation of the cytotoxic effects of Bis-2-ethylhexyl-phthalate on HL-60 cells

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Abstract:
Introduction: Within the phthalates, bis-2-ethylhexyl-phthalate (DEHP) contains the highest toxic effect on the reproductive system. DEHP is not bound to plastic material and therefore can leach, migrate or evaporate, which makes it ubiquitous. Contact occurs by inhalation, dermal, oral or intravenous exposure. DEHP is classified as group 2B (possibly carcinogenic to humans) by the International Agency for Research on Cancer. Therefore, in this study cytotoxic effects of DEHP on cells were examined.
Material and Methods: HL-60 cells were treated with 100 µg/ mL (256 µM) DEHP in dimethyl sulfoxide (DMSO) (n = 3). The negative control was treated with 1% DMSO correspondingly and the positive control with 6 µM camptothecin. The treatment lasted two or 24 hours, respectively. To detect cytotoxic effects of the plasticizer, cells were stained with the two fluorescent dyes NucView 488 caspase-3 substrate and annexin V (CF568 conjugate) and examined with inverse fluorescent microscopy.
Results: In this setting, a significant increase of apoptotic cells could be observed when treated with 100 µg/mL (256 µM) DEHP for 24 hours or two hours compared with cells of the negative control. For example, cells treated for two hours showed an amount of apoptotic cells of 11 ± 3% without DEHP and 27 ± 12% with DEHP ($p < 0.05$). When treated for 24 hours, cell showed an amount of apoptotic cells of 13 ± 10% without DEHP and 43 ± 8% with DEHP ($p < 0.05$).

Conclusion: The cytotoxic effect of DEHP seems therefore to occur immediately and increase related to the exposure time. This clarifies the necessity, to search for better plasticizers to replace DEHP from more products like storage containers of red blood cells and find a suitable equivalent. It also should be acknowledged when using products containing DEHP as plasticizer.

P062

Is magnesium sulfate suitable as in vitro anticoagulant in the hematological routine laboratory?

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Introduction:
EDTA-induced pseudo-thrombocytopenia (PTCP) is a rare (0,1 – 2,0 %) but clinically relevant in vitro phenomenon, which was also, but less frequent reported from citrate anticoagulated blood. Another disadvantage of EDTA is the time-dependent platelet swelling. The anticoagulant in vitro properties of magnesium sulfate (MgSO4) were described for the first time by Bizzozero more than hundred years ago but fell into oblivion when EDTA became introduced as standard anticoagulant for the automated measurement of CBC and LDC (ICSH guidelines from 1993).
We recently have shown in patients with PTCP that anticoagulation with MgSO4 efficiently avoids platelet aggregation (Schuff-Werner 2013). Users of the in the meanwhile commercially available MgSO4-anticoagulated test tubes (ThromboExact, Sarstedt, Nümbrecht, Germany) are increasingly asking whether magnesium anticoagulated blood is suitable for additional examinations in the hematological routine laboratory. Therefore we conducted the here presented study.
Methods:
CBC and LDC were measured in 100 blood samples, anticoagulated in parallel with EDTA, citrate and MgSO4, using a XE 5000 hematology analyzer (Sysmex Europe, Norderstedt, Germany). Blood smears were analyzed by computer-assisted digital microscopy (Diffmaster DM 96, Cellavision) and also by conventional microscopy. The time-dependent stability of CBC and LDC up to 24 hours was tested in differently anticoagulated blood samples drawn in parallel from 9 volunteers. Statistical analyses were performed by Spearman rank order correlation and Wilcoxon signed rank test.
Results:
The anticoagulation with EDTA, citrate and MgSO4 resulted in definitely comparable CBC and LDC, although the platelet counts were up to 10% and MPV approximately 1,0 fl lower in citrate- and MgSO4-anticoagulated blood.
Compared to EDTA, the time -dependent stability of MPV in MgSO4-anticoagulated blood was obviously superior, whereas the automated as well as the microscopic LCD showed that the morphological integrity of leukocytes in citrate and MgSO4-anticoagulated samples was less stable (only up to 4) hours as compared to EDTA (24 hours).
Conclusion:
MgSO4 is suitable for the measurement of CBC and LDC up to 4 hours after blood sampling. In contrast to EDTA-anticoagulation, MPV and platelet counts were shown to be stable up to 24 hours.

P063

Increased VAP-1 concentration and activity are associated with liver fibrosis in chronic Hepatitis C

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Vascular adhesion protein 1 (VAP-1) is a 170 kDa glycoprotein with amine oxidase activity (also called SSAO activity). It is released from various cell types including endothelial cells, muscle cells, adipocytes, and hepatic sinusoidal endothelium. It triggers inflammation non-enzymatically through attraction of leucocytes via the secretion of chemokines and adhesive functions as well as through an enzymatic pathway via the conversion from amines in aldehydes and hydrogen peroxide.
VAP-1 concentration and, in part, activity have been reported to be modified in various disease conditions such as atherosclerosis, diabetes mellitus and chronic kidney injury. VAP-1 concentration is also elevated in chronic liver diseases (CLD). It has been suggested that VAP-1

represents a marker for inflammation and fibrosis in liver. Blocking VAP-1 activity is currently being tested for the treatment of inflammatory diseases.
Our aim now was to study the role of plasma VAP-1 in chronic liver diseases and thus, we measured plasma VAP-1 concentration and activity in 322 patients with chronic hepatitis C virus infection in different stages. We also assessed the association between VAP-1 and liver stiffness (transient elastography) and biopsy results (n = 92 patients).
We found that the VAP-1 concentration correlated strongly with liver stiffness (r = 0,599; p = 8,6e-33). Additionally, stratification of the patients according to this measure of liver fibrosis showed a significant increase of VAP-1 concentration with progressive disease. The same pattern was seen with histologically confirmed stages of fibrosis (n = 92). In linear regression analysis including liver function tests, age, BMI and enzyme concentrations, VAP-1 concentration remained the strongest influencing variable for liver stiffness and thus evolved as independent noninvasive predictor of fibrosis severity.
ROC analysis showed that a cut-off of 541 ng/mL VAP-1 predicted histologically confirmed cirrhosis (sensitivity 73,7%; specificity 72,2%; AUC 0,791).
In contrast, the VAP-1 activity correlated only modestly with the Fibroscan values (r = 0,395; p = 2,1e-13) and displayed only a relatively small increase in advanced fibrosis stages.
To our knowledge, this is the first study on VAP-1 in chronic hepatitis C, that shows an association between VAP-1 and fibrosis severity. Our results confirm that VAP-1 concentration, but not activity, increases with the severity of fibrosis progression. Our results lead us to speculate that the non-enzymatic effects of VAP-1 may be more relevant for monitoring fibrogenesis than its enzymatic activity. This finding may lead to new therapeutic strategies for VAP-1 inhibition. Further studies on VAP-1 concentration are warranted to see whether VAP-1 is a reliable biomarker in CLD.

P064

Monitoring of peripheral blood stem cells for apheresis with the Sysmex XN 20 automated blood cell analyser compared to flow cytometric enumeration of CD34+-cells

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Introduction:
For an optimal stem cell yield the timing of stem cell apheresis is of major importance. The gold standard method of flow cytometry is expensive and time consuming. We therefore assessed the XN-HPC of the Sysmex XN 20 in comparison to our standard CD34+-cell counting method. The Sysmex XN 20 delivers results much quicker and thus offers more flexibility for medical treatment. Our goal was to assess if the results delivered were as valid as those of the CD34-method.
Methods
We evaluated 175 blood samples from 74 patients with different haematologic malignancies and 8 healthy donors. The samples were used from the routine CD34+-monitoring workload. They were anticoagulated with K2EDTA and tested within 2 hours from blood collection. All samples were measured by using the standard CD34+-enumeration procedure (BDTM Stem Cell Enumeration Kit, FACSCanto II, Becton Dickinson) as gold standard in comparison to the XN-HPC value of the automated haematology analyser (XN series, Sysmex).
Statistical analysis was performed using Coefficient of determination and Fisher's exact test.
Results
Analysing all monitoring results (n = 175) we observed a moderate correlation between CD34-results and XN-HPC (R2 = 0.8509, slope 0.7715 intercept 12.001). Considering only the data less than 50 CD34+-cells/µl (n = 95) the correlation reduced to R2 = 0.5424, slope 0.8898, intercept 5.0682.
German regulatory guidelines recommend a cut-off of at least 10 CD34 cells/µl for apheresis start. During our statistical analysis (Fisher's exact test) we came to the conclusion that for the XN-HPC the most comparable value to 10 CD34 cells/µl is a value of 15 XN HPC cells/µl. To answer our initial question concerning decision to apheresis start we looked at the predictive value of all results getting a positive predictive value of 0.97 and a negative predictive value of 0.69 at the cut-off-value of 15 XN-HPC/µl.
For 160 samples both testing methods led to the same medical decision. In 15 cases the results differed, all of them being at the lower measurement limit. In our clinical setting only 6 patients remained as being treated differently.
Conclusions
With the XN-HPC measurement the Sysmex haematology analyser could provide a rapid and cost effective method for the timing of apheresis. In order to minimise uncertainties we propose to set a cut-off point for apheresis start at greater than 30 XN-HPC cells/µl comparable to 20 CD34 cells/µl. Over the last years this limit has proven to provide a sufficient stem cell product. Below these cut-off points a single patient decision has to be made.

P065

The Hevylite ratio, a prognostic marker for survival of patients with IgA type multiple myeloma

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Objectives: For monitoring patients with multiple myeloma (MM), the quantification of the M-protein in the serum protein electrophoresis is the most important laboratory parameter. Alternatively, IgA subtypes can be quantified by Hevylite® Test, an immunoassay measuring both light-chain restricted immunoglobulins separately (IgAκ and IgAλ). The aim of the study was, to evaluate the significance of the HLC ratio (IgAκ/IgAλ) for the estimation of the survival of patients with multiple myeloma.
Methods: The diagnostic value of the initial HLC ratio is assessed by means of its impact on the survival using a Cox regression model. Hereby, the sample size is n = 70 patients and the number of events (death) is n = 24. Additionally, the relation between the initial HLC ratio and a second measurement is quantified by means of a linear regression based on a sample size of n = 53 patients (after removal of patients with missing data in the second measurement).
Results: The initial HLC ratio observed at the date of prognosis (t = 0) is used after a log-transformation as predictor in a Cox proportional hazard model. The logarithms of the HLC ratios are approximately symmetric around zero such that the absolute values are regarded as sufficient measures for the deviation from the neutral zero. The impact of the initial HLC ratio on the hazard rate is significant based on the likelihood ratio (p = 0.022). The median survival time for abs(log(HLC ratio)) = 0 (i.e. HLC-ratio = 1) is predicted as 1775 days. The median survival time drops down to 390 days for a HLC ratio = 4409, which corresponds to the maximum value observed. The log HLC ratios of the second versus the first measurements are depicted in a scatter diagram and analysed using linear regression. The means of the first and second measurements, 4.07 and 3.04, respectively, differ significantly (t-test yields p = 0.02). The linear regression gives an intercept of 1.4 (se = 0.50) and a slope of 0.41 (se = 0.11). Thus, the initial log HLC ratios less than 2.36 lead to increased second measurements due to the positive intercept and decreased values otherwise due to the moderate slope.
Conclusion: The HLC ratio is a prognostic marker for survival of patients with IgA type multiple myeloma.

P066

Hyperactivity of mTOR in Acute Lymphoblastic Leukemia might be associated with polymorphims in the mTOR 3'UTR

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Acute Lymphoblastic Leukemia (ALL) is the most common malignant disease in childhood. Though the outcome under current chemotherapy regimens has improved, there are still numerous patients suffering from relapse and progressive disease even after hematological stem cell transplantation. mTOR is involved in multiple cellular processes like proliferation and cell growth. By controlling translation, mTOR can generate growth of neoplastic cells when its activity is enhanced. Especially high risk ALL is associated with mTOR hyperactivity, but the pathophysiologic reason for this hyperactivation remains still unclear. Mutations in regulatory gene areas like promoters and 3'UTR can result in protein overexpression and therefore hyperactivity. To determine the putative impact of SNPs in the mTOR promoter region and the 3'UTR on ALL, a series of pediatric ALL patients was genotyped and compared to healthy Chinese and Caucasian blood donors.
Genotypes of the promoter polymorphisms rs2295080 (c*-3162 A > C) and the neighbouring rs2295079 (c*-3099 C > G) were determined by Restriction Fragment Length Analysis. The 3'UTR SNPs rs79536981 (c* 7834 C > T), rs12139042 (c* 8167 G > A) and rs2536 (c*8600 A > G) were analyzed by Pyrosequencing. The assessment of the functional impact of the SNPs rs12139042 and rs2536 was done by in silico analysis and measurement of mRNA stability by Luciferase reporter assays.
The two promoter SNPs are in complete linkage disequilibrium. Minor allele frequency (MAF) was 0.193 in the Chinese cohort, whereas the Caucasian (MAF 0.305) and the ALL cohort (MAF 0.324) have similar frequencies. The 3'UTR SNP on position c* 7834 showed only the C allele in all cohorts. The 3'UTR SNPs c*-8167 and c*-8600 are in complete linkage disequilibrium. We detected the highest MAF in the Chinese cohort (MAF 0.092), with lower rates in the Caucasian cohort (MAF 0.016), but compared to that increased rates in the ALL cohort (MAF 0.033). The in silico analysis of the mTOR 3'UTR revealed a differing folding structure dependent on genotypes. A significantly higher reporter activity of c*8600 G compared to c*8600 A was detected (p = 0.049). Constructs containing both SNPs (c*8167 A and c*8600 G) also had a higher reporter activity (p = 0.042). Though further investigations are necessary, these results suggest that the 3'UTR SNPs could lead to an altered mRNA stability and affect ALL risk. This could be an important finding for diagnostic and therapeutic approaches in the future.

P067

Difficulties in diagnosing antiphospholipid syndrome in patients undergoing DOAC therapy

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Objective: Dabigatran, rivaroxaban and apixaban are now common as therapy for atrial fibrillation or prevention of deep vein thromboembolism. These direct oral anticoagulants (DOACs) affect haemostaseologic laboratory assays, which makes the diagnosis of thrombophilia in patients who receive those drugs difficult. The aim of our study was to investigate the effect on two different dilute Russell viper venom time (DRVVT) assays and the lupus sensitive PTT in spiked samples from healthy volunteers and in samples from patients receiving a DOAC.
Methods: Spiking experiments were performed with the original substances of these drugs at plasma concentrations of 0, 10, 30, 50 and 100 ng/mL. In order to measure the plasma levels of the drug we used ultra-performance liquid chromatography coupled with electrospray ionization-tandem mass spectrometry (Kuhn et al, 2015). The DRVVT assays we performed were from Instrumentation Laboratory (IL) and Stago, while the lupus sensitive PTT was from IL.
Results: Samples spiked with apixaban showed no effect on the ratio of DRVVT assays whereas between 7 % and 11 % of samples from patients receiving apixaban showed values above the cut-off. Up to 71 % of dabigatran spiked samples (100 ng/mL) showed pathological ratio values. By contrast, no effect could be detected in the patients' samples. For rivaroxaban, the DRVVT assays were affected in both the spiked and patient samples.
Conclusion: LA/APS testing during DOAC therapy is a difficult and unreliable way of thrombophilia diagnosis. Due to interferences of the assays with the drugs, LA/APS testing should only be performed before the next DOAC intake (trough level) or during a longer interruption of DOAC intake. However, positive results could indeed still turn out to be false positive.

P068

Development and clinical application of LC-MS/MS multi-analyte methods for monitoring of direct oral anticoagulants

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Aims: The direct acting oral anticoagulants (DOACs) have been developed and introduced as alternatives to vitamin K antagonists (VKAs) and are now widely used in clinical practice. DOACs are being administered in fixed-dose regimens and routine coagulation monitoring is not required. As the anticoagulant effect of DOACs is considered largely concentration dependent, inadequate exposure to DOACs may increase the risk of bleeding or thromboembolic events.
Methods: Two LC-MS/MS assays for the simultaneous quantification of apixaban, dabigatran, edoxaban and rivaroxaban with use of stable isotope labeled analogues (13C,2H7-apixaban, 13C6-dabigatran, 2H6-edoxaban, 13C6-rivaroxaban) as internal standards were developed and validated according to international guideline recommendations. Sample preparation for one method consisted of protein precipitation with acetonitrile and centrifugation, while sample clean-up for the other assay was based on commercially paramagnetic microparticles (MagSIMUS-TDMPREP Type II kit; MagnaMedics Diagnostics BVGeleen, The Netherlands) and subsequent magnetic depletion. Clinical samples were obtained from routine services at the University Hospital of Cologne. In patients treated with rivaroxaban and scheduled cardiac catheterization, peri-procedural plasma concentration results obtained by LC-MS/MS were compared to those determined by a CE-labeled chromogenic anti-FXa assay (COAMATIC® Heparin, Chromogenix Instrumentation Laboratory, Bedford, USA).
Results: LC-MS/MS assays enabled linear quantification of DOACs across the concentration range of 1-500 ng/ml and 2-500 ng/ml ($R^2 > 0.99$ for all analytes) within a run time of 2.5 and 2 minutes, respectively. Inter- and intra-day precisions and trueness results remained within the acceptance criteria ±15%. The LC-MS/MS methodology was introduced into clinical practice and its applicability demonstrated in clinical cases with documented overdose, non-adherence to medication, and use of DOACs in patients with severe obesity. Compared to results obtained by LC-MS/MS, the anti-FXa assay showed inaccuracies at low rivaroxaban concentrations and in heparin containing plasma samples.
Conclusions: LC-MS/MS multi-analyte methods offer considerable advantages. The fast and easy methodology enables simultaneous quantification of several DOACs within one analysis and can thus serve as a screening assay when it is not known if or which particular DOAC was taken by a patient. LC-MS/MS assays have excellent sensitivities and are less likely to suffer from reagent variability. Due to direct quantification of analytes, DOACs can be quantified by LC-MS/MS in other matrices than plasma, e.g. breast milk or forensic samples. Monitoring of DOACs may be useful in special situations, e.g. before planned interventional procedures or suspected non-adherence to medication. Future studies are needed to assess the benefits of monitoring of DOACs.

P069

Diagnosing and classifying von Willebrand disease: Testing for platelet-dependent von Willebrand factor activity as essential element of the diagnostic repository

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Objective:
Von Willebrand disease (vWD) is the most common inherited bleeding disorder. Acquired von Willebrand disease (avWD) comprises a deficiency or defect in von Willebrand factor (vWF). Diagnosing vWD is a complex and not always straightforward process accompanied by limitations in test procedures. It suffers from over- and under-diagnosis as well as misinterpretation in classification.
The historical assay for evaluating the platelet-dependent vWF function (vWF:RCo) is known for a high imprecision and low sensitivity. The new generation assays offer more reliable results. According to the new nomenclature they differentiate between vWF:GPIbR (ristocetin-induced binding of vWF to a recombinant wildtype GPIb fragment) and vWF:GPIbM (spontaneous binding of vWF to a gain-of-function mutant GPIb fragment).
Methods:
In 441 routine samples vWF-Antigen (vWF:Ag), vWF-Collagen-binding-activity (vWF:CB) and vWF-Ristocetin-induced-binding (vWF:GP1bR) were determined applying HemosIL AcuStar-Assays (Instrumentation Laboratory, Werfen Group, Kirchheim) including the calculation of vWF:GP1bR /Ag- and vWF:CB/Ag-ratio.
The vWF-binding in the vWF: GP1bR-Assay is proportionally compared to the former ristocetin cofactor activity. The assay overcomes the limitations of the vWF:RCo-Assay, based on the agglutination of formalin-fixed normal platelets in presence of ristocetin.
Results:
The samples contained 402 normal constellations as well as 11 low von Willebrand-Factor (LWF), 7 type 1, 4 type 2A, 1 type 2B, 11 type 2M and 5 avWD cases. In all cases with normal, LWF and type 1 vWD the vWF:GPIbR /Ag- as well as vWF:CB/Ag-ratio was >0,7. In type 2A and type 2B cases both ratios were ≤ 0,7. In cases with type 2M constellation either vWF:GPIbR /Ag- or the vWF:CB/Ag-ratio was ≤ 0,7. In avWD cases the pattern of the ratios varied.
Conclusion:
The results are in concordance with the algorithmic approach published by Favaloro and Leebeek & Eikenboom. They underline the importance to include a test covering the glycoprotein GP1b-activity of von Willebrand factor within the primary test-panel.
In 2 cases the constellation of the new assay panel stressed the requirement to reassign the classification, changing 1 case from type 1 as well as 1 case from type 2A to type 2M. This relates to reports from other groups on the requirement of re-classification in certain instances.
The secondary differentiation of type 2A and 2B cases with an identical pattern of vWF:CB/Ag- and vWF:Ac/Ag-ratio could be achieved by light transmission aggregometry applying low- and high-dose ristocetin.
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P070

A novel Cys486Trp mutation in the integrin β3 leads to the disruption of an intramolecular disulfide bridge

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Background: Glanzmann thrombasthenia (GT) is an autosomal recessive bleeding disorder that is caused by quantitative or qualitative abnormalities in the integrin αIIbβ3. The patients show an absence of platelet aggregation to all physiological agonists except Ristocetin. Normally routine diagnostics and the functional analysis by light transmission aggregometry (LTA) are sufficient for the distinct diagnosis. Additionally, flow cytometry and DNA sequencing are the methods of choice for a more detailed analysis.
Methods: For the molecular genetic analysis we studied six patients whose diagnosis was consistent with GT. Genomic DNA was isolated from EDTA-anticoagulated whole blood. The respective gene regions were amplified by PCR using the HotStar Taq DNA polymerase. For DNA sequencing we used the Sanger method. Sequencing was performed in an Applied Biosystem sequencer.

Results: In the GT patient population we found two mutations already described. Furthermore, the sequence analysis revealed a novel homozygous C to G transversion in the Exon 10 of the ITGB3 gene. This mutation resulted in a Cys to Trp amino acid substitution on position 486. The normally expressed Cys486 is localized in a cysteine rich, proteinase-resistant core of the integrin β3 and usually forms an intramolecular disulfide bridge with the Cys473. The patient with the Cys486Trp mutation showed the for GT typical pathognomonic aggregation. Conclusions: We found a novel mutation in the integrin β3 that implicate a Cys to Trp substitution, which leads to the disruption of an intramolecular disulfide bridge. We suggest that the GT phenotype is the result of this Cys486Trp mutation.

P071

p45 NF-E2 deficiency results in background dependent embryonic lethality in mice

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p45 NF-E2 deficiency results in background dependent embryonic lethality in mice
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Objective: p45 NF-E2 is a transcription factor and has been shown to play a role in megakaryocyte maturation. It has recently been shown that the absence of p45 NF-E2 in placenta is associated with excess syncytiotrophoblast formation and intrauterine growth restriction (IUGR) in humans and mice. Intriguingly, p45 NF-E2-/- mice survive to birth and reproduce normally on a 129/Sv background, but display embryonic lethality on C57/Bl6 background. We therefore aim to characterize the embryonic lethality and related genetic modifier in these mice.
Methods: p45 NF-E2 mice were backcrossed for 10 generations on 129/Sv and C57/Bl6 backgrounds and post-natal survival of these mice was assessed. We then performed timed matings with p45 NF-E2 mice on C57/Bl6 background and placental and embryonic tissues were obtained at day 12.5, 14.5 and 16.5 p.c. The embryos were visualized under the microscope for visual abnormalities and further genotyped. Placental vascularization was studied by H&E staining. RT-PCR for markers (Tpbpa, Pl-II, Esx-1 and Gcm-1) was done to evaluate trophoblast differentiation. In order to study background associated effects, microsatellite markers from different backgrounds are assessed using PCR.
Results: We observed that the p45 NF-E2-/- mice are embryonic lethal on C57BL6 background. Furthermore, these embryos develop normally until day 12.5 but display vascular abnormalities starting at day 14.5 and are absent at day 16.5 p.c. The placenta of p45NF-E2-/- showed altered vascularization and differentiation compared to p45 NF-E2+/+ littermates which would result in impaired nutrient uptake. We are currently investigating the role of SNPs and microsatellite markers on different genetic backgrounds.
Conclusion: Embryonic survival in p45 NF-E2-/- mice is dependent on genetic background of the mice. Deficiency of p45 NF-E2 results in vascular defects on a C57/Bl6 background. Further studies evaluating potential SNPs and microsatellite markers regulating embryonic survival in 129/Sv background are required.

P072

„Role of the TM-PC System in the regulation of the tubular regeneration in diabetic nephropathy“

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Background
Diabetes mellitus (DM) is a worldwide increasing health problem. Secondary to its long-term vascular complications it causes a burden for patients as well as for health systems. Diabetic nephropathy (dNP) which is a microangiopathic sequelae of DM is the main cause of end-stage renal failure in western societies. The pathophysiological relevance of tubular cells in dNP is increasingly recognized. Tubulointerstitial fibrosis is closely associated with the decline in renal function and chronic kidney disease (CKD). In dNP cells undergo a proliferative arrest and senescence. These pathological changes are associated with a higher risk for and impaired outcome in acute kidney injury (AKI). The underlying mechanisms remain obscure, hampering the development of specific biomarkers and therapeutic approaches. Considering the potent nephroprotective effects of the coagulation protease activated protein C (aPC) we hypothesized a role of coagulation protease dependent signalling for tubular injury in dNP.
Methods
Persistent hyperglycaemia, reflecting type 1 DM, was induced in wild type (Wt) mice using streptozotocin (STZ) and maintained for 16 weeks. In diabetic and non-diabetic control mice with and without aPC treatment we induced acute renal injury using the ischemia reperfusion injury (IRI) model. Subsequently ex-vivo analysis was performed (albuminuria, BUN, PAS-staining, immunofluorescence staining, colony-formation-assay, western blot).

Results
Following IRI albuminuria (albumin creatinine ratio, ACR) and blood urea nitrogen level (BUN) were increased in diabetic mice compared to non-diabetic mice, reflecting more severe acute kidney injury in diabetic mice. Pretreatment with aPC for 2 weeks in advance of IRI ameliorated the diabetes induced worsening. Histological analyses of PAS-stained sections revealed improved outcome following aPC pretreatment, as reflected by reduced tubular dilatation, tubular lysis and cast formation. Proliferation, as indicated by Ki-67 immunofluorescence staining, was reduced in diabetic mice, reflecting impaired regenerative tubular capacity. Pretreatment with aPC dramatically increased the proliferation capacity. Protein expression of the proliferation marker PCNA was upregulated in the kidney lysates of aPC pretreated mice compared to DM control and DM IRI. While the tubular injury marker KIM-1 was reduced indicating tubular protective effects of aPC. Consistent with these findings, isolated tubular cells from aPC pretreated mice showed more colony formation capacity.
Conclusion
These findings suggest a protective effect of aPC in acute-on-chronic renal injury through enhanced tubular regeneration. Studies to further delineate the underlying mechanism are ongoing.

Neue analytische Methoden

P073

A bioluminescence-based ATP assay for the rapid detection of multidrug-resistant Gram-negative bacteria

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Objective:
Accurate detection of multidrug-resistant (MDR) Enterobacteriaceae constitutes a major healthcare problem and a laboratory diagnostic challenge. Aim of the present study was to develop a rapid phenotypic assay for the detection of MDR Enterobacteriaceae.
Method:
We developed a bioluminescence-based assay to detect ATP levels which correlate proportionally with the number of bacteria. Bioluminescence was generated by BacTiter-Glo microbial viability assay. ATP levels were measured after incubation of the bacteria with 1mL AST broth with and without antibiotics (ciprofloxacin, ceftazidime/cefotaxime, meropenem and piperacillin). Light emission (measured in relative light units (RLU)) was detected after 2.5h of incubation at 37°C by the GloMax Integrated Luminescence System (Promega) and relative induction was calculated. The BD PhoenixTM was used as reference method for antibiotic susceptibility testing. For the evaluation of the ATP assay, inter alia, 60 genotyped carbapenemase-producing isolates were used. The investigated study population consisted of 185 Enterobacteriaceae isolates with different phenotypic resistance patterns.
Results:
180 out of 185 (97%) investigated isolates were correctly identified by our ATP assay. All of the antibiotic susceptibility tests (AST) performed with ciprofloxacin and ceftazidime/cefotaxime were in accordance with the Phoenix AST results. In two cases the results for meropenem by the ATP assay were incorrect (2/88; 2%), once false positive and false negative. However, the incubation period for piperacillin susceptibility was extended to 4h to gain sensitivity and specificity. The results of the ATP assay for 94 out of 97 isolates were in accordance with those of Phoenix AST. We also performed our assay for positive blood cultures testing meropenem susceptibility and revealed 100% concordance to the PhoenixTM AST results (n=20).
Conclusion:
Our bioluminescence-based ATP assay enabled accurate and rapid detection of the most common antibiotic resistances in Enterobacteriaceae within 2.5h and is an additional option for microbiological laboratories to identify MDR in Enterobacteriaceae within few hours.

P074

Analytical performance and diagnostic accuracy of a new fully automated androstendione chemiluminescence immunoassay

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Objective: Androstendione is an androgen produced as an intermediate product of the biosynthesis of testosterone and estradiol in the testicles, in the ovaries and also in the adrenal cortex. Measurement is used for diagnosing and differentiating hirsutism and virilisation, enzyme deficiencies of the steroid hormone biosynthesis and in suspicion of androgen-producing tumors.

Methods: Specimens included de-identified residual serum specimens submitted for routine testing and banked adult and pediatric sera. Samples were measured with tandem mass spectroscopy, two automated immunoassays, the newly developed DiaSorin LIAISON androstendione assay and the Immulite assay, and a radioimmunoassay (Beckman Coulter) each according to the manufacturer's protocol. Performance characteristics evaluated included method correlation, analytical sensitivity, linearity and precision. Diagnostic accuracy was evaluated by verifying the respective reference ranges of the different assays and receiver operating characteristic (ROC) analysis.
Result: Passing-Bablok regression of the LC-MS/MS versus the LIAISON was y = 0.65x-0.01, Pearson's coefficient of correlation r = 0.875 (n = 175); LC-MS/MS versus Immulite was y = 0.60x + 0.21, r = 0.746 (n = 125), LC-MS/MS versus RIA was y = 0.75x + 0.01, r = 0.956 (n = 80); LIAISON versus Immulite was y = 0.96x + 0.25, r = 0.745 (n = 135); LIAISON versus RIA was y = 1.17x + 0.01, r = 0.954 (n = 79); and Immulite versus RIA was y = 1.29x-0.34, r = 0.810 (n = 40). The RIA showed the highest analytical sensitivity (0.09 ng/mL) followed by LC-MS/MS (0.10 ng/mL), LIAISON (0.3 ng/mL) and Immulite (0.51 ng/mL). Repeatability CV's were 1.0 – 2.6% with LIAISON, 1.8 – 3.5% LC-MS/MS, 3.4-7.5% with RIA and 4.0 – 9.8% with Immulite respectively. Within laboratory CV's were 2.4 – 9.7% with LIAISON, 3.6 – 9.9% LC-MS/MS, 4.8 - 11.3% with RIA and 6.4 – 15.2% with Immulite, respectively. All methods exhibited good linearity in dilution over the respective analytical measuring range. The area under the curve of the ROC analysis for the correct diagnosis of a hyperandrogenism was 0.96 with the LC-MS/MS, 0.94 with RIA, 0.93 with LIAISON and 0.85 with Immulite, respectively.
Conclusion: Due to the methodology LC-MS/MS demonstrated the highest analytical specificity, good performance and excellent diagnostic accuracy. The best agreement was found with the RIA method. Although, the coefficient of correlation between LC-MS/MS and LIAISON is lower, the assay demonstrated the best analytical performance and a similar diagnostic accuracy. These data suggest that the LIAISON may be a suitable alternative for the measurement of androstendione with all advantages of a fully automated assay. In contrast the Immulite showed the worst correlation with a systematic difference to the other assays, poor performance data and diagnostic accuracy.

P075

Analytical performance of the first fully automated immunoassay for the determination of intact fibroblast growth factor 23 (FGF-23)

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Objective: There is growing interest in measuring plasma fibroblast growth factor 23 (FGF23) concentrations in a number of clinical settings. However, a reliable assay with acceptable performance is lacking. For the first time a fully automated immunoassay was deveoped for the DiaSorin LIAISON immunoassay system.
Methode: Plasma samples of healthy adults and patients with different stages of chronic kidney disease were used to compare the precision, recovery, linearity and the pre-analytical stability characteristics of a new fully automated FGF23 (intact) assay with a commercially available FGF23 (intact) ELISA. Method agreement was evaluated, reference and stage-specific ranges for kidney disease were established.
Result: The intermediate imprecision for FGF23 plasma pools between concentrations of 18.2 – 2687.6 pg/mL ranged from 5.8 to 7.1% CV using the CLIA method whether the corresponding CV´s for the ELISA ranged between 6.9 and 19.2% CV. Limits of blank (LoB), detection (LOD) and quantification (LOQ) of the immunoassay were 1.0 pg/mL, 4.7 pg/L and 5.9 pg/L for the LIAISON XL CLIA and 1.9 pg/mL, 12.8 pg/mL and 15.4 pg/L for the ELISA assay, respectively. Both methods demostrated high levels of linearity. The coefficient of correlation between the expected theoretical values was 0.9997 for the LIAISON CLIA and 0.9995 for the Immunotopics ELISA method, respectively. The LIAISON XL FGF23 CLIA and the manual Immunotopics ELISA assay displayed a strong correlation (Spearment rank correlation at 0.997) The Passing-Bablok regression analysis resulted in a slope value from 0.78. The LIASON FGF23 measurements showed up to 22% higher concentrations as compared to the Immunotopics FGF23 (intact) ELISA not only over the entire measuring range (4.7 – 5000 pg/mL) but also in diluted samples up to 35.000 pg/mL. For the LIAISON XL FGF23 CLIA no high-dose hook effect was observed in samples with concentrations up to 35.000 pg/mL whether the Immunotopics ELISA assay showed noticeably reductions of the true concentrations in samples higher than 10.000 pg/mL. The FGF23 (intact sample stability showed a variation The FGF23 (intact) sample stability showed a variation of less tha 6% between 0 and 16 h at room temperature, 3 days at 2-8° C or 90 days frozen stored at -20° C for EDTA plasma. The median FGF23 (intact) values measured with the LIAISON XL CLIA were 52.6 pg/mL and 57.9 pg/mL respectively for females and males aged between 18 and 93 years with a normal GFR. No significant difference between both genders was observed. The medians in the patient groups with CKD were 98 pg/mL, (stage 1), 133 pg/mL (stage 2), 176 pg/mL stage (3), 239 pg/mL (stage 4) and 855 pg/mL (stage 5) respectively.
Conclusion: The new fully automated FGF23 (intact) assay demonstrates excellent analytical performance data. As a consequence, the availability of this assay will represent a robust, fast and precise alternative to manual FGF23 testing.

P076

Novel Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) Protocols for Metal Imaging and Visualization in Experimental and Clinical Wilson's Disease

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Background: Metal intoxication may be the consequence of inherited disorders (e.g. hemochromatosis, Wilson's disease), chronic metal intake, or acute intoxication. There is an urgent need for analytical methods for determining precise metal content within a tissue [1]. LA-ICP-MS is an innovative bioimaging diagnostic method with multi-element capability, granting the simultaneous analysis of a large variety of biological materials with high spatial resolution even at extremely low concentrations [2]. However, the mapping and visualization in tissue specimens by LA-ICP-MS is technically challenging. We have previously established LA-ICP-MS protocols for trace metal imaging in livers and brains of wild type and Atp7b deficient mice representing an experimental model of human Wilson's disease [3].
Methods: Novel standardization and normalization methods were established for accurate metal measurements. Heat maps of individual metals and digital image overlay were visualized with a novel software tool (i.e. Excel Laser Ablation Imaging, ELAI) that is based on Microsoft Excel Visual Basic for Applications [4-5].
Results: The progressive cerebral copper overload in Atp7b-deficient mice is simultaneously associated with alterations in iron and zinc concentrations. Similar results were found in human liver samples obtained from patients suffering from genetically proven Wilson's disease. In addition, comparative analysis of hepatic metal content in healthy and diseased liver samples showed that the copper concentration in diseased liver was approximately 10 fold higher than in specimen taken from healthy donors (500 μg/g tissue vs. 50 μg/g tissue), while the concentrations of iron and zinc showed individual variances ranging from 100 to 400 μg/g and 30 to 70 μg/g liver tissues. The generated images prepared with ELAI show that cerebral copper mainly accumulates in defined cerebral regions. Moreover, our data show that digital image overlay in ELAI is highly helpful for visualization of regional trace metal accumulation and in interpreting of metal-induced tissue damage.
Conclusions: LA-ICP-MS is a highly powerful analytical technique for metals quantification in experimental and clinical metal overload diseases such as Wilson's disease. It allows metal measurements with high sensitivity, spatial resolution, specificity, and quantification ability. The digital overlay of metal maps into a composite will be an important add-on in visualization and interpretation of coherent or opposite metal quantities within tissue samples. This methodology will have tremendous impact on biomedical and clinical research.
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P077

Isoform-specific quantitation of human growth hormone via a bead-based MSIA assay

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Introduction: Human growth hormone (hGH) is a key player in the age-adjusted development of infants, children and adolescents. By virtue of alternative splicing, hGH is expressed in various isoforms whose detailed functions are still unknown since commercially available immunoassays cannot perform an exact quantitation of these hGH isoforms. Additionally, such immunoassays are known to suffer from interferences with growth hormone-binding protein and may have a lack of specificity due to antibody cross-reactivity.
Consequently, we developed a Mass Spectrometry ImmunoAssay (MSIA) for a reliable concurrent quantitation of total hGH and its isoforms.
Methods: In a targeted proteomics assay U-15N-labeled analogues of 20 kDa hGH and 22 kDa hGH were used for internal standardization. hGH isoforms and internal standards were enriched from 200 μL human serum applying polyclonal rabbit anti-hGH antibodies coupled to Protein A-modified magnetic beads. Omitting an elution of captured hGH isoforms, basic proteomics sample preparation steps comprising denaturation, reduction, alkylation and in solution tryptic digestion were performed. Subsequently, samples were purified in an on line SPE prior to reversed-phase chromatographic separation performed at 1.5 mL/min resulting in a total run time of 5 min. A hybrid triple quadrupole mass spectrometer was used for MS detection.
Results: The single steps of the complex sample preparation procedure including bead incubation, alkylation and tryptic digestion were optimized for the application in a 96-well format facilitating a highly sensitive high throughput application. The methodology proved to be linear as low as a total hGH concentration of 0.5 ng/mL. In a first comparison with a commercial immunoassay (hGH from IDS-iSYS) it could be shown that in average by 30% higher concentrations were determined by our LC-MS/MS approach. For this comparison values of hGH

ranged from 0.9 ng/mL to 70 ng/mL and a Pearson correlation coefficient of 0.995 was obtained. In a ring trial for MS-based hGH quantitation the target value was missed by only 22%.
Conclusions: The presented MSIA hGH assay enables a sensitive quantitation of hGH by the combination of polyclonal anti-hGH antibodies and highly specific MS/MS detection. Furthermore, it allows the detailed investigation of hGH isoform functions in growth disorders.
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P078

Investigation of saliva as an alternative matrix for general unknown screening of drugs of abuse and medication

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Objective
Drug screening in patients undergoing substitution or abstinence therapy is predominantly performed with urine samples using immunoassays. To prevent manipulation, sampling under visual inspection is required. In recent years immuno testing has not been sufficient in many cases, therefore chromatographic methods became more important. In comparison to urine, saliva is much easier to obtain under supervision. In this study, general unknown (GU) screening in saliva samples using a automated LC-MSn based spectral library approach is investigated.
Methods
250 saliva samples for routine drug screening of patients mainly from substitution or abstinence therapy (62% male, 27% female, 11% unspecified) were investigated after anonymization. For sample collection Greiner Bio-One Saliva Collection System pH 4.2 was used according to the manufacturer's instructions.
An aliquot of the saliva sample was mixed with internal standards and concentrated 1:20 after protein precipitation and LLE. Samples were measured by two different LC methods using an LC-MSn ion trap (Toxtyper, Bruker). Acquired mass spectra were automatically analyzed with the spectral library of Bruker and the Maurer/Weber/Wissenbach "LC-MSn Library of Drugs, Poisons and their Metabolites" (Wiley-VCH, 2014).
Results
The detected compounds were categorized into the following classes: amphetamines, benzodiazepines, cocaine, opiates, opioids/analgesics, antidepressants, beta-blockers, neuroleptics and others. THC is poorly detectable in saliva with this approach, and was not considered.
Amphetamines: In 10 patients (4%) 4 different amphetamines could be detected 13 times. Benzodiazepines: 9 different benzodiazepines were detected 61 times in 36 patient samples (14%). Cocaine: Cocaine or its metabolite was positive in 14 patients (6%). Opiates: 4 different opiates were detected at least once in 31 patients (12%). Opioids/analgesics: 9 different substances were detected 69 times in 63 patients (25%). Of this amount, paracetamol was detected 36 times and naloxone 8 times. Antidepressants: In 81 samples (32%) 14 different antidepressants were 101 times positive. ß-blocker: In 18 patients (7%) 7 different substances could be detected 21 times. Neuroleptics: In 55 patients (22%) 12 different neuroleptics were detected 70 times. Other compounds: 37 additional compounds of toxicological or forensic relevance could be detected 91 times in 75 patient samples (30%).
In total 99 different compounds were detected. Methadone was present in 135 patients (54%) and buprenorphine in 55 patients (22%). Twelve samples (5%) were completely negative for xenobiotics.
Conclusion
95% of the investigated samples were positive for at least one substance. 99 different substances were detected a total of 686 times. Thus, GU screening in saliva samples appears to be a good alternative to urine. An indeterminate percentage of the substances had certainly been prescribed by the doctor and should not be classified as drug abuse.

P079

Clinical validation and comparison of serotonin analysis in various blood fractions for the follow-up of neuroendocrine tumor patients

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Introduction. Serotonin, an endogenous neurotransmitter and paracrine agent, is used in the diagnosis and follow-up of neuroendocrine tumors (NET). We recently developed a LC-MSMS based method for serotonin analysis in serum and platelet-rich plasma (PRP). Here, we study the clinical application of various blood fractions for serotonin analysis used for the follow-up of NET patients.

Methods. 94 patient samples obtained from 78 patients visiting our NET outpatient clinic were collected. Furthermore blood samples of 112 healthy volunteers were used for determination of the upper limits of normal for serum and PRP serotonin. Serum and PRP serotonin concentrations were determined using the LC-MSMS method and whole blood serotonin analysis was performed using the Chromsystems HPLC-ECD method. Method comparisons were performed using Passing-Bablok regression and Spearman's correlation analysis. Furthermore serotonin concentrations of the healthy volunteers, 14 NET patients without evidence of disease and 51 NET patients with evidence of disease were compared.
Results. All obtained correlation coefficients were 0.98 and the slope of the whole blood versus serum regression was not significantly different from 1. The slopes obtained when comparing whole blood and serum serotonin with PRP serotonin were 0.74 and 0.71 respectively. NET patients with confirmed evidence of disease had significantly higher whole blood, serum and PRP serotonin concentrations when compared to NET patients without evidence of disease and healthy volunteers.
Conclusion. Our results suggest that as long as serotonin is expressed per platelet, serotonin results obtained from whole blood, serum and PRP seem to be interchangeable and a similar clinical performance can be expected.

P080

Next Generation Sequencing (NGS) des Mikrobioms als innovative Methode im Routinelabor zur Detektion bakterieller Dysbiosen in Darm-, Vaginal- und Oralflora

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Hintergrund und Ziel:
Die konventionelle mikrobiologische Diagnostik zur Detektion bakteriellen Dysbiosen basiert auf kulturellen Methoden. Ihre Aussagekraft wird durch die unterschiedliche Kultivierbarkeit der verschiedenen Bakterien stark eingeschränkt. Die meisten Bakterien sind Anaerobier, die sich sehr schwierig kultivieren lassen. Molekularbiologische Methoden werden für diese Fragestellung im Routinelabor bisher kaum eingesetzt. Unser Ziel war es herauszufinden, ob Next Generation Sequencing (NGS) auf Basis einer qualitätsgefilterten Datenbank mit ca. 8000 Keimen als Methode zur verbesserten Detektion von Dysbiosen in unterschiedlichen Untersuchungsmaterialien im Routinelabor geeignet ist.
Methode:
Die Protokolle zur DNA-Extraktion wurden für das jeweilige Untersuchungsmaterial optimiert. Nach der DNA-Extraktion erfolgte eine Multiplex-PCR zur Amplifikation der bakteriellen variablen Region V3 und V4 der 16S rRNA und der pilzspezifischen ITS 2 Region. Die Sequenzierung der beiden Amplicons erfolgte auf einem MiSeq® (Illumina). Die bioinformatische Auswertung und taxonomische Klassifikation der Sequenzen wurde mit dem Programm MiSeq Reporter® 2.5.1 durchgeführt. Zur Auswertung wurde die Datenbank des Programms für eine parallele Auswertung von 16S rRNA und ITS2 Sequenzen angepasst. Das Protokoll und die bioinformatische Auswertung wurden unter dem Aspekt der Anwendbarkeit in einem Routinelabor angepasst.
Ergebnisse:
Die parallele Analyse der Proben unterschiedlichen Ursprungs lieferte Ergebnisse auf allen Taxonomie-Stufen (Stamm, Klasse, Ordnung, Familie, Gattung, Art). In den untersuchten Proben wurden bis zu 500 Bakterienarten identifiziert, darunter auch eine Vielzahl schwer kultivierbarer Anaerobier. Je Taxonomie-Stufe wurde ein semiquantitatives Ergebnis in Form der relativen Häufigkeit in Prozent der Bakterien ausgegeben. Aus den Daten konnten neue Bioindikatoren wie Diversität und organbezogener Flora-Typ generiert werden. Für DNA-Extraktion, zwei PCRs, anschließende ca. 64-stündige Sequenzierung und Datenauswertung vergingen bis zur Ergebniserstellung ca. 3-4 Tage, was etwa der konventionellen Diagnostik entspricht. Der Materialverbrauch war vergleichsweise gering und vor allem durch die DNA-Extraktion bedingt. Allerdings sind die Kosten für eine NGS-Analyse zurzeit noch erheblich höher als die Kosten der konventionellen Analytik.
Zusammenfassung:
Der Einsatz der NGS-Technologie zur Detektion bakterieller Dysbiosen im Routinelabor liefert im Vergleich zur konventionellen mikrobiologischen Untersuchung ganz erheblich präzisere und objektivere Daten. NGS ist unabhängig von der Kultivierbarkeit der Bakterien, bringt neue Erkenntnisse über die Zusammensetzung der bakteriellen Flora und ermöglicht so eine deutlich bessere Bewertung als bisher. NGS nimmt ähnlich viel Zeit wie die konventionelle Diagnostik in Anspruch und zeichnet sich durch einen geringeren Materialverbrauch aus, ist jedoch gegenwärtig noch vergleichsweise teuer.

P081

Einsatz des Next Generation Sequencing (NGS) zur Detektion einer bakteriellen Dysbiose der Darmflora bei einem Kind mit lange bestehender, chronischer Diarrhoe - Ein Case Report

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Hintergrund und Ziel:
Wir untersuchten die Darmflora eines 13 jährigen Mädchens, das seit ca. 12 Jahren an einer chronischen Diarrhoe (bis zu 5 Stühlen am Tag) litt, mittels Next Generation Sequencing (NGS). Die Diarrhoe setzte laut Anamnese der Eltern nach einer antibiotischen Behandlung im Alter von ca. einem Jahr ein. Umfangreiche konventionelle Untersuchungen konnten die Ursache der Diarrhoe bisher nicht erklären. Das Ziel der Untersuchung war es, herauszufinden, ob eine Dysbiose der Darmflora die Ursache für den langen Leidensweg des Mädchens darstellt und ob diese mittels NGS detektiert werden kann.
Methode:
Die DNA aus einer Stuhlprobe der Patientin wurde analysiert. Zunächst erfolgte eine DNA-Extraktion mittels eines automatisierten Verfahrens (GenoXtract, Hain Lifescience, Germany). Die genetische Information der Bakterien wurde in den variablen Regionen V3 und V4 auf der Länge 464 bp der 16S rRNA Einheit mittels NGS-Protokoll (Miseq, Illumina, USA) analysiert. Das Ergebnis der Sequenzierung wurde mit einer populationsbezogenen Keimverteilung verglichen.
Ergebnisse:
In den Sequenzierungsdaten fanden wir eine reduzierte Diversität (Shannon-Index = 1,87) im Vergleich zum Median der Referenzpopulation (Shannon-Index = 2,94). Die Bakterien aus dem Stamm Bacteroidetes waren mit einer Häufigkeit von lediglich 0,041% kaum nachweisbar. Vergleichend betrug der Median der Häufigkeit in der Referenzpopulation 40,712%. Gleichzeitig fanden wir eine starke Keimvermehrung im Stamm Proteobacteria mit einer Häufigkeit von 21,916% und darin Escherichia coli mit 15,660% (Median in der Referenzpopulation 4,530% bzw. 0,058%). Die mukosaprotektiven Keime Akkermansia muciniphila und Faecalibacterium prausnitzii waren nicht nachweisbar (jeweils 0,000%), wobei der Median in der Referenzpopulation 0,880% bzw. 0,173% betrug.
Zusammenfassung:
Mittels Next Generation Sequencing (NGS) konnte in der Probe der jungen Patientin das Bild einer massiven bakteriellen Dysbiose im Dickdarm nachgewiesen werden. In vielfachen Voruntersuchungen mittels konventioneller Diagnostik wurden keine pathologischen Veränderungen gefunden. Der beschriebene Case Report belegt die deutlichen Vorteile von NGS gegenüber der konventionellen Diagnostik. Mit Hilfe unserer Ergebnisse konnte eine individuelle Therapie durch den behandelnden Arzt eingeleitet werden, die in der Folge zu einer schnellen Genesung der jungen Patientin führte.

P082

Increased accuracy and reproducibility in the determination of 1alpha,25 dihydroxyvitamin D by the use of automated assays

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Background
Due to the very low concentrations in the blood and its lipophilic character, the measurement of 1alpha,25 (OH)2 Vitamin D (1,25(OH)2 VitD) is analytically challenging. Radioimmunoassays reach sufficient sensitivity but are laborious and time consuming. The development of automated assays therefore promises to facilitate and accelerate the measurement of 1,25(OH)2 VitD in patient serum samples.
Methods
Remaining material of 93 serum samples sent for determination of 1,25(OH)2 VitD to our laboratory were measured with two commercially available automated immunoassays (IDS on the IDS iSYS immunoanalyzer and DiaSorin on the immunoassay analyzer LIAISON XL). 1,25(OH)2 VitD results of both immunoassays were compared to those obtained by LC-MS/MS as a reference method. Assay imprecision and linearity was estimated according to CLSI EP15-A3 guidelines.

Results
1,25(OH)2 VitD concentrations measured with the DiaSorin assay showed a strong correlation with the values obtained from measurements by LC-MS/MS (r = 0.967), whereas the IDS iSYS test overestimated 1,25(OH)2 VitD serum concentrations, particularly at higher concentrations. Total imprecision ranged between 3.1% and 5.2% for the DiaSorin test, but reached 20.1% for the IDS iSYS test.
Conclusions
The use of automated assays enables the measurement of 1,25(OH)2 VitD in serum samples within a shorter time frame with good reliability. Due to its high sensitivity, low imprecision and the good agreement with concentrations measured by LC-MS/MS, particularly the DiaSorin test is a valuable analytical option for the determination of 1,25(OH)2 VitD.

P083

Monohydroxy- and Dihydroxycholesterols in Inflammatory Bowel Disease

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Inflammatory bowel diseases (IBDs) are a group of chronic relapsing immune-mediated disorders of the gastrointestinal tract for which the pathophysiology is still badly understood. A set of oxysterol recep-tors have recently been implicated in IBD, namely the Epstein-Barr virus-induced G-protein coupled re-ceptor 2 (EBI2), the Liver X receptors (LXRs) and the RAR-related orphan receptor gamma t (RORγt). We surmised that the oxysterols triggering these receptors may play a role in the pathophysiology of IBD and that there are differences in plasma levels between healthy controls and different IBD forms.
We established a LC-MS/MS (ESI+) method to measure 24(S)-hydroxycholesterol (24(S)-OHC), 25-hydroxycholesterol (25-OHC), 27-hydroxycholesterol (27-OHC), 7α,24(S)-dihydroxycholesterol (7α,24(S)-OHC), 7α,25-dihydroxycholesterol (7α,25-OHC), 7β,25-dihydroxycholesterol (7β,25-OHC), 7α,27-dihydroxycholesterol (7α,27-OHC), and 7β,27-dihydroxycholesterol (7β,27-OHC) in human plasma. The method was subsequently applied to determine the oxysterol profile in plasma of healthy volunteers (n = 22) and patients with Crohn's disease (CD) (n = 19) and Ulcerative colitis (UC) (n = 11) during active and inactive disease stages.
The reported method is linear (r >0.99), sensitive (detection limits ranging from 0,4 nM to 3.0 nM in plas-ma) and precise, with a median inter-day imprecision of 8.2 % and 8.5 % for monohydroxycholesterols and dihydroxycholesterols, respectively. Recoveries for all oxysterols in the inter-day assay ranged between 90 % and 115 %.
With this method, 24(S)-OHC, 25-OHC, 27-OHC, and 7α,27-OHC could be detected in human plasma of most controls and IBD patients. The levels of 27-OHC and its metabolite 7α,27-OHC are lower in CD and UC patients than in controls. The results indicate that IBD patients have lower 27-OHC and 7α,27-OHC levels in the active and the remission phases compared to controls, indicating a role for the oxysterol-LXR- and the oxysterol- RORγt-axis in IBD.

Metabolom, Lipidom, Proteom, Glykom/ Seltene Erkrankungen

P084

Differential expression of eicosanoid pathways in stimulated human blood cultures from asthmatic patients and healthy controls

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Background: Allergic asthma is a chronic inflammatory airway disease with acute exacerbations. Recent research identifies a key role of specific lipid mediators derived from polyunsaturated fatty acids (PUFA) in tissue inflammation. Through cellular enzymatic oxidation by cyclooxygenase (COX), various lipoxygenases (LOX) and cytochrom P450 (CYP450) enzymes PUFAs were metabolized to oxylipids such as eicosanoids which possess biological activity. Aim: To investigate the lipid mediator profile in patients with chronic asthma. Method: We studied the expression of eicosanoids derived from arachidonic acid in zymosan-activated blood culture supernatants from asthmatic patients and healthy controls. Eicosanoids were quantified by a LC-MS/MS method. Results: We found significant differences in the expression levels as well as time kinetics of the COX and LOX-5/-12/-15 and no changes in the CYP450 pathway. After 4h stimulation the expression of

mediators derived from the COX pathway (e.g. PGE2, 11-HETE, TXB2) was lower while mediators from the 5-LOX pathway (e.g. 5-HETE, LTB4) were increased in asthmatic patients. 12-Lox and 15-LOX mediators were lower in asthmatics compared to controls. No differences were found in eicosanoid levels after 4h and 48h in healthy controls. In asthmatic patients after 48h the levels of COX-derived mediators are higher and those of the 5-LOX pathway are lower compared to 4h stimulation. Differences in eicosanoid expression may result from altered activity of the pathway enzymes as no correlation was found between the cellular content of arachidonic acid and eicosanoid levels. Conclusion: The altered expression profile of eicosanoids in asthmatic patients may contribute to the pathogenesis of chronic allergic airway inflammation.

P085

Complementary metabolic profiling reveals new insights into fatty liver disease and associated comorbidities among healthy individuals

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Triglyceride accumulation in the liver, so-called fatty liver disease (FLD) represents a risk factor for metabolic diseases like type 2 diabetes (T2DM) or cardiovascular diseases (CVD). Despite the recognition of impaired insulin signaling the molecular mechanisms involved are still poorly understood. We combined MS and NMR techniques to comprehensively characterize plasma and urine samples from 762 non-diabetic participants of a population-based sample. Liver fat content (LFC) was assessed using quantitative chemical shift encoded MRI. Associations between LFC as well as markers of hepatic damage and the metabolome were assessed by multivariable linear regression analyses controlling for several confounders. An integrated multi-fluid metabolite analysis was facilitated by the use of Gaussian graphical modelling (GGM). A predictive molecular signature of FLD was established using the LASSO embedded in a two-stage cross-validation procedure. As expected alterations in lipoprotein (atherogenic profile) and fatty acid metabolism were pronounced and strongly interrelated, whereby associations to free fatty acids were unique to liver enzyme activities. GGM analyses revealed an enriched cluster of metabolites uniquely associated with LFC, comprising branch-chained and aromatic amino acids. Even associations to urine metabolites were almost unique to LFC. In particular, a part of this urine signature improved the predictive performance for FLD moderately in comparison to classical factors. The application of untargeted metabolomics revealed a metabolic fingerprint of LFC which mimics molecular profiles associated with the progression of T2DM and CVD. Moreover, the comprehensive metabolic profiling applied here allowed for systemic understanding of previously separately presented molecular events associated with liver disease, e.g. an altered phospholipid composition of lipoprotein particles.

P086

Metabolic fingerprints of visceral and subcutaneous adipose tissue among apparently healthy subjects

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Background: Obesity is undoubtedly recognized as one of the important risk factors for cardiovascular and metabolic disease and subsequently mortality. However, a significant portion of obese adults lacks those adverse consequences commonly preceded by known risk factors, like hypertension, dyslipidemia or impaired fasting glucose (i.e. metabolic syndrome). We conducted a comprehensive untargeted metabolome approach in sample of healthy moderately obese subjects to address the so-called “healthy obesity”.
Material and Methods: The study population comprised 491 subjects without hypertension, dyslipidemia or impaired renal function. We associated MRI derived fat amounts (visceral (VAT) and subcutaneous (SAT)) with a broad panel of mass spectrometry derived metabolites in plasma and urine using linear regression analyses. An integrated multi-fluid metabolite analysis was facilitated by the use of Gaussian graphical modelling (GGM).
Results: Significant associations were merely observed with respect to VAT, comprising positive associations with several lipid species, intermediates of branched-chain amino acid metabolism, surrogates of adverse lifestyle and inverse associations with markers of the gut microbiome. The metabolic pattern observed for SAT almost completely covered by VAT. Associations between VAT and cortisol metabolism were unique to women.
Conclusion: Despite a strong sex-specific distribution of VAT and SAT the effect was limited to cortisol derivatives. In general, the accumulation of VAT was associated with an adverse molecular pattern, e.g. previously linked with the onset of type 2 diabetes, thus questioning the metabolically healthy state of asymptomatic obese individuals.

P087

Metabolic profiling of low-grade inflammation: a MS and NMR based study

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Background: Inflammation is a process which occurs as a reaction of the immune system to a stimulus, locally confined or systemic, or pass into a chronic process with the latter being the most unfavorable case. A chronic inflammatory environment represents a crucial pathological mechanism in the development of common diseases, like diabetes or atherosclerosis. In clinical practice laboratory markers like high sensitive C-reactive protein (hsCRP), white blood cell count (WBC) and fibrinogen are used to reveal inflammatory processes. In order to gain deeper insights in the inflammation-related changes in metabolism, the present study aimed to investigate metabolic patterns associated with alterations in inflammatory markers.
Methods: Based on mass spectrometry and nuclear magnetic resonance spectroscopy we determined a comprehensive panel of 1200 metabolites in plasma and urine samples of 925 apparently healthy individuals. Associations between inflammatory markers, namely hsCRP, WBC and fibrinogen and metabolite levels were tested by linear regression analysis controlling for common confounders. Additionally, we tested for a discriminative signature for an advanced inflammatory state using random forest analysis.
Results: HsCRP, WBC and fibrinogen were significantly associated with 71, 20 and 19 plasma and 22, 3 and 16 urine metabolites, respectively. Identified metabolites were related to the bradykinin system, involved in oxidative stress (e.g. glutamine or pipecolate) or linked to the urea cycle (e.g. ornithine or citrulline). In particular, urine 3'-sialyllactose was found as novel metabolite related to inflammation. Prediction of an advanced inflammatory state solely based on 10 metabolites was feasible possible (median AUC: 0.80).
Conclusions: Comprehensive metabolic profiling confirmed the ubiquitous impact of inflammatory processes on human metabolism. The identified metabolites included not only those already described as immune-modulatory but also completely novel patterns. Moreover, the observed alterations provide molecular links to inflammation-associated disease like diabetes or cardiovascular disorders.

P088

Urinary metabolic profiles of longterm changes in biomarkers of glucose homeostasis: an NMR spectroscopy based study

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Background: As known for the last decades, type 2 diabetes mellitus (T2DM) represents one of the major health problems which will become more and more important due to the steadily increasing prevalence of overweight and obesity. During the last decade metabolomics studies were used to gain deeper insight into the pathogenesis and possible treatment of diabetes mellitus with promising but also partly conflicting results. Moreover, longitudinal metabolomics studies of possible subclinical states of disturbed glucose metabolism are limited. Therefore, the aim was to assess the associations between baseline urinary metabolites and long-term changes in markers of glucose homeostasis including fasting glucose, glycated hemoglobin (HbA1c) and homeostatic model assessment for insulin resistance (HOMA-IR) index.
Methods: Urinary metabolite levels were analyzed by 1H-NMR among 3986 participants of the population-based Inter99 study. Linear regression and analyses of covariance models adjusted for age, sex, BMI, LDL cholesterol and blood pressure were used to detect associations between baseline urinary metabolite levels and 5-year changes in markers of glucose homeostasis.
Results: The analyses revealed that several urinary metabolites were found to associate with detrimental longitudinal changes in biomarkers of glucose homeostasis with higher baseline urinary levels of alanine, betaine, N,N-dimethylglycine, creatinine and trimethylamine were related to an increase in HbA1c from baseline to follow-up. In contrast, formic acid and trigonelline levels were associated with a decrease in HbA1c over time. The analyses regarding changes in fasting glucose and HOMA-IR index showed similar findings with high baseline levels of lactic acid, D-glucose-beta, creatinine, alanine and 1-methylnicotinamide related to an increase in both parameters.
Conclusion: Several urinary metabolites were found to associate with detrimental longitudinal changes in biomarkers of glucose homeostasis. The identified metabolites point to mechanisms within betaine and coffee metabolism as well as possible effects of the microbiome. Such knowledge may provide clues of pathogenetic mechanisms, targets for interventions, and might improve risk stratification of the population based on a readily obtainable bio fluid for clinical routine.

P089

Simultaneous Quantification of Oxysterols and Bile Acids in Human Plasma by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry

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Introduction: In humans, the brain contains about 20% of the body's free cholesterol (CH), mostly synthesized de novo. CH itself cannot cross the blood-brain barrier (BBB). Bile acids (BA) as primary products of the CH catabolism and the oxidized CH metabolites collectively known as oxysterols are able to pass the BBB. Through their signaling function and activation of diverse signaling pathways, BAs and oxysterols can regulate lipid, energy and CH homeostasis. Little is known about the pathophysiological role of bile acids and oxysterols in neurodegenerative disease. Here we describe a validated LC-MS/MS method for the quantification of 35 analytes of the CH metabolism, including 12 oxysterols and 17 free and conjugated BAs in plasma without derivatization and enhanced ionization using the positive electrospray ionization mode.
Methods: Tandem mass spectrometric detection was performed on a SCIEX QTRAP 5500 hybrid triple quadrupole linear ion trap mass spectrometer with positive electrospray ionization. Protonated precursor and daughter ions of oxysterols and BA were studied by multiple reaction monitoring. Sample preparation of 45 µL human plasma was carried out by simple protein precipitation in isopropyl alcohol containing 50 µg/mL butylated hydroxytoluene. Centrifugation was performed at 13,000 x g for 10 minutes at 4°C. An eluent gradient of water/acetonitrile/methanol 90:5:5 v/v/v and acetonitrile/water/methanol 90:5:5 v/v/v, containing 0.1 % formic acid each, with a flow gradient of 600 to 1000 µL/min was applied. Seperation was performed after on-line solid phase extraction on a core shell C18 reversed phase column. An external calibration and deuterated internal standards had been used for quantification.
Results: 35 Analytes of the CH metabolism, including 12 oxysterols (e.g. 24S-, 25-, 26-Hydroxycholesterol and Dihydroxycholesterol), 17 free and conjugated bile acids (e.g. the most abundant CA, CDCA, DCA, LCA and UDCA). Mass spectrometric parameters for ionization and analysis via MRM as well as chromatographic seperation within 23 minutes were optimized. In spiked human plasma, inter-day coeffiecients of variation (n = 10) of 3 % - 26 % were determined. The lower limit of quantification with S/N = 10 was 0.2 to 10 ng/mL dependent on the analyte. We achived autosampler storing stability at 4°C for all analytes over 74 hours.
Summary: The simultaneous analysis of 35 analytes of the CH metabolism, including 12 oxysterols and 17 BA in human plasma can be accomplished applying the established novel LC-MS/MS approach, dealing with a simple protein precipitation. Using the more intense and robust positive electrospray ionization mode. This validated method may be applied to comparison studies of disease and healthy cohorts to get knowledge of the possible changes in CH metabolism during the development of the disease. Further method development for the analysis of other sample matrizes e.g. liver or brain tissue is currently under investigation.

P090

Quantitative lipidomic comparison of mitochondria from mouse muscle and liver and integrated analysis of function and protein composition

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Background: Mitochondria are the major sites of energy production through oxidative phosphorylation in all cells, but mitochondrial composition is tailored to the specific needs of different tissues. Little is known on the relationship between mitochondrial function and mitochondrial lipid composition, possibly due to the challenges of isolating pure mitochondria from small tissue samples in a quality and quantity sufficient for a robust high-resolution analysis by UHPLC-MS. We investigated not only function of mitochondria from two different mouse tissues-liver and skeletal muscle-, but also lipid and protein composition.
Objectives: Comprehensive understanding of the molecular and functional differences between mouse skeletal muscle and liver mitochondria on functional, lipid and protein level to provide new insights into tissue-specific mitochondrial function and equipment.
Methods: Differential centrifugation followed by ultracentrifugation was optimized using mouse skeletal muscle and liver tissue to yield highly pure mitochondria. Mitochondrial function was investigated by high-resolution respirometry (HRR, Oxygraph-2k). Using UHPLC-MS lipidomics, we compared the lipid profiles of muscle and liver mitochondria from mice. Tissue-specific abundances of proteins were first investigated in tissue lysates by western blot analysis and are currently being further investigated in mitochondria by a proteomics approach.

Results: In HRR of isolated mitochondria a significantly different substrate preference was detected between liver and muscle mitochondria. With octanoylcarnitine/malate present, muscle mitochondria oxidized significantly more of the complex 1 substrate pyruvate whereas liver mitochondria respired more after addition of the complex 2 associated substrate succinate. In consistency with that, western blot analyses indicated higher abundance of mitochondria-specific proteins involved in not only gluconeogenesis but also β-oxidation in liver. Proteomics data, which are currently being processed, will provide deeper insights into the tissue-specific mitochondrial protein equipment. We detected a total of 278 lipid species in mouse liver mitochondria and 282 lipid species in skeletal muscle mitochondria, both including 28 cardiolipins (signature lipid of mitochondria) in as little as 50 µg (by protein) of mitochondria. The lipid patterns of skeletal muscle and hepatic mitochondria separated into clear clusters in a multivariate PCA plot, indicating distinct tissue-specific differences in the mitochondrial lipid pattern. The acyl chain composition of lipids was distinctly different throughout several phospholipid classes including cardiolipins.
Conclusions: We established and optimized a reliable and comprehensive method to obtain lipid profiles from pure tissue-derived mitochondria. We integrated this tool into a systems biological analysis of isolated mitochondria covering not only lipidomics but also respirometric and proteomics analyses.

P091

A complimentary investigation of the prostate cancer tissue metabolome and lipidome by capillary electrophoresis (CE)- and ultra high performance liquid chromatography (UHPLC)-mass spectrometry (MS)

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Background: Knowledge of metabolic alterations in prostate cancer (PCa) tissue may open up perspectives for novel biomarkers and treatment strategies. In contrast, the current knowledge on metabolic alterations in PCa is still limited.
Objectives: Comparison of PCa and adjacent non-tumor (ANT) tissue using comprehensive metabolomics and lipidomics strategies to discover metabolic pathways altered in PCa.
Methods: CE-MS as well as non-targeted metabolomics and lipidomics by UHPLC-MS were applied to study a broad range of metabolites, ranging from very polar to very apolar, in PCa and ANT tissue samples obtained from 12 patients.
Results: The joint application of lipidomics by UHPLC-MS and metabolomics by CE-MS and UHPLC-MS resulted in the coverage of more than 800 known metabolites and >4000 unknown features. A clear separation of PCa and ANT tissue was achieved based on both lipidomics as well as metabolomics fingerprints in a scores plot of a principle component analysis (PCA). In the lipidome the levels of 89 lipids from various lipid classes showed significant alterations. In the metabolome numerous changes were detected in PCa tissue, in particular alterations in pantothenate and CoA biosynthesis, glycolysis, TCA cycle, methionine metabolism, amino sugar and nucleotide sugar biosynthesis, and cysteine metabolism. The levels of several metabolites of the glycolytic pathway were, remarkably, downregulated, which is in contrast to most other tumour tissues. Taken together, our complementary analytical approach, covering metabolites ranging from extremely polar to extremely apolar, revealed novel, interesting insights into alterations of metabolic pathways in prostate cancer tissue.

P092

CLK3 mediates pathologic growth and metabolic reprogramming in HIF1α driven hypertrophic heart disease

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Background and Aim: Chronic heart failure remains the only cardiovascular disease with increasing hospital readmission rates causing an ongoing drain on health care expenditures. It often results from pathologic stress-induced growth in response to left ventricular pressure overload. Pathologic cardiac growth is preceded by profound metabolic changes, in consequence of hypoxia inducible factor (HIF) 1α driven fundamental reprogramming of the cardiac transcriptome. We have previously shown that HIF1α guided orchestration of transcriptional and post-transcriptional gene regulatory cues plays a vital role in the pathology. In the present work, we aim to characterize the splicing associated kinase CDC2-like protein kinase isoform 3 (CLK3) as an entirely novel player of the HIF1α driven hypoxic response.

Results: Left ventricular specific deletion of Clk3 prevented pathologic stress-induced cardiac hypertrophy, systolic dysfunction, fibrosis and steatosis and protected from energy depletion by maintaining high levels of mitochondrial β-oxidation. Immunoblots from human heart biopsies of patients with ischemic cardiomyopathy or aortic stenosis displayed an increased CLK3 abundance pointing to a vital role of CLK3 in hypertrophic heart disease, potentially mediated by phosphorylating the serine residue in serine-arginine rich splice factors, altering both gene expression and splicing.
Summary: Thus, pharmacological inhibition of CLK3 might provide a promising therapeutic option in the treatment of hypertrophic heart disease.

P093

Abcc6 knockout in mice is associated with disturbed cholesterol and lipoprotein metabolism

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Objectives: Pseudoxanthoma elasticum (PXE) is a rare genetic disease, caused by mutations in the ATP-binding cassette subfamily c member 6 (ABCC6) gene while the physiological function of ABCC6 is still unknown. PXE is characterized by progressive mineralization and fragmentation of elastic fibers in soft connective tissue of the eye, skin and cardiovascular system. Indications like reduced plasma high density lipoprotein (HDL) cholesterol concentrations in Abcc6 knockout mice and increased cholesterol biosynthesis in human dermal PXE fibroblasts reveal that lipid metabolism might be disturbed in PXE. Therefore, we analyzed the lipoprotein and lipid metabolism in Abcc6 knockout mice.
Methods: We performed quantitative real time PCR in healthy and Abcc6 deficient murine liver tissue (n = 21 mice for each group) to investigate the relative gene expression of proteins acting in the lipid metabolic pathway such as abc transporters, apolipoproteins, lipoprotein receptors and transcription factors plus the rate-limiting enzymes of cholesterol biosynthesis 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Hmgcr) and lecithin cholesterol acyltransferase (Lcat). Moreover, using tandem mass spectrometry, we determined concentrations of apolipoproteins A-I, A-II, A-IV and C-I (apoA-I, apoA-II, apoA IV, apoC-I) as well as lanosterol and cholesterol (free and esterified) in serum of one year old Abcc6 knockout (n = 10) and wildtype mice (n = 10).
Results: We observed significantly reduced serum concentrations of apoA-I, apoA-II and apoC-I in Abcc6 deficient mice in comparison to wildtype mice. Moreover, Abcc6 knockout mice showed induced mRNA expression levels of these apolipoproteins in liver tissue. We found increased expression of Lcat, lipoprotein receptors such as sortilin-related receptor (Sorl1), low-density lipoprotein receptor-related protein 10 (Lrp10), low-density lipoprotein receptor (Ldlr) and correlated transcription factors, e.g. sterol regulatory element-binding protein 1 (Srebf1) and sterol regulatory element-binding protein 2 (Srebf2) as well as a higher expression of the Hmgcr, Abca1 and Abcg1 genes in liver tissue of Abcc6 knockout mice. We further detected that both free and esterified cholesterol concentrations were significantly lower in serum of Abcc6 knockout mice compared to wildtype mice. Serum lanosterol concentrations were also reduced in Abcc6 knockout mice although not statistically significant.
Conclusion: The data show that HDL cholesterol metabolism appears to be involved in PXE pathogenesis, which supports the results of our previous studies where we were able to demonstrate the potential pivotal role of cholesterol biosynthesis and lipoprotein metabolism in the development of PXE.

P094

New diagnosis in the context of new available drugs: a case report

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Background:
PCSK9-inhibitors have been introduced as new lipid-lowering drugs for patients who do not response as expected to a standard-of-care therapy. The availability of these new and potent drugs necessitates increased diagnostic awareness to identify patients with familial forms of hyperlipidaemia, as indicated in the following case.
Results:
A 42-year old female patient was admitted to the lipidological ambulance. At the age of 14 she was diagnosed with heterozygous familial hypercholesterolaemia (HeFH) with the differential diagnosis type III hyperlipoproteinaemia. Since then she had been treated with various drugs. At the time of admission she was treated with Atorvastatin 80 mg and Ezetimib 10 mg. Any examination more comprehensive than a standard lipidological blood analysis was never conducted. At the time of admission she was treated with Atorvastatin 80 mg and Ezetimib 10 mg. A detailed

lipidological examination revealed a TC (total cholesterol) of 10,3 mmol/l and a LDL-C of 8,3 mmol/l. Further analyses revealed an Apo-E-phenotype E2/E2 and an increase of Apo B and Lp(a) of 215 nmol/l. Ultrasound analyses revealed carotid plaques on both sides and early aortic valve sclerosis. A family history was only available from the maternal side. Given the complex lipidologic findings we conducted a genetic investigation of the patient and her mother. The results revealed that the mother carries a heterozygous mutation on the LDLR-gene (c.1567G > A; p.(val523Met)). The index patient carried an additional LRLR-mutation (c.798T > A; p.(Asp266Glu)) revealing compound heterozygosity, which needs to be treated like homozygous familial hypercholesterolaemia (HoFH). Upon detection of HoFH we initiated treatment with the PCSK9-inhibitor Repatha (140 mg). Post-treatment, the LDL-C was lowered by 54% to 3,8 mmol/l after 3 months. Additional family screening was initiated.
Conclusion:
The detailed biochemical and genetic analyses revealed a HoFH and thus provided the rationale to initiated PCSK9-inhibitor therapy in this patient. This case illustrates that the availability of new therapeutic options for severe forms of hyperlipidaemia may necessitates a careful re-evaluation of patients to identify those qualifying for more intensive lipid lowering strategies.

Entzündung / Immunologie/ Neue Biomarker

P095

Monitoring of systemic sclerosis disease progression in the clinical course of heart transplantation by quantifying human xylosyltransferase-I activity

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Background: The human isoenzymes xylosyltransferase (XT)-I and -II catalyze the rate-limiting step in proteoglycan glycosylation. An increase in XYLT1 mRNA expression and XT activity goes along with fibrotic tissue remodeling and myofibroblast differentiation, and is mediated by transforming growth factor-β1. Serum XT activity reflects the proteoglycan synthesis rate and is increased in fibrotic disorders. Although a link of XT with fibrotic disorders exists, specific regulation pathways have not yet been fully clarified.
Here we report about two female patients suffering from systemic sclerosis. In both cases severe right heart failure in the absence of pulmonary hypertension entailed a heart transplantation, which, so far, represents a very rare approach in systemic sclerosis treatment. We evaluated the suitability of three putative serum fibrosis biomarkers (XT activity, galectin-3 level and growth differentiation factor (GDF)-15 level) to evaluate whether it is possible to monitor the fibrotic remodeling in the course of heart transplantation therapy.
Methods: Quantification of fibrosis biomarker serum levels was performed by radiochemical assay (XT activity) or ELISA (galectin-3; GDF-15), respectively. To determine levels of hepatitis C virus (HCV) viral load a pcr was performed, while procalcitonin was quantified via a chemiluminescence immunoassay. Fibrotic remodeling in the explanted hearts was analyzed histochemically.
Results: Both heart transplantations were performed successfully. Patient 1 received a HCV positive donor heart, but excellently recovered from viral transmission after administration of a direct-acting antiviral regimen. Patient 2 unfortunately died for sepsis unrelated to SSc or transplantation in the clinical course. By quantifying different serum SSc biomarkers, it turned out that only XT activity precisely monitors fibrotic remodeling. Besides, it could be shown that increased XT activity correlates with HCV infection or sepsis.
Conclusions: Our results define serum XT activity as a suitable systemic sclerosis biomarker, which does not only reflect disease progression but also displays a suitable therapeutic target in the future and therefore improves our insights into systemic sclerosis pathomechanisms. We also demonstrate that heart transplantation is a valid but underestimated therapeutic option to treat certain systemic sclerosis sub-patient groups suffering from SSc heart involvement in the absence of strong systemic progression or PAH. Furthermore we could show that successful treatment of HCV viral transmission using recently admitted direct-acting antivirals displays an innovative opportunity to also consider HCV positive donor hearts for transplantation.

P096

Intrathecal immunglobulin synthesis: Sensitivity of Kappa free light chains in cerebrospinal fluid to identify patients with oligoclonal bands

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Objective: Intrathecal immunoglobulin synthesis can be present in inflammatory diseases of the central nervous system. The gold standard with highest sensitivity for intrathecal immunglobulin G synthesis is the measurement of oligoclonal bands (OCB). There is rising evidence that the determination of a kappa free light chains (KFLC) index has a similar sensitivity in the diagnostics of diseases associated with detection of OCB in CSF. While detection of OCB is time consuming and the qualitative result relies on experienced evaluators, KFLC measurement is a quantitative method that can be rapidly performed in a highly automated setting. In the present prospective study, we aimed to determine a nonselective optimal cut-off value for determination of the KFLC index in routine clinical CSF analysis.
Methods: 300 consecutive routine serum and CSF samples were measured on the Siemens BN Prospec using the Siemens N Latex FLC kappa Kit on the BN Prospec. We evaluated the diagnostic performance of KFLC for the prediction of OCB in CSF by quantile regression and ROC analysis. The assessed reference limits were prospectively validated in 100 consecutive samples as well as retrospectively in 50 OCB positive paired patient samples.
Results: Using quantile regression for establishing the 95% percentile of KFLC quotient in relation to Albumin quotient in OCB negative patients (n = 266) revealed a sensitivity of 88% and a specificity of 95%. The optimal cut-off value for KFLC index for OCB detection using ROC analysis was 4.93 with a sensitivity of 94% and a specificity of 91%. After extrapolation for highest sensitivity to prevent false negative results the cut-off value for KFLC index was 3.56. Validation of this value prospectively in a second cohort of 100 patient samples revealed 88% sensitivity and 93% specificity. Testing this value retrospectively in 50 OCB positive patients revealed 94% sensitivity.
Discussion: The KFLC index cut-off value 3.56 has a high diagnostic accuracy for detecting intrathecal immunglobulin G synthesis in comparison with the OCB. We conclude that KFLC index determination is suitable for a complementary diagnostic step before applying OCB detection as control and as qualitative evaluation of intrathecal immunglobulin G synthesis. By using automated KFLC tests laboratory work flow is optimized. Multi center studies and comparison of different methods are required to confirm the results of this monocentric study.

P097

Circulating bile acids in patients with acute respiratory distress syndrome

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Objectives: Acute respiratory distress syndrome (ARDS) is common in intensive care units and besides a lot of research and a clear treatment strategy still associated with a high mortality rate and huge sequela for survivors. There are hints in the literature, that bile acids (BAs) may be harmful for the lung, impairing the integrity of the alveolar surfactant system and therefore could be causing or aggravating respiratory distress. Due to the lack of clinical data in this field, our aim was to evaluate circulating BAs in patients with ARDS.
Methods: Specimens were taken from 73 patients suffering from ARDS, who were recruited for a multicenter trial (ClinicalTrials.gov NCT02637011) in one of the centers after consent was obtained. Serum BA profiles were analyzed by an in-house LC-MS/MS method on day 1, 3 and 5 after diagnosis.
Results: Mean total BAs increased in a time dependent manner in patients with severe ARDS, mainly due to an increase of primary BAs and their respective taurine and glycine conjugates. Interestingly, total BAs not only remained stable, but they were actually higher in mild and moderate ARDS compared to severe ARDS on the first day of diagnosis. Furthermore, patients with initially higher GCA levels, regardless of the severity of the disease (Berlin definition), had a better outcome in terms of survival.
Conclusions: Our data suggest that serum BAs might have important implications for stratification of patients and possibly even to predict outcome of ARDS by assessing them early in the course.

P098

Stabilization of glucose concentration in the new VACUETTE® FC Mix blood collection tube for diagnosis of diabetes

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Introduction: Reliable glucose test results are required in case of transport times of blood collection tubes up to 48h. The use of tubes with an additive composed of citrate, EDTA and NaF to effectively stabilize glucose levels is recommended by German Diabetes Association.
Objectives: The aim was to demonstrate the long term stability of initial glucose concentration in specimens spun directly after collection and compared to whole blood specimens stored at room temperatures.

Patients and Method: A study was done at Faculty Hospital (Pilsen, Czech Republic) using VENOSAFE FC Mixture vs. VACUETTE FC Mix tubes. Altogether, 100 subjects (50 healthy, 50 diabetics) were recruited. IC was given by all donors and the study was approved by Czech EC. Venous blood was drawn into four tubes (two tubes each tube type). One tube of each type was spun directly after blood collection according to IFU and the second one after whole blood storage for 48h at RT. Following collection, plasma was measured immediately after centrifugation to obtain initial values (fasting) and after 48h for evaluation of glucose stability on a COBAS c702 (Roche). Statistical evaluation was done by STATISTICA.
Results: Evaluation of all clinical results for glucose and any deviations was done on basis of maximal allowed deviation for a single value (for glucose 11%) according to the guidelines of the German Association of Laboratory Testing (Rilibäk). The utilization of both tubes did not reveal any clinically nor statistically significant deviations ($p < 0.05$). The values resulted in an initial highest deviation of 5.7% (diabetics), and after 48h 7.3% (healthy). Comparable highest deviations for initial values in relation to 48h values were obtained for VENOSAFE (7.5%), and VACUETTE tubes (7.69%). The storage of whole blood specimens for 48h showed no significant deviation.
Conclusion: The FC Mix tube is suitable for reliable determination of blood glucose, one of the most frequently measured analytes and of primary importance in diagnosis, monitoring and diabetes therapy. Stability of glucose in whole blood specimens stored up to 48h at RT has been proven. The use of this tube improves the stability of glucose during extended transport times, which is more common with centralization of laboratory testing and negates the need for sample aliquotting.

P099

C-terminal alpha-1 antitrypsin peptide (CAAP48) - A promising sepsis biomarker with immunomodulatory function on human monocytes

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Objectives: Sepsis is a life threatening condition caused by a dysregulated host response to infection and is the leading cause of mortality in the critically ill. Although some aspects of the sepsis pathophysiology have been elucidated in the past, numerous unknown mediators contribute to the progression of this complex disease. Therefore, understanding the pathophysiology may lead to better diagnostics and new therapeutic strategies. In a previous study, using mass spectrometry, we identified a proteolytic fragment of alpha-1-antitrypsin, designated CAAP48 (C-terminal peptide of alpha-1-antitrypsin with a mass of 4.8kDa), as potential sepsis biomarker. The peptide was observed to be present in 3-4 fold higher concentrations in sepsis patients than in control patients. In a current study we analyzed the pathophysiological function of this biomarker on immune cells, particularly on human monocytes.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood of healthy volunteers using Ficoll density gradient centrifugation. After 3h of incubation the cells were washed and adherent monocytes were cultivated for 24h at 5% CO2 and 37°C. Monocytes were incubated with different concentrations of synthetic CAAP48 and several control peptides and the expression of activation markers were analyzed by flow cytometry. Cytokines secreted by monocytes were measured in collected supernatants using a commercially available Luminex assay. The chemotactic response was examined using disposable Boyden chambers. The viability was determined based on Annexin V and propidium iodide staining.
Results: Monocytes were activated by CAAP48 as demonstrated by increased expression of CD86, CD14 and CD16. Furthermore, the peptide induced a dose-dependent release of the cytokines MIP-1α and IL-8 and stimulated cell migration. There was also an effect of CAAP48 on cell viability in concentrations up to 100 μM.
Conclusion: Based on our previous results we propose that CAAP48 is a promising sepsis biomarker, with immunomodulatory functions, particularly on human monocytes, supporting its role in the pathobiology of the sepsis host response. Ongoing studies will address the immunomodulatory effects on other immune cells like natural killer cells or T cells, the underlying molecular mechanisms of CAAP48 uptake, the receptor binding and the signal transduction.

P100

Fat tissue specific microRNA expression in individuals with metabolic syndrome following lifestyle-induced weight loss

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1. Objectives:
Lifestyle-induced weight loss is regarded as efficient therapy to reverse metabolic syndrome (MetS) and to prevent type 2 diabetes and CVD in individuals with MetS. Underlying mechanisms that promote reversibility of adipocyte dysfunction during lifestyle-induced weight loss are supposed to be regulated by adipose tissue specific miRNA (miR) expression. Aim of this study was to identify specific miR and miR regulated pathways in adipose tissue of patients with MetS that are affected by lifestyle-induced weight loss.
2. 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 was obtained. Gene expression was analyzed by mRNA array analysis in all paired sample sets, meaning adipose tissue RNA from a specific patient at baseline and after 6 months. miR expression was analyzed in a subgroup of 6 participants of each arm by a qRT-PCR approach covering 1100 human miR species in quadruplicate. Clinical and laboratory parameters as well as body composition were determined. Obtained miR data were normalized by global mean normalization
3. Results:
We identified 54 differently expressed genes in patients of the treatment arm following lifestyle-induced weight loss. Some of these genes are known to play a significant role in adipocyte function, lipid metabolism or in macrophage function (CD163). According to our analysis 7 miR were differently regulated following weight loss. As no mouse orthologues exist for 4 of the identified miR, barely noting is known about these miR in regard to adipocyte function or adipocyte-macrophage interaction. Target prediction analysis revealed potential binding sites for identified miR in identified differently expressed genes.
4. Conclusion
lifestyle-induced weight loss is associated with specific alterations of adipose tissue specific mRNA and miR expression. Results of our analysis suggest that identified miR are relevant for observed differences of adipose tissue gene expression following lifestyle-induced weight loss. We now investigate if modulation of identified miR may affect adipocyte function in regard to adipogenesis, insulin sensitivity, ER stress and mitochondrial dysfunction. Results of this study will be presented and discussed.

P101

Identification of MARCKS as a potential regulator in TNF-tolerant monocytes

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Objective. The precise regulation of the inflammatory process is essential for its termination and dysregulations may result in acute/chronic inflammatory diseases or immune paralysis. Different mechanisms are involved in the coordinated termination of inflammation including several forms of tolerance. Based on the identification of two forms of TNF tolerance, our aim was to identify potential regulatory key molecules in tolerant monocytes by (phospho-)proteome analysis.
Methods. For the isolation of primary human monocytes from blood samples of healthy donors, a negative cell isolation protocol was applied. Monocytes were incubated ± 400 U/ml TNF for 48 h, protein extracts were prepared (2 donors each, n = 4), and phosphorylated proteins were enriched via IMAC and TiO2 columns. Subsequently, a systemic analysis of the (phospho)proteome was performed using liquid chromatography/mass spectrometry. (Phospho)proteins were identified using MaxQuant; for data analysis and visualization Perseus software was used.
Results. (Phospho-)proteome analyses revealed 569 significantly regulated phophopeptides in TNF-treated samples including 377 peptides with increased and 192 peptides with decreased phosphorylation (cut off: 2- and 0.5-fold induction, respectively). With the concurrently performed proteome analysis, the amount of total proteins was assessed. One of the most promising candidate proteins differentially phosphorylated following long-term TNF incubation is the myristoylated alanine-rich protein kinase C substrate (MARCKS; Phos-Ser101: 40-fold, Phos-Ser77: 23-fold induction), a protein participating in the regulation of several immunological functions and transport processes such as phagocytosis and oxidative burst. Unphosphorylated MARCKS is known to be located to the plasma membrane thus supporting the stability of the cytoskeleton and sequestering phosphatidylinositol 4,5-bisphosphate which excludes it from signaling. Both functions are reversed by phosphorylation.
Conclusion. Phosphorylated MARCKS is a potential key regulator of processes determining or contributing to the tolerant state in long-term TNF-incubated monocytes. The further characterization of MARCKS-dependent mechanisms may provide additional diagnostic and therapeutic aspects to specifically diagnose certain aspects of inflammation and specifically modulate them.

P102

A physiological role of NLPR3 inflammasome activation for trophoblast differentiation and embryonic development

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Objectives: Pregnancy is associated with an altered immune system which is critical for maintaining a homeostatic balance. Indeed, successful placentation and trophoblast remodeling, which are required for normal development, are associated with a pro-inflammatory state of the placenta. Yet excessive inflammation results in pregnancy complications such as preeclampsia (PE) and intra-uterine growth restriction (IUGR). We have recently shown that excess sterile inflammation via the NLRP3 inflammasome activation causes PE. However, it remains to be shown whether the inflammasome has a physiological function in placental and embryonic development. We aimed to study the physiological role of NLRP3 inflammasome activation during development.
Methods: Timed matings were performed in WT (C57/Bl6) and NLRP3-/- mice and embryonic development was assessed at day 12.5, 14.5 and 16.5 p.c. and 1 day post-natal. IUGR was assessed by measuring embryonic height and weights. Impaired vascularization in placenta was studied by H&E staining. Inflammasome markers (NLRP3, cleaved caspase-1 and cleaved IL-1β) were assessed in WT placentae by immunoblotting. RT-PCR for marker genes (PL-II, Tpbpa, Esx-1 and Gcm-1) was performed in WT and NLRP3-/- to evaluate trophoblast differentiation. Human trophoblast cells, BeWo and mouse trophoblast stem (TS) cells were used to study differentiation and inflammasome activation in vitro. IL-1 receptor antagonist (Anakinra) and NLRP3 inhibitor (MCC 950) were used to address mechanistic questions.
Results: NLRP3-/- mice were smaller at birth compared to WT mice indicating IUGR. Timed mating showed that the IUGR of NLRP3-/- embryos first occurs at day 14.5 p.c. and is readily detectable at day 16.5 p.c. Furthermore, NLRP3-/- placentae showed impaired differentiation compared to WT placentae. In addition, WT placentae displayed time-dependent placental inflammasome activation. Treatment of WT mice with Anakinra resulted in IUGR and impaired trophoblast differentiation showing that these effects are IL-1 dependent. In vitro studies in human and mouse trophoblast cells showed that trophoblast differentiation was paralleled by activation of inflammasome markers. Treatment with either Anakinra or MCC 950 suppressed in vitro trophoblast differentiation corroborating the in vivo findings.
Conclusion: Our results uncover a physiological function of the NLRP3 inflammasome for trophoblast differentiation and embryonic development. Based on these insights, therapies limiting inflammasome activation may be harmful during pregnancy. In addition, monitoring biomarkers of inflammasome activation may enable diagnosis of placental failure and IUGR.

P103

The relation of uremia-induced inflammasome activation and K+ dyshomeostasis

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Background:
Chronic kidney disease (CKD) causes cognitive impairment and dementia. Alteration such as uremic toxins affect neuronal function and survival. The molecular mechanisms causing neuronal dysfunction remain unknown. We hypothesized that sterile inflammation is mechanistically linked with impaired cerebral function in CKD. In detail, we hypothesize that CKD and associated uremic toxins cause K+-dyshomeostasis, which contribute to inflammasome activation within the CNS and thus to neuronal impairment.
Aim:
We aim to decipher the mechanistic relevance of K+-dyshomeostasis in the CNS for sterile inflammation in neuronal cells. We intend to determine the mechanistic relevance of K+-dyshomeostasis for inflammasome activation and neuronal dysfunction. Finally, we wish to identify the relevant cell-type in which the inflammasome is activated. Furthermore, we intend to explore the temporal dynamics of K+-dyshomeostasis and inflammasome activation in different cerebral cell types.
Methods and materials:
We used thallium-autometallography (Tl-AMG) to monitor cellular K+-metabolism. The potassium analogue thallium is taken up simultaneously with K+ via the same mechanisms from the extracellular space and can be fixed in situ and visualized by means of an autometallographic method. Cells which have been characterized for Tl+-flux and therefore for K+-metabolism can be stained with immunohistochemistry and further investigated with respect to cell type or expression of markers of inflammasome activation. CKD in mice was induced by 5/6 nephrectomy (5/6 NX). To study cell-activation in the CNS in vivo we conducted two-photon intravital microscopy. We used double transgenic mice expressing EGFP under the control of the CX3C chemokine receptor 1 (CXC3R1) promoter (labeling microglia) and YFP under the control of the Thy-1 promoter (CD90, labeling neurons). NLRP3 knock-out mice (NLRP3-/-, C57BL/6 background) were used to determine the mechanistic relevance of inflammasome activation.
Results:
Mice with CKD revealed altered K+-metabolism in the CNS and increased expression of caspase-1 and IL-1ß. Two-photon microscopy revealed activation of microglia and neurons in mice with CKD. Analyses of wild type C57BL/6 and NX NLRP3-/- mice six weeks after inducing CKD revealed a comparable number of thallium positive activated microglia, indicating that the uremia-induced reactive shift and K+-dyshomeostasis in microglia is a process which is independent from NLRP3 inflammasome activation. However, in 5/6 NX NLRP3-/- mice the decrease of neuronal thallium uptake in the CA1 region and somatosensoric cortex was abolished, indicating that the NLRP3 inflammasome is indeed involved in CKD-induced K+-dyshomeostasis of neurons, hence neuronal dysfunction. To support these findings, we used caspase-1knockout mice (CASP-1-/-) to block this down-stream target of NLRP3 activation. Similar to NLRP3-/- mice, we found an increased number of activated microglia but normal thallium-uptake by neurons.

Conclusion:
These data suggest that in microglia K+ is an initial step occurring prior to inflammasome activation and neuronal dysfunction. Furthermore, these data indicate a functional relevance of NLRP3 – caspase-1 activation secondary to K+-dyshomeostasis in microglia, which subsequently promote inflammasome activation and neuronal dysfunction. Hence, we propose that activation of microglia is pivotal in the pathophysiology of CKD-induced impairment of brain function. These results may lay ground for new biomarkers in CKD-associated neuronal dysfunction and therapeutic approaches to combat this malady.

P104

Identification of the target antigen of the first macrophage-derived antibody

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Objectives:
Very recently, we have first described that subpopulations of human macrophages are capable of recombining immunoglobulin variable heavy and light chain genes and express antibodies. However, neither structure nor antigen specificities have been reported at a single cell level. Here we have cloned, engineered and expressed a fully functional antibody (designated DER1G10) from a single oesophagus tumour-associated macrophage and describe the identification of its target antigen.
Methods:
We used immunoprecipitation techniques to isolate potential target antigens from cell lysates of a variety of human tissues including PBMCs, erythrocytes, oesophagus tumour and healthy oesophagus tissue. The precipitated proteins were further analysed and identified by Western blotting and nanoLC-MS/MS mass spectrometry, respectively.
Results:
Mass spectrometric analyses of immunoprecipitation samples revealed the specific precipitation of erythrocyte band 7 integral membrane protein (stomatin) by DER1G10. As compared to negative controls, no other proteins were detected in similar abundance and frequencies. Specific binding of DER1G10 to stomatin was further confirmed by using commercially available polyclonal and monoclonal antibodies as well as by precipitation of purified recombinant stomatin from solution.
Conclusion:
Our results clearly demonstrate that the macrophage-derived antibody DER1G10 targets stomatin, an ubiquitously expressed membrane protein that usually is entirely located on the inside of the cell membrane. However, there is evidence in the literature that a major C-terminal portion of stomatin can be translocated in a flip-flop manner to the outside of the cell membrane, thereby constituting a target for the antibody. This work demonstrates for the first time that macrophages produce antigen-specific immunoglobulins suggesting involvement in adaptive immune responses. To investigate the unclear biological function of macrophage-derived DER1G10 in the context of cancer, further experiments are currently being conducted to map its epitope specificity and also investigate potentially differential features of stomatin in normal and cancerous tissues.

P105

EVALUATION OF THE DIAGNOSTIC PERFORMANCE OF A NOVEL ANTIPHOSPHOLIPID ANTIBODY IMMUNODOT: LATENT CLASS ANALYSIS TO CIRCUMVENT THE LACK OF A REFERENCE STANDARD

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Background: Diagnosis of antiphospholipid syndrome (APS) requires the fulfilment of clinical as well of laboratory criteria. Anti-cardiolipin (aCL) and anti-β2-glycoprotein I (aβ2GPI) antibodies constitute part of these laboratory criteria. In clinical routine, aCL and aβ2GPI are commonly measured using ELISA methods. Nevertheless, with autoimmune diagnostics being on its way to more and more multiplexed analyses, new assay formats are evolving. Reference materials for antiphospholipid antibodies (aPL), however, are controversially discussed and results of different ELISA systems may vary considerably. As such, determining the diagnostic performance of new aPL assays turns out to be challenging. In the presented study, we apply the statistical method of latent class analysis (LCA) in order to investigate the diagnostic performance of a novel immunodot for aCL and aβ2GPI, which is now commercially available.

Method: Sera from 53 APS patients and 34 healthy volunteers were collected with the APS patients fulfilling the Sydney criteria. The novel Anti-Phospholipid 10 Dot (Generic Assays, GA) as well as four established immunoassays (Alegria from Orgentec (ALE), Acustar from IL (ACU), Unicap from Phadia (UNI), Aeskulisa from Aesku (AES)) were used to determine aCL and aβ2GPI IgG and IgM. LCA was performed based on the results of the ALE, ACU, UNI, and AES system, and the respective manufacturers' cut-offs. In case of this work, "antibody-positive" and "antibody-negative" constitute the unknown (latent) classes of subjects to which the observed test results are assigned to. Finally, sensitivities and specificities of the immunodot (GA) were calculated with respect to this LCA-derived "gold standard".
Results: For aCL IgG and aβ2GPI IgG, the sensitivity of the immunodot from GA was significantly higher than the UNI and the AES system, respectively. With respect to specificities, significantly lower values were found for the immunodot for aCL IgG as compared to the ALE and for aβ2GPI IgG as compared to the UNI and the AES systems. Beyond that, sensitivities and specificities of the immunodot were not significantly different from the four other assays included in the study.
Summary: Sensitivities and specificities for aCL and aβ2GPI IgG and IgM of the immunodot were demonstrated to be similar to those of established immunoassays. Due to the fact that definite reference material and method are not available for aPL, LCA as a statistical concept was applied to evaluate the diagnostic performance of the immunodot. LCA enabled calculation of these assay performance data with proper statistical methods even in the challenging setting of aPL. It therefore can be considered as prototypic approach readily lending itself to the evaluation of other autoantibody assays.

Varia

P106

Tools for Investigating and Recognizing Uncommon Body Fluids

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Requests for investigation of uncommon body fluids may be infrequent, but not insignificant. Good and well known reasons for investigation of uncommon body fluids are that clinicians want to have certainty as to the origin of material collected from the body or wish more precise knowledge about the process going on in the cavity that gave rise to the fluid. The results of the laboratory investigations often have direct consequences. The information may be needed for diagnosis, or intended or on-going treatment. Laboratory analysis of uncommon body fluids requires special attention as it is distinct from routine analysis. In general, it is recommendable to seek distinct consultation with the requesting physician, to know the background of the patient and the fluid as is presented, and appreciate the significance of the answer pursued.
More than 30 different human body fluids can be distinguished. As an aid to recognize fluids and discriminate them from others an overview was produced in which characteristic constituents of the body fluids are listed. In addition markers are listed that can be used to characterize the pathological process taking place in the fluid. Recognizing body fluids and discriminating from others is often possible with common constituents for which tests are available at 24/7 basis. Our experience is that there are generally more possibilities for fruitful investigation than apparent at first sight. Recommendable is both to test constituents that discriminate fluids from each other and constituents that verify a fluid's supposed identity. For a sensible approach towards investigation of uncommon body fluids a number of characteristics and considerations when interpreting the results have to be taken into account. These are presented as a number of practical recommendations related to investigating and identifying uncommon body fluids.
Janssens PMW. Recognizing and differentiating uncommon body fluids: Considerations and tools for a proper practical approach. Clin Chim Acta 2017; 471:6-11 [Epub ahead of print], doi: 10.1016/j.cca.2017.05.005.

P107

Comparison of serum to liquor quotient and light chain measurement between BN prospec and Cobas c501

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Objective:
Health economics require process optimization, resulting in a minimum of measurement platforms. In our laboratory, the nephelometric BN prospec was used for measurement of low concentrated proteins to obtain Reiber diagrams as well as light chains in serum samples. We

compared the measurement of IgA, IgG, IgM and albumin in serum and liquor as well as kappa and lambda light chains in serum between a nephelometric and a turbidimetric assay to elucidate, whether there is still a clinical requirement for nephelometry in the routine laboratory.
Material and Methods:
We used serum and liquor samples out of the daily routine. All samples were measured on a BN prospec (Siemens) and on a cobas c501 (Roche). Measurements, which were below or above the measuring range and without the possibility to run a dilution due to limited material on either of the systems were excluded. In detail, this was n = 104 for kappa and n = 101 for lambda light chains as well as n = 89 serum-liquor pairs for albumin, n = 86 for IgG, n = 23 for IgA and n = 30 for IgM. Statistics were done in R. Beside linear regression, Passing-Bablok regression was used as a parameter free statistical approach from package "mcr". Slope, intercept and linearity as pearson's r are shown as determined by Passing-Bablok regression with x = BN prospec and y = cobas c 501.
Result:
Correlation of measurements between the two methods was y = 1.09 * x -0.21, r = 0.974 for kappa light chain and y = 1.12 * x - 0.12, r = 0.979 for lambda light chain. Comparing the single parameters of the serum-liquor pairs, we found differences particularly for albumin in serum and liquor. Correlation was y = 1.23 * x - 3.01 g/l, r = 0.923 for serum and y = 1.18 * x - 34.53 mg/l, r = 0.996 for liquor samples. However, the serum to liquor quotient correlates well between the nephelometric and turbidimetric method, with exception of the IgA-serum to liquor quotient. Correlations for the quotients were y = 1.03 * x - 0.75, r = 0.996 for albumin, y = 0.97 * x - 0.14, r = 0.997 for IgG, y = 0.93 * x + 0.04, r = 0.801 for IgA, and y = 1.22 * x - 0.1, r = 0.994 for IgM.
Conclusion:
Detection of light chain concentrations as well as serum to liquor-quotients of immunoglobulins and albumin with the turbidimetric cobas c 501 method showed comparable values to the nephelometric method. An obvious difference was seen for a single IgA measurement pair, which was most probably due to a measurement error on BN prospec considering the value pattern. Due to limited patient material, only few patients with high IgM or IgA values could be included. As there is for some parameters a significant difference between both methods we recommend to measure all parameters for the calculation of the Reiber-diagram with the same method.

P108

Comparative evaluation of analytes susceptible to hemolysis in serum and heparin plasma under defined blood withdrawal and pre-analytical conditions

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Objective: The pre-analytical phase comprises blood withdrawal, applied collection tubes, material transport, centrifugation conditions, and sample aliquotation. These aspects are significantly less standardized than analytical processing and measurement. However, they majorly impact on material quality directly affecting validity of generated data, and thus usefulness for e.g. biobanks. Especially differences in blood withdrawal conditions, including underfilling of blood sample tubes frequently observed in clinical routine settings, has not been analyzed in great detail, in particular with respect to erythrocyte lysis parameters, such as potassium and free hemoglobin. Further, lactate dehydrogenase (LDH) is a cell lysis-susceptible parameter frequently used for evaluation and therapeutic risk stratification of patients with malignant disease. Here we analyzed systematically the effects of underfilling of blood tubes, blood withdrawal additives (serum, heparin plasma), and prolonged pre-analytical time prior to tube centrifugation on hemolysis-susceptible parameters.
Methods: Venous blood was withdrawn from healthy volunteers (N = 20; age range 29-64 a, mean 49.8 a) in vacuum blood collection tubes (4 serum, 4 heparin plasma (HP) from each volunteer). Tubes were either filled completely or filled to ~50% and processed directly or stored at room temperature for 4 h prior to centrifugation. Nine parameters were analyzed per tube: sodium, potassium, chloride, LDH, creatine kinase, total cholesterol, along with indices for hemolysis, icterus, and lipemia. Thus, a total of 72 measures were determined in each volunteer.
Results: LDH activity was significantly enhanced in HP compared to serum (+9.6%; means 221 ± 48 U/L vs. 201 ± 46 U/L, respectively). Further, underfilling led to significantly higher LDH activities (serum: +23.2%; HP: +27.1%) and hemolysis index (serum: +237.6%; HP: +170.1%), as well as – to a lesser extent – potassium (serum: +5.6%; HP: +4.5%). Total cholesterol, creatine kinase, sodium and chloride largely remained unchanged. Interestingly, the lipemia index was also enhanced in underfilled serum tubes only (+27.0%). Both hemolysis index (-13.2%) and potassium concentrations (-6.5%) were expectedly reduced in HP, surprisingly contrasting lower LDH levels in serum. Assuming that blood cell shear stress was aggravated by underfilling, we measured filling velocity. The first half volume of blood tubes was filled at ~1.14 mL/s, while the second half at ~0.24 mL/s.
Conclusion: While it remains unclear, whether an active process is the underlying mechanism of higher LDH activity, or the vacuum per se impacts on cells, underfilling of blood collection tubes should be considered in clinically implausible high LDH levels. Further, it seems relevant to distinguish between serum and heparin plasma collection tubes when using LDH as a risk stratification parameter, since the latter produce significantly higher results.

P109

Pseudohypernatremia, a common instrument-dependent analytical problem in critically ill patients

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Objective:
Accurate sodium measurements are key to initiate appropriate treatment of dysnatremia in critically ill patients. Sodium concentrations in samples with aberrant protein and lipid concentrations are erroneously increased or decreased when measured on routine chemistry instruments. An analytical phenomenon known as pseudonatremia. In the present study, we analyzed the extent of pseudohypernatremia in relation to the protein concentration in ICU patients and compared the magnitude of pseudohypernatremia of five different routine chemistry instruments.
Method:
Sodium measurements of 382 ICU patients were analyzed between January and June 2015. The indirect ion selective electrode (ISE) technique on a chemistry instrument (Dimension Vista 1500, Siemens) was compared with the direct ISE technique on a blood gas analyzer (Rapidpoint 500, Siemens). The delta of both measurements (ΔNa) was evaluated in relation to the total protein concentration. 36 samples with varying protein concentrations were re-analyzed for sodium on four additional chemistry instruments with different dilution factors (Siemens Advia1800; Abbott c8000; Beckman DXC800; Roche Cobas8000).
Result:
The ΔNa between the indirect and direct ISE technique was inversely correlated with the total protein concentration. Low and very low total protein concentrations (40-50g/L and 30-40g/L) caused high average ΔNa of 4,8mmol/L and 6,5mmol/L, respectively. The comparison of 5 different routine chemistry instruments revealed analyzer specific proportions of pseudonatremia with highest discrepancies on the Dimension Vista1500 (7,2mmol/L at TE 30-50g/L).
Conclusion:
Hypoproteinemia in ICU patients causes erroneously elevated sodium measurements of up to 6,5mmol/L on routine chemistry instruments putatively leading to inappropriate management of the delicate sodium-water balance. The magnitude of the ΔNa differs between instruments but does not correlate with the analyzer specific dilution. Direct sodium ISE techniques on blood gas analyzers are the method of choice for ICU patients.

P110

Influence of a triathlon race on markers of myocardial damage and cardiac insufficiency

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Introduction:
For the diagnosis of myocardial damage and acute myocardial infarction as well as myocardial insufficiency, there are highly sensitive and specific markers available, namely cardiac troponins (cTn) and natriuretic peptides. Additionally, copeptin, the C-teminal propeptide of the antidiuretic hormone, is a promising new parameter to rule out an acute myocardial infarction in the earliest phase. In endurance sports, there is a high demand on the heart of the athlete with possible leakage of the myocardial cells and subsequent release of markers of myocardial damage and insufficiency, as well as stress, leading to a release of copeptin. In this study, we examined the short-time influence of a triathlon on the concentration of cardiac troponin T (cTnT), N-terminal proBNP (NT-proBNP) and copeptin.
Materials and Methods:
In 50 participants (43 men, aged 28 – 64 years and 7 women, aged 30 – 48 years) of the Ironman 70.3 Kraichgau, we collected blood specimen before and within 15 minutes after finishing the triathlon (1.9 km swimming, 90 km cycling, and 21.1 km running). In all serum samples hs-cTnT, NT-proBNP (both Roche Diagnostics) and copeptin (Thermo-Fisher) were measured. For statistical analysis we used Excel.
Results:
Compared to the values measured before the start of the race, where one athlete showed an elevated value for copeptin and two for NT-proBNP, there was a significant increase of cTnT and NT-proBNP in all participants and of copeptin in 47 of 50 participants. In average this increase was 25-fold for hs-cTnT from 4.16 pg/mL to 63.1 pg/mL, 8.5-fold for NT-proBNP from 42.14 pg/mL to 252.6 pg/mL and 12.7-fold for copeptin from 5.5 pmol/L to 48.4 pmol/L.

Conclusions:
A triathlon leads to a significant increase of parameters of myocardial damage and insufficiency with elevated values shortly after the race. They indicate a possible leakage of the cardiomyocytes and physiological stress. This significant increase above the diagnostic cut-off for acute myocardial damage could complicate the detection of possible pathological myocardial injury or insufficiency in those athletes.

P111

A targeted optimization strategy for improving preanalytical sample quality and minimising the volume of blood collected

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Aim of the study
A preanalytical phase (PAP) audit revealed non-compliances during hospital blood collections, compromising sample quality. Based on these findings a multistage plan was educed in order to improve preanalytical quality, to establish a contemporary patient blood management process and to increase overall hospital efficiency.
Materials and Methods
Using BD Laboratory Consulting Services®, we observed 54 blood collections in 6 hospital departments to investigate preanalytical errors. Also, blood sample quality was visually inspected (n = 544). Blood collection was then converted to BD Vacutainer®, with tubes having a lower fill volume. Staff received training on those parts of the PAP where non-compliances had been identified. The audit was repeated a year later. The number of tubes per patient per day was determined as was the total volume of patients' blood collected in 1 month. The blood collection volume of 32 ICU patients before and 33 patients after tube conversion (stay > 1 week) was compared.
Results
The number of several PAP blood collection non-compliances had decreased substantially 1 year after conversion (prolonged tourniquet time (-38%); incorrect puncture site disinfection (-49%); insufficient mixing of tubes (-40%); incorrect label placement (-47%)). Clotting towers in serum samples were eliminated, enabling to introduce serum tubes with lower blood volume. Blood volume per collection, as evaluated by the tubes used, decreased by 42.5% for geriatric patients. The mean number of tubes used per patient per day in the entire hospital decreased by 13%. The overall volume of blood collected for all hospitalized patients decreased by 17.5 % (177 L) per year. Data from the audit after the 1 year for 33 ICU patients (stay > 1 week) showed a decrease of 27.2% (30 mL) per week in collected blood volume.
Summary
A targeted approach, analyzing the current situation to identify optimization potential and then implementing a holistic improvement project, enables improvement of preanalytical practices efficiency and so patient care.

P112

Human adult cardiac stem cells - novel relation to the neural crest

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Purpose
Cardiovascular diseases are the major cause of death worldwide and main clinical treatments are still palliative, emphasizing the necessity to establish suitable in vitro models to better understand adult human cardiac cell biology. Although the adult heart was considered as a terminally differentiated organ, rare populations of cardiac stem cells (CSCs) have been found recently. Although neural crest-derived stem cells (NCSCs) displaying an extraordinary broad differentiation potential can be found in various tissues of adult human body, a neural crest origin of CSCs has only been described in the murine heart. The present study aims to investigate the differentiation potential and developmental origin of human CSCs (hCSCs) in more detail, particularly regarding a potential relation to the neural crest. In addition, expansion steps of CSCs after isolation have to be optimized to gain sufficient amounts of cells also for extending potential clinical usability.
Methods
Human heart auricles were routinely removed during heart surgery and processed for explant culture or immunohistochemistry. All patients participating gave their informed consent according to local and international guidelines. Successfully isolated cells were characterized using reverse transcriptase-PCR, flow cytometry and immunocytochemistry in terms of expression of stemness and neural crest markers. Proliferation assays were performed to investigate the effects of human blood plasma on hCSCs.

Results
Here, we show cells positive for neural crest-derived stem cell markers nestin and S100 in adult human heart auricle tissue. Nestin + /S100 + cells could be successfully isolated by explant-culture and kept their expression of Nestin and S100 on mRNA and protein level during in vitro cultivation. Validating their neural crest origin, human CSCs isolated from independent donors showed strong expression of characteristic stem cell- and neural crest markers like Snail, Twist, cMyc and Sox10. We found the transcriptional profile to be similar to an adult human neural crest-derived stem cell population from the head and neck region. Additionally, we applied a human blood plasma-derived 3D-matrix for cultivation of hCSCs and demonstrated a significantly increased proliferation of hCSCs during blood plasma-based cultivation, thus providing a new expansion system for human CSCs.
Summary
hCSCs can be easily obtained by explant-culture of human heart auricles. The expression of neural crest-derived stem cell markers provides a strong evidence for a neural crest-related developmental origin of hCSCs, promising a wide differentiation potential. In addition, culture conditions can be improved by the application of human blood plasma, which was shown to support the proliferation of hCSCs, thereby extending potential clinical usability. Consequently, our findings gain deeper insights into the developmental origin and biology of human heart stem cells for potential use in regenerative medicine.

P113

FXR-agonism protects against diabetic tubulopathy -add-on effect on top of ACE-inhibition

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Objective: The importance of tubular damage in diabetic nephropathy (dNP) is increasingly recognized. However, established therapies for dNP delay, but do not prevent its progression. This may reflect their lack to target the tubular compartment. The chemical chaperone/bile acid derivative; TUDCA ameliorates maladaptive Endoplasmic reticulum (ER)-stress signaling and dNP. Additionally, TUDCA activates farnesoid X receptor (FXR), which is highly expressed in tubular cells. We hypothesized that TUDCA ameliorates maladaptive ER-signaling via FXR-agonism specifically in tubular cells. Moreover, the renin–angiotensin system (RAS)-inhibition is thought to primarily provide glomerular protection. Whether TUDCA targets a pathomechanism independent of RAS-inhibition and hence provides an added value on top of RAS-inhibition in dNP remains unknown.
Methods: 16-weeks old db/db mice were treated with TUDCA in the absence (T) or presence of the FXR-antagonist Z-guggulsterone (T + Gu), or in-vivo knockdown of FXR, using Morpholino (T + FXR-MO) for 6-weeks. Other subsets of 16-weeks old db/db mice were treated with PBS (control, C), angiotensin converting enzyme (ACE)-inhibitor; enalapril (E), TUDCA (T) or a combination of both (E + T) for 6-weeks.
Results: TUDCA induces expression of FXR-dependent genes (SOCS3 and DDAH1) specifically in tubular, but not in other renal cells. In-vivo, TUDCA reduces glomerular and tubular injury in db/db mice. FXR-inhibition (Z-guggulsterone or morpholino targeting FXR) diminished the ER-stabilizing and reno-protective effects of TUDCA. Intriguingly, these in-vivo approaches abolished tubular, but not glomerular protection by TUDCA. Combined intervention with TUDCA and ACE-inhibitor enalapril in 16-weeks old db/db mice reduced albuminuria more efficiently than either treatment alone. While both therapies reduced glomerular damage, only TUDCA ameliorated tubular damage. Thus, FXR-agonism conveys specific protection of the tubular compartment in dNP, providing reno-protective effects on top of ACE-inhibition.
Conclusion: This study demonstrates that interventions protecting the tubular compartment, such as FXR-agonism by TUDCA, may provide additional protection on top of RAS inhibition in dNP, via ER-dependent and independent mechanisms.

P114

The method for creatinine measurement affects the in-hospital diagnosis of acute kidney injury

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Introduction: The diagnosis of acute kidney injury (AKI) in hospitalized patients is associated with adverse short- and long-term outcomes. AKI is diagnosed by a defined increase of blood creatinine over a period of time. Creatinine can routinely be measured by two procedures, the compensated kinetic Jaffé reaction or an enzyme-based assay. It is not known so far, whether the method for creatinine measurement itself can affect the diagnosis of AKI.
Methods: In a prospective observational study creatinine was measured simultaneously by both assays (Jaffé and enzymatic method) in patients from a tertiary care hospital using Abbott Reagent Kits. An algorithm that bases on the most recent guideline for AKI (KDIGO 2012) was applied to detect potential cases of AKI in both creatinine measurement methods.

Results: From 4761 patients 839 met AKI-criteria by measurement of either assay. 635 were diagnosed by both assays while 103 and 101 were detected exclusively by the Jaffé or enzymatic method respectively. Compared to the Jaffé method, the number of AKIs diagnosed by the enzymatic method was significantly higher in patients under 70 years (372 vs 348) and in patients with an eGFR >60 ml/min/1.73m^2 at admission (403 vs 364). Correspondingly, urea at admission was significantly higher in those patients exclusively diagnosed by the Jaffé method compared to the enzymatic method (8.6 vs 5.6 mmol/l). The incidence of a composite endpoint including mortality, dialysis treatment and ICU-stay after AKI was significantly higher in those patients diagnosed by both methods compared to patients diagnosed by exclusively one assay with no significant difference between Jaffé and enzymatic method (29.8% vs 8.8% vs 10.1%). Of note, samples from AKI patients diagnosed by exclusively one assay did not differ significantly from each other regarding levels of haemolysis, bilirubin, CRP, CBC or HbA1c that was used as a surrogate parameter for potentially high levels of glucose.
Conclusion: A considerable number of AKI patients are exclusively diagnosed by either the Jaffé or the enzymatic method and these patient cohorts have a reduced risk for adverse outcomes compared to the AKI cohort recognized by both assays. Patients diagnosed by the Jaffé method differ significantly from those diagnosed by the enzymatic method regarding renal function and age. These discrepancies seem to be systematic and independent from known interferences and might explain at least partly discrepancies between published studies as well as affect planning of future multi-centre AKI studies.

P115

Comparision of two statistical methods (Reference Limit Estimator vs. Quantile Quantile Plot) to determination of reference intervals for serum creatinine, serum urea and eGFR

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Background: The establishing of intra-laboratory reference limits is an essential element of quality management. The gold standard for determination of reference limits in clinical laboratories is described in the guideline (EP28-A3c) of CLSI. This guideline states that laboratories should pay more attention to verification and identification of reference range. Many laboratories cannot use the gold standard on the grounds of the high costs of determination. As alternatives, newer retrospective methods were developed. Based on the large data pools of laboratory information systems, these methods provide intra-laboratory reference limits.
Aim and Method: This work aimed to evaluate the quality of cost-free programs such as the Reference Limit Estimator (RLE, a program developed by DGKL) and the Quantile Quantile Plot Method in R (QQ Plot). Both programs were employed in the determination of reference intervals for the parameters serum creatinine and serum urea taking into consideration the estimated Glomerular Filtration Rate (commonly abbreviated as eGFR), and results were then compared with the available literature. Moreover, the effects of gender and age on reference limits were investigated. For the calculations, 93,482 data sets were made available by the Center for Laboratory Medicine and Microbiology GmbH.
Results: The paper demonstrates that the RLE and QQ Plot programs are generally suitable methods for the determination of reference intervals. However, the programs face difficulties when confronted with parameters dependent on other influencing factors. Furthermore, they confirm that there exist both age and gender-specific differences in reference intervals. In the case of serum creatinine, the reference intervals determined through the two methods are approximately the same, and congrue with the intervals encountered in the available literature. Additionally, in the Quantile Quantile Plot method, it is shown that, as the age of the patients increases, the eGFR has to be taken into account for the determination of the reference intervals. As for the parameter serum urea, it was found that both retrospective methods do not provide optimal results for the reference intervals.
Conclusions: The retrospective, bimodal concepts of the RLE and Q-Q plot are adequate for the calculation of intra-laboratory reference limits in some cases. At present, there are issues with parameters which, in addition to gender and age, have further influencing factors. Nevertheless, a clear advantage of these methods over the gold standard is the possibility to determine reference intervals on the basis of already collected laboratory data. However, for some parameters, bimodal retrospective methods can not replace the gold standard.

P116

Reference intervals for serum indices in seniors: results from the prospective SENIORLAB study

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Background and aim: A majority of resources in clinical medicine is spent on seniors. The correct interpretation of laboratory results plays an important role for accurate diagnosis and therapy. Preanalytical assessment of sample quality can be done with determination of serum

indices. So far there not much is known, whether age has an influence on serum indices. SENIORLAB study set out to define reference intervals in seniors for a variety of laboratory parameters. The present analysis aims to estimate reference intervals for serum indices for Caucasian individuals aged 60 years and more.
Background and aim: A majority of resources in clinical medicine is spent on seniors. The correct interpretation of laboratory results plays an important role for accurate diagnosis and therapy. Preanalytical assessment of sample quality can be done with determination of serum indices. So far there not much is known, whether age has an influence on serum indices. SENIORLAB study set out to define reference intervals in seniors for a variety of laboratory parameters. The present analysis aims to estimate reference intervals for serum indices for Caucasian individuals aged 60 years and more.
Methods: From a total of 1467 study participants reporting subjective wellbeing at baseline examination in the SENIORLAB study, serum samples were drawn in fasting state and processed under optimal and standardized preanalytical circumstances. The following individuals were excluded from analysis: death at first follow-up for participants <80 years of age (mean follow-up time 3.7 +/- 0.7 years), survival of less than 3 years between age 80-84, survival of less than 2 years between age 85-89, and survival of less than 1 year for age 90 and older. Serum indices (hemolysis index, icteria index, lipemia index) were determined by commercially available reagents on a Cobas Integra 800 (Roche, Rotkreuz, Switzerland). Double sided 95% reference intervals together with the 90% confidence intervals (CI) were evaluated according to CLSI guideline EP28-3c.
Results: A total of 630 study participants (290 males, 340 females, age range 60-95, median age 73 years) had measurements available and were included into the present analysis. In contrast to the lipemia index ($r = -0.17$, $p < 0.001$) there was no significant correlation between age and hemolysis index or icteria index. Further, no sex differences could be observed among all indices. An age and sex independent non-parametric reference interval was 4 90% CI [3,4] to 14 90% CI [13,16] for hemolysis index, and 11 90% CI [10,11] to 29 90% CI [28,30]. Even if there was an association of age and lipemia index, stratification into different age classes was not justified. A common non-parametric sex and age independent reference interval for lipemia thus spans from 8 90% CI [8,9] to 17 90% CI [17,18]. Hemolysis index ($r = -0.08$, $p = 0.04$) and icteria index ($r = -0.25$, $p < 0.001$) were significantly associated correlated with haptoglobin. Lipemia index was lower in individuals without lipid lowering therapy (median 12 interquartile range, IQR [11,14] vs. 13 IQR [11,14]; $p = 0.002$).
Conclusion: In this carefully selected reference population, reference intervals for serum indices could be established. There is no need for sex and age-specific stratification of serum index reference intervals in seniors of Caucasian origin. The present study provides benchmarks, which can be used to determine e.g. the percentage of preanalytically normal samples in a medical laboratory.

P117

COMPARISON OF THE HEAVY/LIGHT CHAIN ASSAY TO OTHER QUANTITATIVE METHODS IN THE DIAGNOSIS OF IGA MULTIPLE MYELOMA PATIENTS

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Background
The heavy/light chain (HLC) immunoassays (Hevylite®, The Binding Site UK) separately identify and quantify the immunoglobulin isotypes like IgA-Kappa and IgA-Lambda. This enables the calculation of the heavy/light chain Kappa/Lambda ratio (HLCR) which provides additional information about the underlying disease. The HLC assays are used for diagnosis, monitoring, response assessment and prognosis of monoclonal gammopathies.
Objective of the study
The aim of the study was to assess the correlation between HLCR and other standard laboratory methods. Results were compared retrospectively with serum immunofixation electrophoresis (sIFE), total IgA, total light chains and serum free light chains (sFLC).
Patients and Methods
Sera from 31 IgA myeloma patients, selected on the basis of positive sIFE results, were studied. sFLC (Freelite®, The Binding Site UK) and HLC were quantified using polyclonal antisera assays on the BNTMII nephelometer (Siemens Healthcare Diagnostics Inc). Total IgA and total light chain measurements were analyzed on the BNTMII nephelometer. sIFE was performed using the Sebia Hydrasys 2 system.
Results
An abnormal IgA-HLCR was present in 100 % of the IgA samples with a positive sIFE result. Total IgA was abnormal elevated in 74.2 % and total light chain ratio was abnormal in 90.3 % of the cases. An abnormal sFLC ratio was found in 100 % of the sIFE positive samples. Two patients with normal values of total IgA and normal total light chain ratio but abnormal HLCR had been identified. One patient presented with a tri clone (IgA-Kappa, IgA-Lambda and IgG-Kappa) in which HLC measurements indicated IgA-Lambda (suppressed) as the minor clone. The HLC assay allowed the quantification of IgA monoclonal proteins which were not accurately measurable by serum protein electrophoresis.
Conclusion
A 100% agreement with positive sIFE was found with an abnormal IgA-HLCR as well with an abnormal FLC ratio. HLCR was more sensitive than total IgA or total light chain ratio. HLC assays provide a quantitative measurement, even when IgA M-proteins co-migrate with other serum proteins. The separate analysis of involved and uninvolved HLC could give additional information about the major/minor clones in case of oligoclonality.

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Bioactive Lipids, Inflammation, and Coronary Artery Disease

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Objectives: The aim of this study is to investigate the relationship between the eicosanoid metabolism and atherosclerosis. Eicosanoids are bioactive lipids, which regulate the initiation, progression, and resolution of inflammatory processes. While eicosanoids of the arachidonic acid metabolism are mainly proinflammatoric, metabolites such as resolvins produced from omega-3 fatty acids regulate the resolution of inflammation.
Methods: Plasma of patients of the LIFE Leipzig heart study without coronary stenosis (n = 1000), patients with a stenosis greater or equal 50% in all three coronary vessels (n = 1000), as well as patients with acute myocardial infarction (n = 1000) will be investigated. The highly standardized sample collection has already been completed. The detailed phenotyping of the patients includes coronary angiography, carotis sonography, and examination of the leg vessels, as well as the acquisition of anthropometric data. Further, the data of the established markers of coronary artery disease and extensive genotyping are available. We developed a method applying liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to quantify 94 eicosanoids as well as 7 polyunsaturated fatty acids (PUFAs) mainly of the proinflammatoric arachidonic acid metabolism. This method is currently being extended by the proresolute metabolites of omega-3 fatty acids such as resolvins.
Results: In a subcohort of 500 patients of the LIFE Leipzig heart study we already found 6 PUFAs and 20 eicosanoids, which derived mainly from the arachidonic acid pathway with a high interindividual variation (from 16 % for 5,6-dihydroxyeicosatrienoic acid (5,6-DHET) to 77 % for 13-hydroxyoctadecadienoic acid (13-HODE)). In a multivariate linear regression model, we identified the time of the last food intake to be the most prominent covariate for PUFA and eicosanoid concentration in plasma. Further, we found a significant positive association of arachidonic acid ($p = 0.019$) and a negative association of 5,6-DHET ($p = 0.036$) and 13-HODE ($p = 0.023$) with atherosclerosis through a multivariate logistic regression model.
Summary: For the first time it is possible to determine the pro- and anti-inflammatory as well as proresolute lipids, which act as agonists and antagonists in the inflammatory process, from a well characterized collective of patients. The relationship between PUFAs, eicosanoids, and coronary artery disease and other atherosclerotic vascular changes such as carotid plaques will be investigated. The prognostic value of the identified biomarkers will be determined with regard to the stability of the atherosclerosis and other cardiovascular events.

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Origin of antiphospholipid antibodies

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The antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by the occurrence of either venous or arterial thrombosis or recurrent pregnancy complications combined with the persistent presence of antiphospholipid antibodies (aPL). It is well accepted that aPL cause the development of the clinical manifestations of APS. Although several signaling pathways leading to APS have been identified, the precise pathogenesis of APS is still a matter of debate. Moreover it remains unclear how aPL develop. In this context the question arises why aPL occure spontaneously in some individuals while others develop a chronic aPL titer. The typical clinical manifestations are only detectable in the latter group of patients.
A common hypothesis is that bacterial and viral antigens have structural similarities to the autoantigens of the aPL, resulting in the development of infectious-associated autoreactive aPL (molecular mimicry). Appropriate structural similarities have been described. Some authors suggested that aPL belong to a repertoire of natural antibodies that are produced by b1 b-cells. This assumption could explain an interesting mouse model in which an increased aPL IgG production could be detected only one week after immunization with aPL as antigen.
Such a rapid IgG production after immunization with an “unsuitable” antigen can only be explained by the activation of preformed B1 cell clones.
The aim of the proposed project is to investigate how pathogenic aPLs are generated. In particular, it should be examined whether the hypothesis that aPL belong to the natural antibody repertoire can be confirmed. Furthermore, it should be examined whether pathogenic and non-pathogenic aPL are released from the same or from different B cell clones. If the B lymphocytes can be distinguished, this would represent a new diagnostic target. Otherwise, we will focus on the underlying mechanisms leading to aPL secretion. For this purpose, a mouse model is established in which the secretion of aPL can be specifically induced. First experiments have already identified TLR7 as a potential candidate molecule, which appears to be essential for the release of aPL. Thus, the specific inhibition of TLR7 could represent a new therapeutic approach to suppress the release of aPL.

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Mechanism and diagnostics of Cytomegalovirus-driven inflammation in neonatal and adult mice

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Congenital infection with human cytomegalovirus (HCMV) remains the most prevalent infectious cause of permanent abnormalities in newborns such as sensorineural hearing loss, liver and lung damage. In addition, postnatal HCMV infection can be life-threatening and may lead to long-term sequelae. In general, neonates are more susceptible to a variety of infection as compared to adults and it is believed that this is due to altered immune responses in the early life. HCMV infection is one example where the host's age has major effects on the clinical outcome. Despite the high seroprevalence of HCMV infection the majority of infected humans remain asymptomatic.
The murine cytomegalovirus (MCMV) - the mouse homologue to HCMV - has been used for a long time to study CMV infection in the mouse model and has unmasked many aspects of the complex interplay between this pathogen and the host, with predictive value for humans. We will utilize this infection model to identify and functionally characterize proteins that are involved in the inflammatory response to virus infection in the adult versus the neonatal host to unmask factors that explain the higher susceptibility of neonates to virus infection. By differential quantitative proteomics proteins will be identified, which contribute to the inflammatory response to CMV infection and are involved in antiviral immunity. In summary, this approach may allow to define critical molecules involved in control of CMV infection and new diagnostic markers for virus infection and systemic inflammation.

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Identification of novel biomarkers for systemic inflammation based on metabolomics studies in population-based cohorts

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Inflammation is characterized by comprehensive metabolic adaptions and is currently monitored using circulating markers like C-reactive protein (CRP). A persisting inflammatory condition is a known pathophysiological mechanism of widespread disease, like type 2 diabetes and a risk factor for mortality. CRP concentrations are too unspecific to mirror the global role of inflammatory processes in human metabolism. Comprehensive profiling of the small molecule content of plasma and urine (Metabolomics) is a suitable alternative to overcome this drawback. It allows for the determination of an altered energy metabolism or necrotic products as well as immunemodulatory molecules. Up to now, Metabolomics-studies regarding inflammatory conditions are limited to small patient cohorts neglecting the systemic relevance for associated diseases. The utilization of population-based studies could bridge this gap. The present proposal is designed to utilize the advantages of large scale metabolic profiling in population-based cohorts to address inflammatory conditions from a systems biological context. First results from the Study of Health in Pomerania (SHIP) revealed several potentially novel biomarkers. Among them urine 3-sialyllactose was of particular interest. It represented a completely novel finding with respect to inflammatory conditions. The detection in urine is further a promising alternative to ease clinical routine. Data about 3-sialyllactose in literature is quite sparse and the present proposal attempts to expand the knowledge using genomic data, genotypes as well as expression levels, obtained within the SHIP. To sum up, the present proposal addresses three key points: 1) The utilization of Metabolomics-studies to analyze inflammation from a systems biological context. 2) The derivation of novel biomarker to potentially refine therapy and diagnosis. 3) The establishment of a nuclear magnetic resonance (NMR)-based technique to determine urine 3-sialyllactose in several other large cohort studies which were already characterized using NMR spectroscopy. In brief, the proposal aims to use a systems biological approach to derive relevant biomarkers that allow for a refinement of diagnosis and treatment of low-grade chronic inflammatory conditions.

Autorenverzeichnis