Abstracts*)

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M1: Mikroskopierkurs: Hämatologie - Schwerpunkt Anämien

M1

Mikroskopierkurs- Schwerpunkt Anämien M1

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Anämien sind häufige Erkrankungen und betreffen jedes Lebensalter. Die Erkennung einer Anämie mittels Blutbildbestimmung ist einfach, die weitere diagnostische Zuordnung kann jedoch eine große Herausforderung darstellen. In vielen Fällen ist die zytologische Beurteilung des nach Pappenheim gefärbten Blutausstrichs hilfreich, da Anämien häufig mit spezifischen Veränderungen der Erythrozytenmorphologie einhergehen. Die mikroskopische Blutbilddiagnostik stellt einen wesentlichen Bestandteil der Stufendiagnostik von Anämien dar. In einigen Fällen kann eine zusätzliche Knochenmarkzytologie hilfreich sein. Der Schwerpunkt des Seminars liegt in der eigenständigen Mikroskopie ausgewählter Blut- und einiger Knochenmarkausstriche aus dem Themenkreis der Anämien. Darüber hinaus werden ergänzende, zur Diagnosestellung notwendige Untersuchungen vorgestellt und lehrreiche Informationen zur Physiologie der Erythropoese und zu den einzelnen Krankheitsbildern vermittelt.

W1: Workshop Durchflusszytometrie - Anfänger

W1

“Flow cytometry for beginners”

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Flow cytometry plays a major role in the diagnosis of various hematological disorders. It is therefore of great importance to achieve a standardized work.

This includes an understanding of the principle of the technical bases, such as the device setting, the presentation of the results and the interpretation of results.

This requires a diversity of quality control and a targeted fault analysis.

Content of the workshop are:

- principle of flow cytometry
- Design and Machine Setting
- presentation of the results and the quality control
- investigation procedure based on an immune status
- measurement and interpretation of results

W3: Orientierende Personalbedarfsermittlung

W3

Orientierende Personalbedarfsermittlung

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Der Arbeitsbereich der MTLA hat sich in den letzten Jahrzehnten massiv gewandelt. Ursache ist die fortschreitende Mechanisierung und Automatisierung der Labordiagnostik, so dass mit dieser Entwicklung Einflüsse auf den Personalbedarf gegeben sind. Im Gegensatz zum niedergelassenen Bereich hat ein Krankenhauslabor mit der Sicherstellung der 24-stündigen Laborversorgung für die stationären Bereiche jedoch zusätzliche Aufgaben. In dem Workshop werden die Grundlagen für die Berechnung des Personalbedarfs vermittelt, Praxisbeispiele gesammelt und Anwendungsbeispiele gerechnet. Zu berücksichtigen sind die tägliche Besetzung der Laborroutine, klinik- und

laborindividuelle Gegebenheiten, Sonderdienstzeiten (Besetzung von Nachtund Wochenenddiensten) sowie nicht-analytische Tätigkeiten, um daraus eine aufwandsgerechte Besetzung der Abteilung abzuleiten.

W4: Durchflusszytometrie Fortgeschrittene

W4

"Flow cytometry for experts"

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The advanced course deepens the understanding of the measurement sample and it provides knowledge in lymphoma and leukemia diagnosis using standardized measurement methods.

To assess the measurement of flow cytometric sample always include the microscopic assessment of blood and bone marrow

Content of the workshop are:

- Technology: Indexes of blood and bone marrow (Gatingstrategien)
- Case studies from their own practice (laboratory)
- Quality control, proficiency testing

W5: Massenspektrometrie (Theorieworkshop mit Anwendungsbeispielen)

W5

Massenspektrometrie (Theorieworkshop mit Anwendungsbeispielen)

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Mass spectrometry is an analytical technique which separates molecules according to their mass: charge ratio and provides molecular spectra for subsequent analysis. Mass spectrometry can be used in many fields ranging from medicine to doping controls, pharmacokinetic and pharmacodynamics and "omics- technologies", such as metabolomics and proteomics. Furthermore, mass spectrometry is highly suitable to identify and characterize a wide range of molecules.

In this theoretical workshop "Massenspektrometrie (Theorieworkshop mit Anwendungsbeispielen)", participants will learn how different analytical questions or research hypotheses can be solved by using mass spectrometry.

Different ionization techniques depending on sample matrix and type of analyte will be presented and advantages and disadvantages of different mass analyzers and hybrid mass spectrometers will be discussed. The interpretation of spectra and the detection of hidden information from a spectrum will also be a part of this workshop. In addition, participants will have the opportunity to present and discuss their own case studies.

W8: Clinical Reasoning in der BMA-Ausbildung

W8

Uncovering Decision-making processes: Clinical Reasoning as a Framework for biomedical scientists in training and practice.

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Background
Decision-making plays an important role in our everyday professional working life where decisions are usually based on personal expertise, experience, traditions and emotions. Reflective practice and well-founded transparent decision-making processes are however a prerequisite

to establish professional practice. Theoretical models to guide this process in vocational training, further education and practice are available in Nursing and the therapeutic professions. To date an appropriate model for biomedical scientists is missing.

Learning objectives

The objective of this workshop is to clarify to which extent the model "Clinical reasoning", applied by therapists, can serve as a heuristic for biomedical scientists in order to reflect and communicate decision making processes. We will explore the models potential to promote professional expertise for biomedical scientists in vocational training, further education and everyday practice.

Contents

15 min: Introduction 15 min: Key note 1: What is Clinical Reasoning (CR)? 15 min: Key note 2: Learning experience with CR in the bachelorprogramme „Interprofessionelle Gesundheitsversorgung B. Sc.", Medizinische Fakultät Heidelberg. [Interprofessional Health Care B.Sc., Medical Faculty, University Heidelberg 45 min: Group work: Typical decision-making processes will be collected and assigned to the correspondending types of Reasoning within the CR-Modell. 45 min: Consolidation and discussion of the findings 30 min: Summary of significant statements for a discussion paper 15 min: Reflection and Feedback

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W9: Mentoring Praktiker Workshop "Mentoringkonzepte" "Schulung, Kompetenzprüfung und Lernaufgaben"

W9.1

Part I Training in laboratory medicine laboratory

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Keywords: Rili-BÄK, accreditation, PDCA cycle "training and competence",
MIQ concept, qualified admission, new employment field

Legally required by New RiliBÄK (Guideline of the German Medical Association):

"Rules should be established and documented, how and by whom must be the training of new employees in new analysis systems or lab tests."

Certified and accredited laboratories (DIN EN ISO 9001 and 15189) "shall retain appropriate documented information as evidence of competence."

The workshop factors and conditions are explained and illustrated practical examples of training conditions. There should be taught that they as an employee entitled to qualified admissions by specially trained. Mentors or quality manager could process oriented assume planning (mentoring concept) and coordination of the interested parties on behalf of the Head of Laboratory - a new employment field for more educated analysts in the laboratory management?

W9.2

Introduction to the development of learning tasks

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Keywords: learning process, acquisition of skills and abilities, problem solving, reflection

The didactic-methodical instrument of learning task has his beginning already in the nineties in the industrial, technical and commercial-administrative area. Experiences from the nursing sector confirm that a use of learning tasks in real professional situations promotes the acquisition of skills and abilities as well as the individual learning process in particular.

The work processes in the field of biomedical analytics undergoing currently enormous changes, so an implementation of learning tasks in the practical part of the education in the area of biomedical analytics and their education paths (z. B. POCT-Coordinator, POCT-Manager) can be used to optimize learning processes of this branch of profession.

The principal purpose in the use of learning tasks is the development of professional action competence, within the meaning of knowledge-based problem solving ability. The knowledge necessary for problem solving and the necessary competence arise from the respective action situation. Knowledge and experiences are activated and develops individual concepts for problem solving.

The protection as well as the deepening of the qualification provides the opportunity to link old with new content. Moreover, the achieved learning success is visible through the instrument of reflection and allows the learner to develop new strategies or modify known strategies.

W11: Crashkurs Biostatik with R

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[6]Helmholtz Centre for Infection Reseach, Bioinformatics and Statistics, Braunschweig, Deutschland

The workshop will be organized by the DGKL working group Bioinformatics. One of the goals of this group is to establish biostatistics and bioinformatics in the further education in clinical chemistry and laboratory medicine. As a common and very powerful tool, the program package shall be introduced and trained.

The workshop consists of two parts:

1. Basic introduction into R, using the graphical user interface R Commander (Rcmdr)
2. Expanded functionality with adaptated R functions and special R Packages.

Preparation

In advance, each attendee should install R on his or her own laptop. Alternatively, the tutors offer access to their personal laptops for a limited number of participants.

The installation is simple and needs just a few minutes: open www.r-project.org, select an appropriate CRAN mirror server from the download section, and choose for example “Download R for Windows” / “Install R for the first time”. Execute the installation and accept all proposals of the program with “OK” and “Continue”. Instructions for Mac OS and Linux are also available.

In addition, the Rcmdr package is needed: Click on the menu item “packages | install packages”, choose first the country, then “Rcmdr”. After completion of the download, start Rcmdr with “load package” and follow the instructions with “Yes” and “OK”. For an initial test enter any calculation in the upper field (e. g. 3 +5) and click on “execute command”.

If you have problems with the installation, you may send an e-mail to georg.hoffmann@trillium.de, andreas.bietenbeck@tum.de, or frank.klawonn@helmholtz-hzi.de.

Agenda

In part 1, the attendees will learn how to read, check and modify data from various sources (Excel, text).

Data evaluation will include the graphical representation (e. g. histograms and boxplots) as well as common procedures for univariate and bivariate analysis (normal distribution check using QQ plots and Shapiro Wilk test, significance of difference using the *t*-test, correlation of two measurands using scatter plots and regression analysis). All methods will be practically applied and critically discussed.

In part 2, three typical routine applications will be partially performed, partially demonstrated:

- method comparison and quality control
- estimation of reference intervals and data normalization
- classification and discriminatory power

The attendees will learn how to expand the functionality of the R Commander with their own commands. Some of the more than 1,000 additional packages, which are available on the Internet, will be demonstrated.

S1 Seminar: Grundkurs Molekularbiologie

S1

Molecular diagnostics

personalized medicine, quality management, and the German gene diagnostic act in the daily routine of a hospital laboratory

In the last 20 years molecular diagnostics have arrived in the clinical laboratory. Since that time the analytical requirements have continuously increased. The RiliBÄK, the German gene diagnostic act, and the DAkkS define the mandatory standards. Modern medicine demands patient adjusted therapies.

This workshop outlines the advantages and disadvantages of various molecular techniques. Furthermore, experiences in establishing in-house methods, performing proficiency tests, and addressing the requirements of the German gene diagnostic act and the RiliBÄK will be presented and discussed from the viewpoint of hospital laboratory routine.

S3: Harnsedimente

S3

Urinary sediment

J. Neuendorf[1]
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The urine sediment diagnosis in recent years experienced a huge boom and a certain reevaluation: The ability to get an insight into the qualitative and quantitative composition of the urine sediment very fast by simple laboratory equipment (centrifuge and microscope) qualifies the urine sediment diagnosis as an important and relevant method of examination.

The detailed and accurate morphological differentiation (isomorphic or dysmorphic erythrocytes, renal tubular-, transitional-, squamous epithelial cells, cell casts etc.) of the urinary sediment components makes possible a clear statement concerning the origin of the ingredients. This allows for a reliable diagnosis – for example of renal or post-renal diseases. Thus the modern urine sediment examination provides a safe diagnostic method for physicians and patients.

Especially the use of a phase contrast microscope facilitates the morphological differentiation of urine sediment components. The advantages of phase contrast microscopy over the brightfield technology are presented in this workshop. Numerous practical hints provide assistance in dealing with brightfield and phase microscopy.

Proper preparation and preanalytics of the urine sample are crucial for high quality urine sediment diagnostics and for reproducible results. Typical questions will be answered like: Which centrifugal RPM adjustment must be used? How quickly disintegrate the cellular components? What should be considered in correctly preparing an urine sample?

Morphological characteristics of urinary sediment components are illustrated and explained based on many digital images (brightfield and phase contrast) as well as short films.

The chemical testing using urine reagent test strips must be underpinned by the urine sediment diagnosis to get valid and meaningful results. Discrepancies between urine test strip and urinary sediment results will be explained.

This workshop will also address the current Guidelines of the German Medical Association (RiliBÄK) for urine analysis.

Talks

Tumortarget Identifizierung für Therapie und Diagnostik

V03 - Talk Naumann

Identifizierung neuer Zielmoleküle beim Magenkarzinom

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An einem Magenkarzinom erkranken in Deutschland jährlich ca. 20.000 Patienten. Die Kolonisation des Magenepithels mit dem humanpathogenen Keim *Helicobacter pylori* ist ein Risikofaktor für die Entstehung von Magenkarzinomen. Die relativen 5-Jahres-Überlebensraten beim Magenkrebs liegen bei 31 % für Frauen und 35 % für Männer. Hinsichtlich der Identifizierung von Biomarker zur Diagnostik von Frühstadien

des Magenkarzinoms existieren nur wenige Kenntnisse. Das Carbohydrate Antigen CA 72-4 dent als grober Serummarker für Magenkarzinome, während Frühmarker in Gewebeproben wenig erforscht sind. Im Rahmen unserer Forschung wurden Modifikationen von Signalproteinen (PKC, PKB) identifiziert, die als mögliche Frühmarker in Gewebeproben von Patienten (Magenbiopsien bzw. Resektionen) mit einer *H. pylori*-assoziierten chronischen Gastritis und Adenokarzinom dienen können. Aktuelle Forschungsergebnisse zu Faktoren (z.B. Deubiquitinylasen), die eine wichtige Rolle bei der Regulation der Überlebens (NF-kB)/Zelltod Signaltransmission im Magenepithel spielen, werden im Hinblick auf die Bedeutung neuer Biomarker und Zielstrukturen diskutiert.

V04 – Talk Poremba

Stratifying of cancer patients for targeted therapies by molecular pathology and CDx

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Companion diagnostics (CDx) is defined as a "product or technique specially designed for determining particular therapies for patients with already diagnosed disease or known predisposition". CDx nowadays belongs to the most important means of personalized medicine. The register "Personalisierte Medizin - in Deutschland zugelassene Arzneimittel", published annually by the "Verband forschender Arzeimittelhersteller (vfa)" comprises 41 active components for which CDx is mandatory before use for treatment. Among the latest substances is Olaparib (approved by the end of 2014) for the therapy of relapsed BRCA-mutated high-grade serous ovarial carcinoma and Osimertinib (approved in spring 2016) for inhibition of T790M-mutated EGFR. In approximately 60% of patients with non-small cell lung cancer (NSCLC) the EGFR T790M-mutation is the cause of resistance for therapy acquired resistance against tyrosin-kinase inhibitors of the 1st and 2nd generation. Even though the term CDx sounds new, it is a "good old friend" for pathologists: immunohistochemical analyses for estrogen- and progesteron receptor expression in the context of anti-hormonal therapy for breast cancer is nothing else than CDx! In solid malignant tumors, pathologists mainly use CDx in breast cancer, gastric cancer, colorectal cancer, NSCLC, malignant melanoma and ovarial carcinoma. Hematopathologists (often together with hematological oncologists) in addition deal with CDx in leukemia and some lymphomas. The broad technical spectrum for CDx in pathology and molecular pathology comprises immunohistochemistry (e.g. ER-, PR-receptor, Her2/neu), in situ hybridization (FISH and/or CISH), PCR techniques and sequencing, including NGS. Financial analysts expect that the global CDx market will almost triplicate from approximately 3 billion US-$ in 2014 to nearly 9 billion US-$ by the end of the decade. But more important than economic aspects will be the benefits for cancer patients: certified quality insurance among the institutes of pathology all over Germany provides state of the art CDx and molecular diagnostics leading to more therapeutic success and less side effects, and finally to less health care costs.

from bench to beside: Nephropathie

V09 – Talk Speer

Lipoproteine bei Nierenkranken - von der Modifikation zur Funktion

T. Speer[1]
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High-Density Lipoproteine (HDL) und Low-Density Lipoproteine (LDL) stellen neben ihrer Funktion als Lipid-Carrier zentrale Regulatoren der Endothelfunktion, Inflammation, Koagulation und Zellhomöostase dar. Entscheidend werden diese Funktionen der Lipoproteine durch deren Protein- und Lipidzusammensetzung sowie zusätzlich durch posttranslationale Proteinmodifikationen beeinflusst. In den letzten Jahren konnte nachgewiesen werden, dass die Funktionalität von HDL und LDL sehr heterogen ist. So konnte eindrücklich gezeigt werden, dass "protektive" HDL bei Patienten mit chronischer Nierenerkrankung nicht nur ihre positiven Eigenschaften auf die Zellen des Gefäßsystems verlieren, sondern sogar in schädliche Partikel transformieren. HDL von Nierenkranken induzieren eine endotheliale Dysfunktion, fördern eine endotheliale Inflammation und wirken prokoagulatorisch. Passend dazu waren in klinischen Studien bei Nierenkranken höhere HDL-Cholesterin Serumspiegel nicht mit einer niedrigeren Mortalität assoziiert sind, wie dies bei Gesunden der Fall ist. Gleichzeitig konnte nachgewiesen werden, dass sich HDL-Partikel von Patienten mit Niereninsuffizienz strukturell von den denen gesunder Personen unterscheiden. Insbesondere spielt eine veränderte Protein- und Lipidzusammensetzung der HDL-Partikel eine wichtige Rolle bei der Veränderung der vaskulären HDL-Effekte. Zudem weisen die HDL-assoziierten Proteine bei Nierenkranken posttranslationale Modifikationen auf, die zusätzlich die protektiven Eigenschaften der HDL negativ beeinflussen. Gleiches gilt auch für die LDL von Patienten mit chronischer Nierenerkrankung. Deren Funktionalität wird insbesondere durch eine Carbamylierung von Lysin-Resten des Apolipoprotein B moduliert.

Insgesamt besteht deshalb eine klare Beziehung zwischen der Struktur der Lipoproteine und deren funktioneller Rolle bei verschiedenen physiologischen Prozessen. Bei Patienten mit chronischer Nierenerkrankung stellen sowohl HDL als auch LDL entscheidende Faktoren bei der Entstehung und Progression einer mit der Niereninsuffizienz assoziierten Atherosklerose und Inflammation dar. Es zeigte sich zudem, dass die einfache Bestimmung von HDL-C und LDL-C keine sinnvolle Messgröße zur Abschätzung deren Funktionalität darstellt.

V10 – Talk Scherberich

Selected "biomarkers" to assess early stages of renal diseases

Association of disturbed structure and function as shown by serum levels of uromodulin, a kidney specific antigen.

J. Scherberich[1]

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Patients suffering from chronic renal failure usually do not reveal any characteristic symptoms, even in late phases of the disease. Thus, a mayor goal of laboratory analysis is to better diagnose renal injury at an early stage, e.g. by assessing urinary components (protein, Hb, blood, leucocytes, etc.) and cellular constituents (casts, tubular cells) through microscopic analysis of the urinary sediment, especially to exclude the socalled "active sediment" indicating a progressive kidney disease. Basic diagnostic procedures also include estimation of serum creatinine and calculated glomerular filtration rate (eGFR MDRD, eGFR-Epi). Unfortunately, these endogeneous filtration parameters, not related to a defined kidney structure, excessively lack accuracy and precision, and depend on variable muscle mass, inflammation, malnutrition. eGFR based on cystatin-C is superior to eGFR based on creatinine although cystatin –C levels also vary by inflammation, haematological/lymphatic diseases, and pregnancy (placental synthesis of cystatin). Nontraditional "renal biomarkers" should overcome these drawbacks, their clinical utility is still a matter of debate, however.

Biomarkers in urine (U):
Potential "renal biomarkers" lack reliability due to high variations of urine volume, osmolality, salt content, enzymatic digestion etc. "Protein excretion patterns" to detect tubular and glomerular lesions apply the markers IgG, albumin and α1-microglobulin related to the urinary creatinine concentration (protein-creatinine index), and are widely used in a clinical routine setting. Biomarkers related to renal tissue components, not kidney specific however, are tubular enzymes (AP, AAP, GGT, ßNAG), NGAL, KIM-1, components of the epithelial cytoskeleton (villin, keratinfragments, actin), membrane proteins as detected by mono/polycloncal antibodies, glomerular antigens (sCD80 in minimal change glomerulopathy), cytokines (IL6,IL18), EGF, MCP1, sCD14) which may be excreted at an increased rate during kidney damage. Urinary components released from the nephron interfere with those shed from compensatory hypertrophic and hypermetabolic remaining nephrons, a fact that dramatically lowers their diagnostic reliability. Tamm-Horsfall glycoprotein (Uromodulin) is the only kidney specific antigen (synthesized by the thick ascending limb of Henle, TAL-segment) and is excreted into urine by an inverse relation to serum creatinine (lowest levels in CKD stage 4-5). However, urinary Uromodulin shows various molecular conformations (oligo/polymers), increased instability due to varying Ca++ concentrations, and proteolytic cleavage of antigenic sites. Nontraditional "kidney biomarkers" still need further evaluation, and have not been introduced until since into clinical routine.

Biomarkers in blood/serum
Novel serum markers of kidney diseases especially of more rare subsets imply antibodies directed against glomerular phospholipase A2 type-1 receptor (PLA2Ra) and "thrombospondin-type 1 domain containing 7A" (THSD7A) presents in patients suffering from primary or secondary membranous glomerulonephritis. The diagnostic value of the soluble urokinase receptor (sUrokinase-type plas- minogen activator receptor, suPAR) to diagnose a focal & segmental glomerulopathy is still completely unclear, since the marker is also associated with a variety of other diseases which are completely unrelated to the kidney. Recently kidney specific uromodulin (see above) was revealed not only to be excreted into urine but also to enter the blood compartment and can be analysed by sensitive ELISA. Serum Uromodulin (sUmod) is a very stable antigen, and serum levels are around 1000 times lower compared to urinary Umod concentrations. Levels of sUmod already decrease in very early stages of renal failure (CKD 0 - CKD1) where serum creatinine, serum cystatin C and eGFR are still within normal ranges. sUmod decreases progressively with CKD stages and is lowest at CKD V and CKD Vd. The TAL-segment, the source of Uromodulin, is a mayor target in various forms of kidney alterations, híghly vulnerable to hypoxia, highly susceptible to various toxins, to ischaemia-reper -fusion injury, and structural changes of the TAL are closely related to the developent of acute renal injury (failure), as revealed by experimental animal studies. Presenting data on sUmod as a novel, sensitive and kidney–specific biomarker will be a main issue of the lecture.

DGKL / DVTA: Update Gerinnung

V12 – Talk Langer

Von-Willebrand-Faktor: erworbenes von-Willebrand-Syndrom trotz normaler Aktivität?

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Acquired von Willebrand syndrome (AVWS) is a rare, but potentially underdiagnosed bleeding disorder caused by quantitative and/or qualitative abnormalities of plasma von Willebrand factor (VWF). AVWS is associated with a variety of cardiovascular, autoimmune, and lympho- or myeloproliferative diseases, and its pathophysiology may include decreased synthesis, accelerated clearance, or increased proteolysis of plasma VWF. Many conditions associated with AVWS, such as hematological malignancies, severe atherosclerosis, aortic valve stenosis, or the use of extracorporeal circulatory assist devices (e.g. ECMO, LVAD), are characterized by dramatically increased plasma VWF. For this reason, patients with AVWS due to increased proteolytic degradation may present with normal or even increased VWF activity levels as assessed, for example, by solid-phase (e.g. VWF:CBA) or low-shear assays (e.g. VWF:RCo, VWF:GPIbM). In these patients, a pathological VWF activity/antigen ratio of < 0.7 may point to a structural defect of plasma VWF, but multimer analysis is usually required, and still considered the gold standard, to demonstrate a complete or relative loss of larger plasma multimers. In this regard, recent evidence indicates that quantification of the VWF propeptide (VWF:pp) and measurement of a proteolytic fragment specifically generated by ADAMTS13-mediated cleavage of plasma VWF provide valuable additional information in the diagnostic work-up of patients with AVWS: while patients with AVWS due to IgG MGUS, who typically responds to high doses of intravenous immunoglobulins, show an increased VWF:pp/VWF:Ag ratio (indicating accelerated immunologic clearance of plasma VWF), the laboratory profile of patients with aortic valve stenosis or left ventricular assist devices (LVADs) is characterized by dramatically increased VWF proteolysis. Understanding the pathophysiology of AVWS in various clinical conditions is not only a prerequisite for early and correct diagnosis of this potentially life-threatening bleeding disorder, but also mandatory for its efficacious management.

V13

Platelet function testing: state of the art

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Dysfunctional platelets cause bleeding or drive thrombosis that may occur as life-threatening complications. In clinical practice several platelet function tests are used particularly for the diagnosis of inherited and acquired platelet function disorders, for diagnostic monitoring of therapeutic platelet inhibition and for quality control of platelet concentrates. In case of a suspected platelet function disorder stained blood smear analysis and light transmission aggregometry are recommended as first-step tests. However, distinct platelet function defects are associated with variable alterations of platelet aggregation or even normal aggregation patterns in response to different platelet agonists. Therefore, complex analyses of platelet function using flow cytometry, lumi-aggregometry, platelet adhesion, thrombin generation and thrombus formation assays as well as functional ELISA assays, combined with immunofluorescence microscopy, electron microscopy, Western blotting, are still necessary for a differential diagnosis. Point-of-care platelet function tests performed in whole blood are not recommended for the diagnosis of platelet function disorders due to limits of sensitivity, but are useful to monitor anti-platelet therapy. Case reports with applied diagnostic approaches are presented to demonstrate strengths and limitations of classical and advanced tests of platelet function.

a.u.la - Update gynäkologische Endokrinologie

V14

Endokrinologische Ursachen von Zyklusstörungen

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Kliniker schätzen bei der Abklärung endokrinologischer Störungen einen zielführenden Laborbefund mit Kommentar. Der Hormonstatus bei Zyklusstörungen der Frau bietet sich für eine systematische Abklärung nach Leitsymptomen und diagnostischen Pfaden an. Klinische

Verdachtsdiagnosen können zwar zielgerichtet mit Laboranalytik ausgeschlossen werden (z.B. Hyperandrogenämie? DHEAS und Testosteron unauffällig. Befund: Keine Hyperandrogenämie), jedoch kann im Allgemeinen nur eine umfassendere Analytik angeraten werden. Um bei dem Beispiel zu bleiben: So finden sich auch ohne klinische Zeichen der Hyperandrogenämie (Akne, Hirsutismus, Haarausfall) nicht selten relevante Erhöhungen von DHEAS und Testosteron, die eine biochemische Hyperandrogenämie belegen.

Als Laborprofil bietet sich in der frühen Follikelphase (3.-5. Zyklustag) die Untersuchung von TSH, Prolaktin, LH, FSH, Östradiol, DHEAS, Androstendion, Testosteron und SHBG an. In der mittleren Lutealphase (21. Zyklustag bei 28tägigem Zyklus) sollten LH, Progesteron und Östradiol bestimmt werden, um Störungen der Follikelreifung zu erkennen, die dann ihrerseits zu einer gestörten Lutealfunktion führen. Der Befundkommentar sollte auf Diskrepanzen zwischen dem lt. klinischer Angabe erwarteten Zyklusstatus und „biochemischer Realität" hinweisen und auf mögliche Ursachen und therapeutische Konsequenzen hinweisen. Dieses setzt ein Verständnis der hierfür wichtigsten endokrinen Krankheiten voraus. Diese werden in dem Vortrag exemplarisch dargestellt: Beurteilung der ovariellen Reserve und der Lutealfunktion, Hyperandrogenämie, Hyperprolaktinämie, Schilddrüsenfehlfunktion.

V15 – Talk Ludwig

Gynäkologische Endokrinologie und Befundung in der niedergelassenen Laborarztpraxis

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In der niedergelassenen Laborarztpraxis werden gynäkologisch endokrinologische Laboruntersuchungen als Auftragsleistung erbracht. Die Interpretation der Laborwerte stellt dabei eine wichtige Schnittstelle zwischen Laborarzt und Gynäkologen dar. Eine Befundinterpretation setzt jedoch anamnestische Angaben wie z.B. den Zyklustag oder die Einnahme von Medikamenten voraus. Außerdem muss eine Fragestellung ersichtlich sein, sonst ist eine Befundinterpretation nicht möglich. Neben speziellen Anforderungsscheinen ist daher auch die enge Zusammenarbeit sowie eine gute Kommunikation zwischen Laborärzten und Gynäkologen notwendig. Der Laborarzt kann mit seinem Wissen und seinen Erfahrungen mit den einzelnen Labormethoden wichtige Hilfestellungen liefern. Auch von den Gynäkologen wird eine enge Zusammenarbeit mit den Laborärzten für eine gute Patientenversorgung immer wieder gefordert.

Im Vortrag wird dargestellt wie der Laborarzt seinen einsendenden Gynäkologen durch eine gezielte individuelle Befundinterpretation eine ausreichende Hilfestellung liefern kann. Es wird gezeigt welche Voraussetzungen notwendig sind und wie sich der Laborarzt das für die Befundinterpretation nötige Wissen aneignet. Häufige Fragestellungen werden ebenso erörtert wie das Erarbeiten von Lösungen für spezifische Fragestellungen anhand von Kasuistiken vor dem Hintergrund eines problemorientierten Lernens. Neben fachspezifischen Fortbildungen können Arbeitskreise und Experten-Netzwerke helfen, sich über komplexe gynäkologisch endokrinologische Fragestellungen auszutauschen und eine individuelle Lösung für eine optimale Befundinterpretation liefern.

Zirkulierende Tumor-DNA - diagnostische Relevanz in der Translation

V22 – Talk Haselmann

Towards Liquid Profiling of tumors in blood - Results of a large melanoma follow-up

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Background: The current standard for determining eligibility of patients with metastatic melanoma for BRAF-targeted (BRAFi) therapy is the tissue-based testing of BRAF mutations. As patients are rarely re-biopsied, the detection in blood in real-time may be advantageous as noninvasive biomarker to reflect the current mutational status of the patient's disease as well as allowing a cumulative analysis of the tumor and metastases with respect to a heterogeneous mutational profile. Furthermore, metastatic melanoma lacks clinically efficient biomarkers for early detection of resistance against systemic treatment with BRAF inhibitors and decision-making for alternative therapies.
Method: Blood samples from melanoma patients in two independent studies were subjected to plasma testing using the OncoBEAM™ V600E assay and tissue-based BRAF mutation analysis using Sanger sequencing of FFPE tumor tissue specimens. In the first study, the tissue-based BRAF mutation status was compared with matched plasma samples from 71 patients diagnosed with progressive metastatic melanoma and being either treatment-naïve or undergoing BRAFi therapy. In the second study, the mutational status in tissue and plasma samples from 136 patients of melanoma stage III or IV was compared in follow-up. Furthermore, a cross-section analysis comprising 440 patients suffering from

melanoma stage I to IV was performed in order to test assay robustness and sensitivity. Additionally, circulating tumor DNA harboring the BRAF V600 mutation was correlated with the clinical course and tumor marker S100 and LDH in 26 metastatic melanoma patients treated with BRAFi.

Results: Overall, results from OncoBEAM™ BRAF V600E plasma testing revealed a high degree of concordance with tumor tissue testing in both studies with 92.3% in study 1 and 92.6% in the second study, respectively. In the second study 2 (out of 4) patients originally classified BRAF-negative in the tumor, tested BRAF mutation-positive in plasma. In these cases, secondary BRAF mutant neoplasia or melanoma was found to be responsible for these discrepant results. Additionally, 2 (out of 5) patients with consistent negative OncoBEAM results in plasma, but with positive test in the tumor tissue, did not respond to BRAFi therapy potentially due to a false positive result. Importantly, dynamic changes of BRAF mutant ctDNA over time correlated with the clinical course and response as well as resistance to treatment. Positivity of BRAF plasma testing was significantly earlier detectable than relapse using radio-imaging. This indicates an important role of BRAF plasma testing to predict response or resistance to BRAFi therapy.

Conclusions: Blood-based testing using the OncoBEAM™ BRAF V600E digital PCR assay favorably compares with standard-of-care tissue-based BRAF mutation testing. These findings support the conclusion that blood-based BRAF testing may be an appropriate alternative for tissue-based testing for mutational characterization of the tumor and during the course of targeted BRAF-therapy. Comparable results are available for other malignancies underlying the usability and potential implications of liquid profiling in future diagnostics. Importantly, blood-based BRAF testing may be used to predict response to treatment and resistance prior to radio-imaging under BRAFi therapy thereby allowing for an improved treatment management.

DGKL/DVTA: Neue analytische Technologie

V25 – Talk Kwiatkowski

Moleculare diagnostics of tissues by picosecond infrared laser ablation

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In clinical routine diagnostics, chemical analysis is limited to body fluids, since molecular diagnostics from tissue samples require a time consuming and costly sequence of processing steps. However, tissue species proteomes of diseased individuals provide diagnostic information. The composition of tissue-proteomes can rapidly change during tissue homogenization and protein extraction by the action of enzymes released from their compartments, thus, resulting in the loss of disease specific protein species patterns. To overcome this drawback new methods for tissue homogenization and protein extraction are needed.

We developed a novel, ultrafast and soft method for cold vaporization of tissue via desorption by impulsive vibrational excitation (DIVE) using a picosecond-infrared-laser (PIRL). The exact chemical composition of proteins was not altered during the DIVE process even preserving theirs enzymatic activity. Due to the ultrafast release of proteins from tissues, we hypothesized that intact protein species are exposed to enzymatic degradation reactions to a less extend than conventional homogenization and extraction methods, thus, providing an improved access to the original composition of protein species in tissues. In order to verify this hypothesis, we compared the tissue proteome composition of condensates provided by PIRL-DIVE and protein extracts provided by conventional homogenization and extraction methods. A significantly higher number of intact protein species was observed in PIRL-DIVE condensates. The ultrafast release and transfer of proteins from tissues into frozen condensates resulted in a significantly lower degree of proteolysis using PIRL-DIVE compared to conventional methods. Furthermore, total number of identified proteins was considerably higher and more reproducible in case of PIRL-DIVE condensates, most probably due the very homogenous condensates that contain almost no insoluble particles. This allows a direct analysis with subsequent protein analytical methods without the necessity of additional processing steps like centrifugation.

Our results show that PIRL-DIVE provides unique access to protein species composition of tissue proteomes *in-vivo* that has not been available up to now with conventional homogenization and protein extraction procedures. Together with the ultrafast release of proteins from tissues and the compatibility of the PIRL-DIVE condensates with protein-analytical methods, PIRL-DIVE facilitates molecular diagnostics of tissues at protein species level.

Update Atherosklerose

V29 – Talk Thiery

"Do we need more precise laboratory diagnostics for the familial hypercholesterolemia?"

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Die kürzliche Zulassung cholesterinsenkender Wirkstoffe wie die PCSK9-Inhibitoren stellt die Präzision der modernen Lipiddiagnostik vor neue Herausforderungen. Die hohe Effektivität der PCSK9-Hemmstoffe in der LDL-Cholesterinsenkung bis zu 80% besitzt ein großes Potential für die Behandlung der familiären Hypercholesterinämie. Allerdings ist der klinische Nutzen der Therapie noch nicht durch kardiale Endpunktstudien belegt, sodass sie bisher nur Patienten mit monogenen Formen der Hypercholesterinämie und für koronare Hochrisikopatienten bei Unverträglichkeit oder nicht ausreichender Wirksamkeit von Statinen vorbehalten bleibt.

Die klinische Diagnostik einer monogenen Form der Hypercholesterinämie ist oft schwierig, da sich der Cholesterinphänotyp mit den Merkmalen einer polygenen Form der Hypercholesterinämie überschneiden kann. Eine genetische Analytik aller Verdachtsfälle (<1200 LDLR-Mutationen mit heterogenem Phänotyp, weitere Mutationen im PCSK9 und ApoB-Gen) würde den finanziellen Aufwand der Diagnostik sprengen. Die funktionelle Heterogenität der Mutationen wird zudem unterschätzt. Es ist daher eine Stufendiagnostik erforderlich, um zur Diagnosesicherung die Zahl genetischer Analysen auf ein Minimum zu beschränken. Es werden heute international verschiedene Algorithmen und Kriterien diskutiert, um die Höhe der Lipidkonzentrationen und klinische Merkmale zu verbinden. Von der European Atherosclerosis Society wird der bewährte Algorithmus des Dutch Clinical Network (DLCN) präferiert. Als Kriterien werden die Familiengeschichte für KHK, Xanthome und Arcus lipoides sowie das Vorhandensein einer KHK in Bezug zur Höhe des LDL-Cholesterins gesetzt. Ein Wert von >8 spricht definitiv für eine monogene FH, ein Wert zwischen 6-8 macht diese sehr wahrscheinlich, ein Wert 3-5 lässt den Verdacht auf eine FH zu. Der DLCN Algorithmus wurde unter Einbeziehung des Alters, niedriger Triglyzeride und stärkerer Gewichtung von Sehnenxanthomen weiterentwickelt (Wales FH Service). Alle vorhandenen Algorithmen zeigen jedoch Limitierungen in der Spezifität, besonders bei kontinuierlichen Variablen. Sie eigenen sich jedoch grundsätzlich um Patienten für eine nachfolgende genetische Analytik zu identifizieren und dann ggf. einer PCSK9 Therapie zuzuleiten. Es bleibt abzuwarten, ob die heute zuverlässige Analytik von Apolioprotein B statt LDL-Cholesterin und weitere Lipidvariablen eine Optimierung der FH-Scores erlauben wird.

V30 – Talk von Eckardstein

Pre- and postanalytical phases of lipid testing: Joint Recommendations of the European Atherosclerosis Society and the European Federation of Laboratory

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A Consensus Panel of the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) critically evaluated the clinical implications of using non-fasting rather than fasting plasma lipid profile, and provided guidance for laboratory reporting of abnormal nonfasting and fasting lipid profiles.

The evaluation of extensive data from observational data studies using random non-fasting and fasting blood samples revealed moderate and clinically not significant impact of prandial status on plasma levels of triglycerides, total cholesterol, LDL cholesterol, and non-HDL cholesterol; concentrations of lipoprotein(a), apolipoproteinB, HDL cholesterol, and apolipoproteinA-I are not affected by prandial status. Neither had prandial status any relevant impact on the performance of cardiovascular disease risk prediction. To improve patient compliance the EAS/EFLM panel therefore recommends non-fasting lipid profiles to be used routinely. Only in patients with non-fasting triglycerides> 4.5mmol/L(>400mg/dL) fasting sampling may sometimes be considered. Laboratory reports should use 'ideal' concentration cutpoints and for non-fasting samples flag abnormal levels as triglycerides ≥2mmol/L(≥180mg/dL), total cholesterol ≥5mmol/L(≥190mg/dL), LDL cholesterol ≥3mmol/L(≥115mg/dL), nonHDL cholesterol ≥3.9mmol/L(≥155mg/dL), lipoprotein(a) ≥50mg/dL(≥80th percentile), apolipoproteinB ≥100mg/dL, HDL cholesterol ≤1mmol/L(≤40mg/dL), and apolipoproteinA1 ≤125mg/dL; for fasting samples abnormal concentrations should be triglycerides ≥1.7mmol/L(≥150mg/dL). Finally, life-threatening concentrations should lead to lipid clinic referral at triglycerides> 10mmol/L(>880mg/dL) for risk of pancreatitis, LDL cholesterol> 13mmol/L for homozygous familial hypercholesterolemia, LDL cholesterol> 5mmol/L for heterozygous familial hypercholesterolemia, and at lipoprotein(a)> 150mg/dL(≥99th percentile) for very high risk of myocardial infarction and aortic valve stenosis.

DGKL / DVTA: Point of Care Diagnostik-Management

V33 – Talk Gässler

New concepts for the organization of POCT

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Point-of-Care-Testing offers huge potential for patient care and for the workflow in the respective area of application. The analysis of blood gases in the operating theatre and the surveillance of blood glucose by the bedside or in the doctor's office are well-known examples for this purpose.

New concepts for the use of POCT are principally involved in problems with pre-analytics, blood volume, transport logistic and laboratory test latency.

Point-of-Care-tests, which are in use, sometimes include very complex processes such as microfluidic systems, cartridges with integrated sample preparation, diagnostic tests on mobile devices and many more. They are often insufficiently or not at all integrated in organizational workflows.

Just like in medical-diagnostic laboratories, it is fundamental for POC-diagnostics to seek an improvement, which compromises:

- prevention of time-consuming sample logistics in favor of a rapid availability
- improvement of turn-around-time (TAT), which is mandatory for DRG-conditions
- logistic of sample transport, request and delivery of findings
- integration of laboratory results of out-patients in a cumulative report.

POCT can be used in a number of different areas like doctor's offices, ambulatories, hospital wards or countries without any laboratory-diagnostic infrastructure.

If one really wants to use these advantages of POCT, it is essential to integrate the test results into an existing infrastructure in order to keep comparable results. With modern communication technology, standardization of laboratory-diagnostic techniques, sufficient quality assurance of analyses and such like, these results should induce a medical therapy recommendation as quickly as possible. Only thus, we can speak of a coherent concept for Point-of-Care-diagnostics.

Or to put in another way, how can it be that users of smartphones are better networked than users of POCT? There is still a lot to be done for the manufacturers of analysis devices as well as for us.

DGKL / DVTA: Point of Care Diagnostik-Management

V35 – Talk Vashist

Point of care testing for the developing nations

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There is an immense need for point-of-care testing (POCT) for the developing nations taking into account the rapidly increasing number of persons affected by diabetes, cardiovascular diseases, infections, and other diseases. Diabetes has already become the global epidemic with 415 million (M) diabetics and about 11.2% of global healthcare expenditure that is spent on diabetes annually. Moreover, there are about 318 M persons having impaired glucose tolerance that are progressing towards diabetes unaware. The situation is further worsened by ~ 46.5% undiagnosed diabetics. Similarly, human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), tuberculosis, and malaria affects a vast majority of the population in the developing nations. However, the mortality due to diabetes (i.e. 5 M) still exceeds the combined mortality of 3.6 M due to HIV/AIDS (1.5 M), TB (1.5 M) and malaria (0.6 M). As many people in developing nations do not have access to central laboratory services and have very limited number of healthcare professionals, there is a adamant need for rapid, reliable and affordable POC tests. Such tests can be used by primary healthcare workers without any requirement for expensive devices and complex sample preparation. Although several prospective POC tests have been developed and commercialized recently for HIV, TB, malaria, febrile illness, respiratory infections, and other diseases, there is a requirement for POC tests for many other diseases/disorders prevalent in the developing world. The evolving trend towards personalized mobile healthcare using smartphones and smart wearable gadgets might further facilitate the POCT in the developing nations. However, the current POCT is mainly driven by the business opportunity, which is dictated by the incidence of the disease. The POC tests for other infectious diseases, TB, malaria, sexually transmitted infections, neglected tropical diseases, cancer screening, etc., still need to be developed. POC tests would gretly facilitate healthcare monitoring and management in the developing nations due to the lack of resources, infrastructure, and healthcare professionals. Therefore, there is a need for consistent research, concurrent funding, harmonized regulatory standards, quality assurance, and capacity building for the development, uptake, and delivery of POC tests. The recent advances in POCT will be discussed together with the rapidly evolving trend towards personalized mobile healthcare and the pending challenges.

DGKL / DVTA: Update Klinische Massenspektrometrie

V37

Mass spectrometric methods in microbiology testing

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Matrix assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) for identification of bacteria is a method that revolutionized clinical microbiology during the last decade. The method is based on the work of Koichi Tanaka and the two German scientists, Franz Hillenkamp and Michael Karas. In the late 1980s they discovered that macromolecules can be ionized without losing their structure when embedded in a matrix containing small organic compounds. Based on this observation an MS technology was established that identifies bacteria based on a characteristic peak profile representing mainly ribosomal proteins of the pathogen. MALDI-TOF MS is nowadays identifying bacteria more rapid, accurate and cost-effective than any traditional method. At present there are three MALDI-TOF MS instruments for routine laboratory use available: The Microflex Biotyper from Bruker Daltonik, the bioMerieux Vitek MS (former Axima from Shimadzu) and the Andromas SAS. Only the former two instruments have FDA clearance and are also the most widely used systems in Europe. Identification is generally performed from single colonies grown on agar plates. Less common and more demanding is the identification of microorganisms directly from patient derived material. Given the 10^5 CFU limit of detection, specimens suitable for direct identification are mainly blood cultures and urine. In blood cultures human blood cells, serum proteins and charcoal particles, used by some manufactures to absorb antibiotic carryover, may complicate the process as they can interfere with the peak pattern of the mass spectrum. Various protocols and a commercial kit are available to isolate the bacteria from the blood culture fluid. The actual success rates for direct identification vary by study but it appears that performance is more reliable for Gram-negative than for Gram-positive bacteria. Direct identification of bacteria from urine specimens has shown to be feasible but due to the complexity of the process is hardly used in clinical routine. Apart from species identification MALDI-TOF MS can be used for further characterization and sub-typing of bacterial isolates. By analysing discreet variations of the spectrum it is possible to identify highly virulent clones or strains with a characteristic resistance profile. To a certain extent epidemiological studies can be performed with MALDI-TOF, but it is significant to note that the clustering is based on ribosomal proteins and thus might give different results when compared with other methods like MLST or serotyping. MALDI-TOF MS might also be a promising tool for rapid determination of antibiotic and antifungal resistance. Several techniques for susceptibility testing have been developed with one functional assay for detection of carbapenemases currently being introduced into routine diagnostics. In the future new applications like nucleic acid-based MALDI-TOF MS for microbial and viral detection might play a role. Another challenge lies in the further implementation of MALDI-TOF MS in an increasingly automated lab workflow.

BDL - INQUAM

V41 – Talk Winter

Learning from aviation- innovative quality management for laboratories by using “IQSmart”

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Objectives:
Challenges of today’s process management are the simplicity and comprehensibility of the process management system in use as well as the way of its presentation for its users. Employees face a variety of legal formality and standard operation procedures (SOP) with diverse histories of modification and changes, which cannot be safely ascertained. Often, redundancy or even worse, contradictoriness between the documents can be observed. The established documentations are most of the time quite extensive, isolated from each other and usually “paper-based” and hence not well controllable for the employees. Another weak point is the missing linkage and verification between the existing SOP’s and the continuously changing legal framework. Therefore, there is a huge demand for a feasible process management tool, which ensures an up-to date process presentation for the employees offering him at the same time a simple but also safe access to all his relevant process information.
Methods:
In 2002 Lufthansa Technik developed an integrated process management system (IQ MOVE). IQ MOVE is not only a platform for process documentation. It also 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

Lufthanse Technik spin off, IQSmart was founded aiming for the development of a salable product for the QM market. Within the German health care market, this was realized in close cooperation with the university Medicine Greifswald and the medical information system provider c.a.r.u.s.

Results and Conclusion:

Exemplary, the process management system IQSmart was introduced within the newborn screening laboratory of the University Medicine Greifswald, which is part of the Institute for Clinical Chemistry and Laboratory Medicine. Besides the process orientated presentations a unification and consolidation of existing SOP's was realized within IQSmart. Additionally, a deep cross-interlocking with existing legal framework of the German newborn screening and the laboratory processes were achieved. With this integration, the periodic regulatory audits in due course of the required certification can be performed in a safe and transparent way. All processes are displayed in a role-based way. Thereby, the employee is able to find his/her relevant process steps, 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. The upcoming certification will be performed with IQSmart instead of paper-based SOP's, which was recommended by the auditors during the last audit.

Vaskuläres Remodeling

V44 – Talk Korff

Adaptive Strukturwandlung des gesunden und erkrankten Gefäßsystems

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Das arterielle und venöse Gefäßsystem ist einer großen Fülle von humoralen und biomechanischen Umgebungsparametern ausgesetzt, die lokal oder systemisch und kurz- oder langfristig wirken können. In Abhängigkeit dieser Einflüsse sind Arterien, aber auch Venen in der Lage, ihre Struktur und funktionellen Fähigkeiten anzupassen. Beispielsweise führt chronische oder repetitive Erhöhung des lokalen Blutflusses zu einer Durchmesservergrößerung der entsprechenden Arteriolen bzw. Arterien – ein Prozess der im Rahmen einer Kollateralbildung als Arteriogenese bezeichnet wird. Eine chronische Erhöhung des systemischen Blutdrucks führt hingegen in Arterien üblicherweise unter Verkleinerung ihres Durchmessers zu einer Verdickung und Versteifung der Gefäßwände, während dies in Venen im Rahmen der Varikose eine Durchmesservergrößerung und deren Insuffizienz bedingt. Alle (patho)physiologischen Anpassungsreaktionen bedienen sich dabei spezifischer durch Umgebungsvariablen geprägte Phänotypänderungen von Endothel- und glatten Gefäßmuskelzellen. Im Rahmen des Vortrags werden Mechanismen vorgestellt auf deren Basis diese Zellen verschiedene Umbauprozesse der Gefäßwand teils unter Einbeziehung des Immunsystems koordinieren. Ein Schwerpunkt wird dabei die bluthochdruckbedingte Gefäßwandumbildung und die Bildung arterieller Kollateralen im Rahmen der peripheren arteriellen Verschlusskrankheit sein.

Kognitionstörungen: Von der Grundlagenwissenschaft zur Diagnostik

V47 – Talk Wirths

Role of N-terminal truncated Abeta peptides in Alzheimer's Disease

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Alzheimer's disease (AD) pathology is intimately linked to the formation of amyloid-ß (Aß) peptides, which represent the main constituents of extracellular amyloid plaques. In addition to the well-studied full-length Aß peptides starting with an aspartate at position 1, a variety of of shorter N-terminal truncated Aß species has been identified in recent years in the brains of sporadic and familial AD subjects. Among those, Aß peptides starting with a pyroglutamate at position 3 (AßpE3-x) and Aß with a phenylalanine residue at position 4 (Aß4-x) represent highly abundant isoforms in hippocampus and cortex of AD patients. Currently, the precise enzymatic activities leading to the generation of the diverse N-terminally truncated variants are not known, but several candidates have been recently proposed. In general, N-terminal truncations result in a higher aggregation propensity due to increased hydrophobicity and extensive *in-vivo* neurotoxicity has been demonstrated in transgenic moouse models that exclusively generate N-truncated Aß species. As there is accumulating evidence that full-length Aß species exert physiological functions, N-truncated Aß variants that appear during AD progression might represent promising targets for therapeutic interventions like passive immotherapy.

Novel aspects of coagulation and inflammation

V49 – Talk Langer

Cancer driven Thrombosis

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In 1865, the French physician Armand Trousseau described the association between superficial vein thrombosis and gastrointestinal cancer. Based on these observations, clinically relevant clotting abnormalities in cancer patients are nowadays referred to as Trousseau's syndrome. Both the clinical presentation and the underlying pathophysiology of Trousseau's syndrome are highly variable. The most frequent manifestation in clinical practice is venous thromboembolism (VTE), a composite of deep vein thrombosis and pulmonary embolism, but cancer patients may also experience more complex clotting abnormalities such as disseminated intravascular coagulation (DIC), excessive fibrinolysis, or thrombotic microangiopathy. Aberrant expression of tissue factor (TF), an integral transmembrane protein that serves as the cellular receptor and cofactor for factor VIIa to initiate the extrinsic coagulation pathway, has been shown to be critically involved in the pathogenesis of cancer-associated VTE and DIC. TF is not only over-expressed by tumor cells and cells of the tumor microenvironment, but also released into the circulation as a soluble molecule (i.e. alternatively spliced TF) or in association with subcellular membrane vesicles, so-called plasma microparticles (MPs). Both cellular TF expression and release of MP-associated TF are thought to be controlled by specific activating or inactivating mutations in oncogenes and tumor suppressor genes, respectively, and coagulation-dependent and –independent functions of TF have been shown to contribute to primary tumor growth, angiogenesis, and hematogenous metastasis. For this reason, TF is still being explored not only as a diagnostic tool and predictive biomarker, but also as a therapeutic target in patients with various types of malignancy. Recent experimental and (pre)clinical evidence also indicates that the intrinsic contact pathway contributes to cancer driven thrombosis. In this regard, activation of factor XII relevant to the pathogenesis of VTE may occur through negatively charged polymers such as polyphosphates or nucleic acids (i.e. RNA, DNA) liberated by bacteria, activated platelets and leukocytes, or (necrotic) cancer cells. Because pharmacological interference with factor XII activation does not increase the risk of bleeding in animal models, further exploration of the role of the intrinsic contact pathway in cancer driven thrombosis may pave the way for safer treatment strategies in patients with Trousseau's syndrome.

V50 – Talk Wiersinga

The role of the gut microbiota in sepsis

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The gastrointestinal tract harbours a complex population of microbes that play a fundamental role in the development of the immune system and human health. Besides an important local contribution in the host defense against infections, it has become increasingly clear that intestinal bacteria also modulate immune responses at systemic sites. These new insights can be of profound clinical relevance especially for intensive care medicine where the majority of patients are treated with antibiotics which have pervasive and long-term effects on the intestinal microbiota. In sepsis, defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, the composition of intestinal microbiome is severely disturbed, which might attribute to the development of organ failure. In this talk these aspects are highlighted with a focus on recent key findings in mice and man addressing the role of intestinal microbiota in sepsis and its impact on intestinal homeostasis in the critically ill. The focus will be on pneumonia-derived sepsis. Potential novel treatment options for sepsis include targeted administration of beneficial commensals or specific microbiota-derived components. Translation of preclinical data into functional characterizations will be essential to understand how our commensals impact on susceptibility and pathogenesis of sepsis.

V51 – Talk Renné

Analysis of body-wide unfractionated tissue data to identify a core human endothelial transcriptome

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Endothelial cells line blood vessels and regulate haemostasis, inflammation and blood pressure. Proteins critical for these specilised functions tend to be predominantly expressed in endothelial cells across vascular beds. Here, we use a systems approach to identify a panel of human endothelial-enriched genes using global, body-wide transcriptomics data from 124 tissue samples from 32 organs. We identify known and unknown endothelial-enriched gene transcripts and use antibody-based profiling to confirm expression across vascular beds. The majority of identified transcripts were detected in cultured endothelial cells from various vascular beds, and we observe maintenance of relative expression in early passage cells. In summary, we describe a widely applicable method to determine cell type-specific transcriptome profiles in a whole organism context, based on differential abundance across tissues. We identify potential vascular drug targets or endothelial biomarkers, and highlight candidates for functional studies to increase understanding of the endothelium in health and disease.

Ausbildungsforum

V53

Development of a graded competence-based framework for biomedical science in Germany, Austria and Switzerland

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Biomedical Scientists (BMS) respectively Medical Laboratory Technologists (MLT) carry out self-responsible performance of tests with biological samples in the fields of analytics and diagnostics in clinical laboratories. The results of their analysis are essential basis for diagnostic and therapeutic purposes. The aim of the project is to develop a graded competence-based framework for different levels of experience of BMS/MLT. A documentary research of (inter-)national rules and standards relevant for the profession was undertaken (Mayring, 2015). According to competence stage models (Benner, 1994; Rauner, 2002) a competence-based framework was developed for seven occupational roles following the CanMEDS-model (Frank, 2005) for different levels of experience. The first version should be verified in focus groups as well as by discussion of this paper with experts from professional practice, education, management and research and if necessary be adapted.

Pathobiochemie und Biomarker zur Diagnostik fibrosierender Erkrankungen

V54 – Talk Ramming

Pathophysiology and potential biomarkers for the diagnostic of fibrotic diseases

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Fibrotic diseases impose a major socioeconomic burden on modern societies and account for up to 45% of deaths in the developed world. Despite the great medical need, anti-fibrotic therapies are not yet available for clinical use. Fibrosis may occur after defined noxious stimuli, but in many cases, no initiating trigger can be identified. Fibrotic diseases can affect virtually every organ system. They can be restricted to single organs, as in idiopathic pulmonary fibrosis (IPF), or may affect multiple organs, as in systemic sclerosis (SSc). The histopathological feature of SSc is an excessive accumulation of extracellular matrix that often disrupts the physiological architecture of affected tissue. Fibroblasts are the principle source of extracellular matrix and have been accused as major culprits of fibrotic disorders. Although the molecular mechanisms underlying the aberrant activation of fibroblasts in SSc and other fibrotic diseases have only partially been unraveled, there is considerable evidence that transforming growth factor-beta (TGF-ß) is a key regulator of fibroblast activation. TGF-ß signaling is activated in fibrotic diseases and fibroblasts display nuclear accumulation of the downstream mediator Smad3 and increased transcription of TGF-ß target genes. Moreover, TGF-ß potently activates fibroblasts and induces an expression profile in resting normal fibroblasts that is reminiscent of SSc

fibroblasts. The central role of TGF-ß signaling is further highlighted by the development of a systemic fibrotic disease in mice with fibroblast-specific overexpression of constitutively active TGF-ß receptor type I. However, the knowledge of the crucial role of TGF-ß has not yet been translated into molecular therapies and effective targeted treatments for fibrosis in SSc and other fibrotic diseases are not available for clinical use. There are promising targets with therapeutic potential that have been extensively characterized in recent years connected with the hope to translate these preclinical results into clinical practice and to improve diagnosis and therapy of fibrotic diseases.

V55 – Talk Faust

Xylosyltransferasen als Marker fibrotischer Erkrankungen

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Fibrotic remodeling of the extracellular matrix (ECM) is associated with nearly 45% of deaths in the developed world. Underlying pathomechanisms include differentiation of resident fibroblasts to myofibroblasts, which excessively synthesize ECM molecules. The accumulation of ECM components and myofibroblast persistence are characteristic hallmarks of fibrosis. However, until today neither a therapeutic option nor a noninvasive fibrosis biomarker is known. Thus, a better understanding of molecular mechanisms is necessary.

Our research focus is on characterizing and unravelling mechanisms of xylosyltransferase (XT) regulation in fibrosis. The isoenzymes XT-I and -II catalyze the rate-limiting step in proteoglycan (PG) core-protein glycosylation in the golgi. In early studies, our group determined the main biochemical properties of both isoenzymes and revealed several differences concerning substrate specificity or tissue expression patterns while both XT could be shown to be secreted in the extracellular space. By establishing an enzymatic assay to quantify XT activity, we examined that serum XT activity reflects the PG biosynthesis rate. As PG are involved in ECM signaling as well as in maintaining matrix integrity, their biosynthesis is upregulated in almost any type of fibrosis. Accordingly, we observed increased serum XT activity rates in liver fibrosis and systemic sclerosis. Furthermore, we were able to show that fibrotic remodeling in dilated cardiomyopathy and arthrofibrosis is associated with increased *XYLT1* mRNA expression.

Recently, we also found that XT activity and increased *XYLT1* mRNA expression are not only associated with manifested fibrotic remodeling but also with the initiating fibrotic event of myofibroblast differentiation. Interestingly, unlike XT-I, XT-II is neither regulated in a fibrotic context nor induced by transforming growth factor-β1 (TGF-β1), the fibrotic key mediator. This observation was reinforced by the identification of an evolutionary highly conserved *XYLT1* promoter fragment, which has not been described in the human *XYLT1* reference sequence before and harbors several transcription factor binding sites being responsible for inducibility by TGF-β1.

Current investigations address the identification of further regulatory molecules as for instance profibrotic proteins, microRNAs and reactive oxygen species which might exert influence on XT. Potentially, an improved characterization of XT regulation will uncover suitable therapeutic targets. Moreover, we analyze to what extent a CRISPR/Cas9-based *XYLT1* knock-out or knock-in might affect ECM remodeling and whether xylosides, nucleotides and several other components might be suitable to inhibit XT activity.

In summary, the results of our work not only provide new insights into XT regulation in fibrosis but also contribute to evaluate XT as a diagnostic biomarker and potential target of antifibrotic therapeutic approaches.

POCT-Management Labormethodenvergleiche Vorstellung des jeweiligen POCT-Managements

V66 – Talk Hug

POCT Management of Labor Berlin

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Background: The guideline of the alliance of the medical association aims to ensure the quality of analyses in laboratory medicine (1). Particularly it guarantees:

– The minimization of influencing variables and disturbing factors in pre analysis

- The professional execution of analyses in laboratory medicine including the identification and minimization of disturbing effects on analyses
- The correct assignment and documentation of test results including the issue of a report

Material and Methods: Labor Berlin has developed and implemented a POCT concept that fulfills these requirements. Required components are:
- Process model
- Quality management system
- Structuring of responsibility, authority and communication
- Management of resources
- Structuring of processes in POC diagnostics
- Specification of measurement, analysis and improvement

Results: The POCT concept of Labor Berlin bases on the process model of the DIN EN ISO 9001:2008 and the RiLiBÄK 2008. Labor Berlin has established central POCT structures in the supervised hospitals and facilities. In addition arrangements inside and outside (subsidiaries) of those hospitals have been implemented. Concerning staff management structures for qualification, training / certification and user identification have been developed. The management of the equipment incorporates the requirements of the different hospitals and facilities. Among other things it includes aspects of user's permission and availability of devices / concept of exchange of devices. Concerning data management Labor Berlin sought to standardize processes. Structures of internal and external quality control have been established to ensure the quality of measurements and analyses. This includes the implementation of a program of quality audits.
Discussion and Conclusion: The POCT concept of Labor Berlin guarantees a highly standardized and efficient way in monitoring the analytical processes in the associated hospitals and facilities according to the guidelines of the alliance of the medical association of quality management of analyses in laboratory medicine.

Literature
(1) Richtlinie der Bundesärztekammer zur Qualitätssicherung Laboratoriums-medizinischer Untersuchungen, Deutsches Ärzteblatt, Volume 105, issue 7, 15. February 2008, page A 341 - 355

Immune modulation in cancer and its therapeutic potential

V72 – Talk Gebhardt

Predictive biomarkers for cancer immunotherapy

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Immune checkpoint-inhibition using monocolonal antibodies targeting PD-1 (pembrolizumab or nivolumab) or CTLA-4 (ipilimumab) is a revolutionary therapeutic approach in metastatic melanoma patients and is characterized by a significant extension of overall survival and long-term responses.

Recently, pre-treatment baseline levels of immune cell subtypes and chronic inflammatory factors have been identified as predictive biomarkers for immune checkpoint-inhibition in melanoma patients.

The presentation will address the challenges in identifying predictive markers of immunotherapy. Moreover, most recent advancements in the list of suitable blood cell subtypes and serum factors as biomarkers will be presented and underlying mechanisms will be discussed.

BDL - Die Gefahr der totalen Vernetzung

V75

The Dangers Caused by Total Cyber-Interconnectedness

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The worldwide electronic interconnectedness of devices and humans, that means "The Internet of Things" ("World of Things"), is a dangerously misguided development. We have to assume that at most all internetworked systems can be watched and controlled from the outside

-- including the possibility of governing and manipulating without being perceived. Security experts are not able to gauge not to mention to limit the risks of crime, spying and sabotage which the population, the economy, the society, the state is exposed to. Interconnectedness is turning to a fatal danger in the case that a certain component exists within the internet which is authorized to start an act of war autonomously if algorithms give order to avert conjectured danger. This component enables the autonomous preventative cyber war. Because of this updated situation of the risk all the critical installations have to be separated from the internet without delay. But for the short term there is a serious consequence of establishing the fully connected "Internet of Things", which urgently runs contrary to german basic law: there will be established conditions to industrial concepts, which are based on expropriation of private data and violation of privacy. If privacy of persons and economic subjects are destroyed, there is no longer any autonomous economic development. In such a way every social community will be destroyed radically. This lecture is asking the question wether we ethically can take the responsibility to develop the "Internet of Things" in view of the dangers of massive interconnectedness of such a system: my concern is to answer this question and discuss the resulting consequences. The ethical basis for discussing this question is the unconditional postulation, that economy, research and development has to serve the people in the spirit of Ludwig Erhard. Given the discordance of European Union we have to trust in the power of the single member of the public; it is the power to turn off evil named devices, and particularly it is the power of speaking in truth. The protagonists of a totally connected "Internet of Things" have to pay attention to do not sink in the quicksand.

Zirkulierende mikroRNAs – diagnostisch relevant?

V81 – Talk Piiper

Pre-analytical and analytical requirements for utilization of circulating microRNAs as diagnostic markers

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Altered levels of microRNAs (miRNAs) circulating extracellularly in the blood in a stable form, have been found in patients with diseases such as myocardial infarct, cancer and inflammatory diseases as compared to healthy subjects. Their levels can be conveniently determined by RT-PCR. Therefore, they are considered as novel class of minimally invasive disease biomarkers that might be valuable in diagnosis, prognosis and treatment response monitoring. For instance, we could show that the serum levels of miR-122, a liver-specific miRNA, in patients with chronic hepatitis correlate with the severity of the disease as well as survival of the patients and reflect response to therapy; miR-1 serum levels are prognostic in patients with hepatocellular carcinoma. Nevertheless, relatively large discrepancies exist among the different miRNA biomarker studies, and mechanistic bases of the postulated biomarkers are often lacking. Insufficient consideration of pre-analytical and analytical pitfalls is another obstacle in the turning of circulating miRNAs into clinically useful disease markers, some of which are considered here. Of obvious importance is that the extracellular miRNA pattern remains unaffected after drawing of the blood from the patients. We found that the miRNAs present in blood serum show differential stability. Encapsulation into lipid vesicles appears to confer higher stability than solely complexation with proteins. Moreover, we found that the degradation of miRNAs in serum can be prevented by RNAse A inhibition. Thus, RNAse Inhibitor might be useful to preserve the extracellular miRNA pattern. Remarkably, stabilization of miRNAs can also achieved by hemolysis, offering the possibility that stabilization of miRNAs not expressed in blood cells this can also be achieved by hemolysis. Another important yet neglected issue concerns the possibility that alteration of the extracellular miRNA composition occurs by differences in the time elapsing between blood drawing and the preparation of blood serum/plasma. Our data indicate that the incubation of EDTA and serum collection tubes for 1-3 hours at room temperature led to an increase in the levels of marker miRNAs expressed in blood cells without signs of hemolysis, and that this release of blood-cell derived miRNAs occurs by release of vesicles containing these miRNAs. Thus, the release of vesicle-associated miRNAs from blood cells can occur in blood samples within the time elapsing in normal clinical practice until their processing without significant hemolysis. Altogether, careful consideration of the pre-analytical and analytical requirements is a pre-requisite for turning extracellularly circulating miRNAs into clinically useful biomarkers.

V82 – Talk Schwarzenbach

Bedeutung Zirkulierender microRNAs in der Diagnostik von Krebserkrankungen

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MicroRNAs are a family of evolutionary conserved, small non-coding RNA molecules that post-transcriptionally inhibit protein expression of their target mRNAs. As microRNAs loci frequently map to fragile chromosomal regions harboring DNA amplifications, deletions or translocations, their expression is often deregulated during tumorigenesis, contributing to tumor progression, metastasis and drug resistance. Apart

from their release by apoptotic and necrotic cells, microRNAs can also be actively secreted into the blood circulation by exosomes. Exosomal microRNAs are thought to play an important role in cell-to-cell communication. Based on their biological functions and the possibility of quantifying microRNAs in patient blood in real-time, these small RNA molecules may be a new promising class of potential blood-based diagnostic biomarkers. Screening of these liquid biopsies may provide information on target molecules of microRNAs and aberrant signaling pathways that can be blocked by a chosen targeted therapy. Consequently, therapy-associated modulations may facilitate treatment decisions. Our findings show the relevance of circulating cell-free and exosomal microRNAs as diagnostic and prognostic tumor markers for breast, ovarian and lung cancer. We detected that the expression levels of microRNAs are associated with a particular biology of carcinomas, favoring tumor progression and metastatic spread. Furthermore, our data demonstrate a specific influence of neoadjuvant therapy on the serum levels of miRNAs in HER2-positive breast cancer patients.

Sterile Entzündung: neue diagnostische und therapeutische Ansätze

V86 – Talk Mulay

Crystallopathies - How crystals kill cells

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Numerous diseases that involve deposition of crystals or crystal-like particles e.g. misfolded proteins, airborne microparticles etc. are collectively referred as crystallopathies. Recent discoveries in the field of crystal biology suggested Nlrp3 inflammasome activation as a unifying inflammatory pathomechanism that underlie these disorders. However, the mechanisms that lead to crystal-induced cell necrosis remain unclear. We demonstrated that crystals of calcium oxalate (CaOx), monosodium urate (MSU), calcium pyrophosphate dehydrate and cystine kill cells via a caspase-independent regulated necrosis pathway that can be blocked by necrostatin-1 and necrosulfonamide. Further, RNA interference studies revealed that the two core proteins involved in necroptosis pathway viz. receptor interacting protein kinase (RIPK) 3 or mixed lineage kinase domain like (MLKL) are essential for crystal cytotoxicity. Consistent with this, *Ripk3*- and *Mlkl*-deficient mice were protected from CaOx crystal-induced acute kidney injury. In addition, we demonstrated that these proteins also critically regulate MSU crystal-induced neutrophil extracellular trap (NET) formation and associated cell death, which is known as NETosis. Either *Ripk3*-deficiency of necrostatin-1 treatment prevented gout tophi formation in the murine air-pouch model. Together, crystal-induced cytotoxicity involves RIPK1, RIPK3 and MLKL, which offer novel molecular targets to limit tissue injury and organ failure.

Update Antiphospholipid-Syndrom

V89 – Talk Canaud

mTOR pathway in the antiphospholipid syndrome related vasculopathy

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Background
Although thrombosis is considered the cardinal feature of the antiphospholipid syndrome, chronic vascular lesions are common, particularly in patients with lifethreatening complications. In patients who require transplantation, vascular lesions often recur. The molecular pathways involved in the vasculopathy of the antiphospholipid syndrome are unknown, and adequate therapies are lacking.
Methods
We used double immunostaining to evaluate pathway activation in the mammalian target of rapamycin complex (mTORC) and the nature of cell proliferation in the vessels of patients with primary or secondary antiphospholipid syndrome nephropathy. We also evaluated autopsy specimens from persons who had catastrophic antiphospholipid syndrome. The molecular pathways through which antiphospholipid antibodies modulate the mTORC pathway were evaluated in vitro, and potential pharmacologic inhibitors were also tested in vitro. Finally, we studied the effect of sirolimus in kidney-transplant recipients with the antiphospholipid syndrome.
Results
The vascular endothelium of proliferating intrarenal vessels from patients with antiphospholipid syndrome nephropathy showed indications of activation of the mTORC pathway. In cultured vascular endothelial cells, IgG antibodies from patients with the antiphospholipid syndrome stimulated mTORC through the phosphatidylinositol 3-kinase (PI3K)–AKT pathway. Patients with antiphospholipid syndrome nephropathy

who required transplantation and were receiving sirolimus had no recurrence of vascular lesions and had decreased vascular proliferation on biopsy as compared with patients with antiphospholipid antibodies who were not receiving sirolimus. Among 10 patients treated with sirolimus, 7 (70%) had a functioning renal allograft 144 months after transplantation versus 3 of 27 untreated patients (11%). Activation of mTORC was also found in the vessels of autopsy specimens from patients with catastrophic antiphospholipid syndrome.

Conclusions

Our results suggest that the mTORC pathway is involved in the vascular lesions associated with the antiphospholipid syndrome.

BDL - Präanalytik

V93 – Talk von Meyer

Methoden zur präanalytischen Überprüfung der Probenqualität und deren Anwendung

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Automation within laboratories has greatly increased through electronic order entry systems and diagnostic analyzers automatically accessing samples. Within the context of automation specific requirements need to be fulfilled to ensure the preanalytic process complies with the regulatory framework.

Correct labeling of correct sample material is paramount as per German „RiliBÄK“ and DIN ISO 15189 Transport within certain time limits and temperature conditions (i.e. temperature) needs to be ensured.

Automated temperature monitoring systems are available and used in the laboratory. These systems need to be adjusted according to the needs and existing infrastructure to cater for automated temperature monitoring during transport.

Similarly filling volume and hematocrit is important for correct interpretation of coagulation testing. Automated sorter or some coagulation analyzer are able to measure these variables automatically. Many automated clinical chemistry analyzer measure interference indices (HIL), which facilitates automated interpretation of results. Setting the thresholds for these indices correctly is important and might vary according to population, setting and clinical scenario

As with all quality measurements complete monitoring and documentation, necessary to meet regulator requirements, is time- and resource-consuming. The use of automated systems either along side existing systems or integrated into analyzers may offer solutions

V94 – Talk Cadamuro

Preanalytical Phase“ for venous blood sampling and its implementation in a hospital setting

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Preanalytics is, although often neglected, still the part within the total testing process in which most errors occur, potentially leading to false test results and sometimes serious consequences for the patient. Besides the medical indication for laboratory analyses and the according order of tests, the preanalytical phase consists mostly of processes before, within and after venous blood collection. Also the updated version of the EN ISO 15189 guideline emphasizes these preanalytical topics.

In the past years many countries therefore have formed working groups and partly also released preanalytical recommendations on a national basis. Additionally there are international recommendations on preanalytics and venous blood collection such as the CLSI guideline GP41-A6 or a WHO guideline. However, these recommendations are not providing clear and unambiguous guidance for all steps during blood collection and some important details may not be considered. Moreover, not all steps are equally important and recommendations should offer some level of critical assessment of the potential risk associated with non-compliance. Therefore the EFLM working group “Preanalytical Phase” (WG-PRE) decided to provide a simple, condensed, risk- and evidence-based recommendation for the venous blood sampling. Besides the necessary requirements for the blood sampling area and key supplies, it covers pre-sampling-, sampling- and post-sampling procedures as well as instructions on implementing and preservation of a standardized blood collection in a hospital setting.

Before publication, the practicability of these documents was tested in a pilot project. At the pediatric department of the University Hospital of Salzburg, Austria, where physicians were in charge of phlebotomy, 282 nurses were trained in blood collection according to the proposed EFLM recommendation. The Power-Point-Presentation as well as the knowledge test, which also are part of the recommendation, were translated, altered slightly to fit local conditions and converted into an e-Learning module. Passing the knowledge test was a prerequisite for phlebotomy on the patient.

Of the 160 nurses who completed the e-learning module and the respective knowledge test within the first month after release, 140 (87.5%) passed the test. The module is accepted very well amongst the nursing staff. An evaluation of a possible improvement of sample quality was not conducted since there was not enough data to validly make a statement on this issue at the time of abstract submission deadline.

Overall we conclude that the EFLM recommendation is a very practicable tool to easily implement a standardized phlebotomy process even in bigger hospitals.

Free Talks

Epigenetik: Von den Grundlagen zur diagnostischen Anwendung

FV01 – Talk Northoff

A Protective Loss-of-Function Mutation in Acvr1c Reduces Atherosclerosis in BALB Mice

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Background: A quantitative trait locus (QTL) of atherosclerotic lesion size in the aortic root was previously identified on mouse chromosome 2 (Chr2) in a F2 intercross of atherosclerosis-susceptible C57BL/6 (B6) and atherosclerosis-resistant BALB/cByJ (BALB) mice on the LDL receptor deficient background (*Ldlr*$^{-/-}$).
Objectives: The aim of the present study was to identify causal genetic variants protecting BALB mice from atherosclerosis and to study its functional role in atherogenesis.
Material and Methods: Fine-mapping of Chr2 was performed in F2 mice using additional single nucleotide polymorphisms (SNPs) thereby narrowing the 84.4 Mb QTL region to 23.6 Mb containing 63 genes. To identify causal genetic variants, chromosome 2 of B6 and BALB F0 mice was enriched and sequenced using next-generation sequencing technology. Knockout mice of the identified candidate gene were crossed on the B6.*Ldlr*$^{-/-}$ background and compared to wildtype mice. Atherosclerotic lesion size was quantified by Oil red O staining and additional associated phenotypes were measured. Furthermore, functional studies of atherosclerosis related mechanisms were performed in murine primary cells.
Results: A nonsense mutation in activin A receptor type IC (*Acvr1c*) was identified in BALB mice co-segregating with the QTL of atherosclerosis. Functionally, *Acvr1c* encodes a type I transforming growth factor-β receptor acting as serine/threonine protein kinase of SMAD2/3 transcription factors modulating cellular functions as apoptosis and proliferation. The identified mutation was located in the kinase domain of *Acvr1c* leading to a truncated protein with reduced kinase activity and reduced SMAD2-phosphorylation in BALB mice.

Tissue profiling showed that *Acvr1c* mRNA was expressed in fat tissues, intestine and aortic tissue of F0 mice with reduced expression levels in all tissues of BALB compared to B6 mice. Differential mRNA expression was confirmed in F2 mice. Immunofluorescent staining showed that ACVR1C was expressed in endothelial cells but not in other cells of the arterial wall.

Acvr1c knockout mice showed decreased atherosclerotic lesion size compared to wildtype mice confirming atherosclerotic effects of F0 and F2 mice. Since no differences in plasma lipid levels or adipokines were found, a causal role of *Acvr1c* in endothelial cells was suggested. *In vitro* studies in endothelial cells using constitutive activated ACVR1C with either B6 or BLAB alleles are currently performed to investigate SMAD-mediated effects on apoptosis.
Conclusion: *Acvr1c* was identified as novel regulator of atherosclerosis. Truncated ACVR1C in athero-protected BALB mice was shown to decrease SMAD signaling. Currently, the functional mechanism of *Acvr1c* in modulating atherogenesis and apoptosis is further investigated *in vivo* and *in vitro*.

Epigenetik: Von den Grundlagen zur diagnostischen Anwendung

FV02 – Talk Al-Dabet

Coagulation protease aPC reverses sustained tubular p21-expression and senescence via a methylation dependent mechanism

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Background: The importance of tubular damage in diabetic nephropathy (dNP) is increasingly recognized. Tubular damage in dNP is characterized by tubular hypertrophy and senescence. One of the regulators contributing to tubular damage is the cyclin-dependent kinase (CDK) inhibitor p21. Protein level of p21 is significantly induced in dNP. At the cellular level, p21 induction is associated with increased tubular damage, senescence and declined tubular proliferation. However the pathophysiological mechanistic relevance and therapeutic structures allowing targeting such mechanisms are lacking. Interestingly, previously we showed that, activated protein C (aPC) ameliorates the glomerular damage in dNP via epigenetic dependent mechanism.
Problem: It remains elusive, whether the tubular damage and senescence can be sustained after normalizing the glucose concentration. The mechanistic relevance of p21 for tubular senescence and damage, and whether aPC would target the underlying mechanism, remain unknown.
Materials and Methods: Type-1 (streptozotocin) diabetic (C57Bl6) mice were analyzed. dNP was validated based on albuminuria. A subset of mice was treated with SGLT-2 inhibitor, aPC, or 5aza-deoxycitidiene. Mice were analyzed after 6 weeks of treatment. Albuminuria, tubular damage (PAS-staining and KIM-1 expression), were analyzed. p21 protein level, as well as SA-βgalactosidase staining were also investigated.
Results: Gene-expression analyses identified p21 as the prominently induced gene in dNP (STZ model). Expression of p21 was further increased in mice with low aPC levels. In-vitro study showed that glucose induced p21 expression remains high after normalization of glucose concentration, suggesting that p21 is epigenetically controlled. Intriguingly, exposure of aPC at time of glucose normalization (25 to 5.5 mM) reversed p21 expression. In parallel, glucose reduced DNMTs activity and DNMT1/3b expression, which remained low despite glucose normalization. These glucose-induced persistent changes were reversed by aPC. Likewise, in diabetic mice (16 weeks of DM) p21 expression in renal tubular compartment remained high despite blood glucose normalization for the last 6 weeks using SGLT-2 inhibitor. aPC treatment during the last 6 weeks abolished hyperglycemia induced sustained p21 expression and protected from tubular damage (histological damage, KIM-1) and senescence (SA-βgal). Concomitant treatment with 5aza-dC abolished aPC's protective effect.
Conclusion: This suggests that p21 induces tubular senescence in dNP, which is sustained despite normalization of glucose level. Importantly, persistent p21-expression and tubular senescence can be reversed by aPC.

From bench to beside: Nephropathie

FV03 – Talk Kohli

Microparticles cause preeclampsia and embryonic growth restriction by platelet-mediated inflammasome activation in the embryonic trophoblast

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Objectives: Preeclampsia (PE) is a placenta-induced inflammatory disease associated with maternal proteinuria, hypertension, inflammation, increased microparticles (MP), platelet-activation and hypercoagulability. A proinflammatory function of platelets is increasingly recognized but is not understood in PE. The association of MP and platelet activation is known but its mechanistic relevance in PE is not studied.
Aim: We aimed to study the pathogenetic role of MP and platelet-activation and the associated inflammatory micro-milieu involved in pregnancy complications.
Methods: MP (endothelial or platelet-derived) were injected into C57BL/6 pregnant mice. Pregnancy outcome and symptoms of PE (renal dysfunction and pathology, increased blood pressure, increased plasma sFlt-1) were studied. MP dependent platelet-activation in the placenta was studied using immunofluorescence. Platelet depletion, aspirin treatment, NFE-2-/- and Gαq-/- mice were used to establish the causative role of platelet-activation. To address mechanistic questions we studied the role of MP on platelet mediated inflammasome activation in murine placenta and trophoblast cells. Genetic and pharmaceutical inflammasome inhibition was conducted to ascertain mechanistic causality. Translational relevance was corroborated by analysis of human PE placentae and trophoblast cells.
Results: MP caused PE along with partial fetal loss and embryonic growth restriction in mice. Following MP-injection activated platelets accumulated within the placental vascular bed and caused ATP-dependent inflammasome activation (elevated expression of NLRP3, cleaved casp-1 and cleaved IL-1β). Platelet depletion and aspirin treatment protected from MP induced PE. NFE-2 -/-mice, which lack functional platelets and Gαq-/- mice, which have impaired platelet-activation, were protected from MP-induced inflammasome activation, pregnancy failure and PE. The pregnancy outcome and renal function was also rescued in NLRP3 or Casp-1 deficient mice. Apyrase and purinergic receptor antagonist treatment rescued the pregnancy outcome indicating the involvement of purinergic signaling. Importantly, inflammasome activation in trophoblast cells was sufficient to cause PE-like phenotype in mice, while the maternal inflammasome was dispensable. MP caused platelet dependent inflammasome activation in human trophoblast cells whereas platelets from donor receiving aspirin treatment failed to induce the inflammasome. Inflammasome activation in association with reduced platelets numbers is likewise observed in human trophoblast cells of PE, but not of control placentae.
Conclusion: Our results uncover a novel thrombo-inflammatory mechanism at the maternal-embryonic interface. MP cause PE through maternal platelet-activation and ATP-mediated purinergic inflammasome activation in trophoblast cells. Based on these results new diagnostic or therapeutic targets for PE may be developed.

DGKL/DVTA: Update Gerinnung

FV04 – Talk Schott

Elucidation of feedback gene regulatory mechanisms controlling the F2 expression

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Prothrombin (F2) plays a central role in blood coagulation. Deregulation of Thrombin (F2a) can result in hemostatic abnormalities, ranging from subtle subclinical to serious life-threatening coagulopathies, i.e. during septicemia. The known F2 20210 G>A mutation leads to a 1,5 - 1,7 fold increase of gene expression, resulting in thrombophilia. Thus, efficiently coordinated dynamics of gene expression, protein biosynthesis or protein secretion of F2 determine the well-balanced equilibrium between thrombosis and blood loss.

Here, we set to define regulatory mechanisms controlling F2 gene expression. To this end, we used a newly generated mouse model system, termed D-Insight, which was established to visualize gene expression and protein secretion of F2 real-time *in-vivo*. Based on fluorescence and luminescence reporters this knock-in mouse allows us to follow-up these processes in a living context by the use of non-invasive optical imaging. Furthermore, we generated a primary hepatic cell line obtained from this mouse model. This cell line permits the elucidation of F2 gene expression-, protein biosynthesis- and secretion dynamics in high resolution and in a high-throughput format with bioluminometry and fluorescence microscopy *ex-vivo*.

Using this tool, we illuminate the (auto-)regulatory feedback-mechanisms underlying the gene expression control of F2. This will help to decipher the intricate role of F2 in steady state condition and in various disease processes accordingly. Additionally, this might help to unveil potential novel therapeutic implications. An update will be presented at the meeting.

Zirkulierende Tumor-DNA - diagnostische Relevanz in der Translation

FV05 – Talk Thöne

Liquid profiling of androgen receptor splice variant V7 RNA in peripheral whole blood predicts response to abiraterone treatment in metastatic prostate cancer patients

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Background. In metastatic castration-resistant prostate cancer (mCRPC) the androgen receptor (AR) has been shown to be still active and promote tumor progression. Novel agents, such as the androgen-biosynthesis inhibitor abiraterone, have been tailored to inhibit AR signalling. About a third of patients, however, show initial resistance towards abiraterone treatment. The AR splice variant 7 transcript isoform (AR-V7) lacks the androgen-binding site but retains stimulation of tumor growth. Recently, high expression of AR-V7 in circulating tumor cells (CTCs) was shown to predict poor treatment response in mCRPC patients treated with abiraterone.
Question. Here, we investigate whether a liquid profiling approach with direct quantification of AR-V7 RNA levels in peripheral whole blood without the need of capturing of CTCs is feasible and can be predictive for response to abiraterone treatment and outcome in mCRPC patients.
Materials and Methods. In a prospective biorepository database, we collected blood samples using 2.7 ml PAXgene blood RNA tubes (Qiagen) from 38 mCRPC patients, before abiraterone treatment was started, and 28 healthy subjects as controls. Total RNA was extracted and converted into cDNA. We developed specific droplet digital PCR assays for both the AR-V7 and the AR full length (AR-FL) isoform. Using these assays, we quantified AR-V7 and AR-FL levels in each sample and calculated the fraction of AR-V7 transcript over total AR (AR-V7 plus AR-FL) transcript.
Results. The fraction of AR-V7 transcript in blood ranged from 0 to 2.0% (median 0.22%). On average, patients had a higher AR-V7 fraction than controls (0.42% vs. 0.21% $p = 0.01$, t-test). Using the maximum observed AR-V7 fraction in controls (0.61%) as cut-off, we dichotomized the patients into an "AR-V7 high" and an "AR-V7 low" group of 11 and 27 patients, respectively. "AR-V7 low" compared to "AR-V7 high" patients had significantly longer progression-free survival (PFS) (median PFS 4.6 vs. 2.5 months, $p = 0.006$, log-rank test) and overall survival (OS) (median OS 13.9 vs. 6.6 months, $p = 0.005$, log-rank test). Notably, treatment response as defined by a maximum PSA decline of 50% or more was observed in 13 out of 23 (57%) "AR-V7 low" patients, but 0 out of 9 (0%) "AR-V7 high" patients ($p = 0.004$, Fisher's exact test). This suggests that patients with a high AR-V7 fraction measured in blood are likely to fail abiraterone treatment.
Conclusion. Accurate and individualized treatment selection are key to a precision medicine approach. We present a cost-effective method that could help, before treatment is initiated, to decide which patients will benefit from AR-targeting treatment such as abiraterone and which will not, using a simple blood test. These promising results merit further prospective validation in larger cohorts.

DGKL / DVTA: Update Klinische Massenspektrometrie

FV06 – Talk Vogeser

Isotope Inversion Experiment evaluating the suitability of calibration in surrogate matrix for quantification via LC-MS/MS – Exemplary application for a steroid multi-method

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Background: In mass spectrometry based assays for the quantification of endogenous analytes, the production of appropriate calibrators is a challenge since analyte-free authentic matrix is usually not available. Thus, calibrators and QC samples are often prepared in "surrogate matrix". However, there is so far no standard experiment that verifies the suitability of a surrogate matrix calibration for quantification of unknown samples in authentic matrix.
Aim: The aim of the present study was the development of a novel validation experiment verifying the correct quantification of authentic matrix samples by means of a surrogate matrix calibration.
Experimental Setup: Key element of this novel experiment, called *Isotope Inversion Experiment*, is the inversion of non-labelled analytes and their stable isotope labelled (SIL) counterpart in respect to their role (non-labelled, endogenous compound → internal standard; SIL compound → analyte). Using this inverse setup, inverse calibrators and inverse controls were prepared and analyzed. In the next step, the obtained response data of the two inverse sets of calibrators are correlated and the accuracy of the inverse controls is calculated. As a proof of concept application, an LC-MS/MS assay addressing six corticosteroids (cortisol, cortisone, corticosterone, 11-deoxycortisol, 11-deoxycorticosterone, and 17-OH-progesterone) was studied.
Results and Discussion: A fast, sensitive, and selective UHPLC-MS/MS method was developed that enables a chromatographic separation of all included isobaric compounds within 7 minutes. In the *Isotope Inversion Experiments* an excellent correlation of inverse calibrators and good accuracy values were found for the SIL compounds of corticosterone, 11-deoxycortisol, 11-deoxycorticosterone, and 17-OH-progesterone. For the SIL compounds of cortisol and corticosterone worse results were obtained. The worse correlation and accuracy values could be traced back to an insufficient suppression of the basal levels of the respective internal standard, i.e. cortisol and corticosterone, in the authentic material. Subtracting the found endogenous amount of cortisol and corticosterone corrected this interference and improved the results. Finally, all observed values were within our pre-defined limits clearly demonstrating that the chosen surrogate matrix calibration is indeed suitable for the quantification of serum based samples.
Conclusion: The *Isotope Inversion Experiment* fills a gap in the validation process of LC-MS/MS assays that quantify endogenous analytes; it can verify the suitability of calibrator matrices which differ systematically from the matrix of clinical samples.

Vaskuläres Remodeling

FV07 – Talk Nicolaou

Adam17-Deficiency Promotes Atherosclerosis in a Mouse Model by Enhanced TNFR2 Signaling

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Background: Expression QTL (eQTL) mapping in atherosclerosis-resistant FVB and atherosclerosis-susceptible C57BL/6 (B6) mice on the Ldl receptor deficient ($Ldlr^{-/-}$) background led to the identification of the metalloproteinase a disintegrin and metalloprotease 17 (Adam17) as a candidate gene of atherosclerosis. Reduced Adam17 mRNA expression and activity of ADAM17 were associated with increased atherosclerosis at the aortic root suggesting that ADAM17 conferred atheroprotection. The metalloprotease ADAM17 is a major sheddase of membrane-bound proteins, such as TNF-α and TNF-receptors 1 and 2.
Objectives: The current study investigated the effect of Adam17 on atherosclerosis development in a hypomorphic Adam17-deficient mouse model and elucidated potential mechanisms of atherosclerosis.
Material and Methods: Since Adam17 knockout mice are not viable, we used hypomorphic Adam17 mice ($Adam17^{ex/ex}$) that have barely detectable levels of Adam17 mRNA in all tissues. Mice were bred onto the $Ldlr^{-/-}$ background and atherosclerosis was quantified at the aortic root. ADAM17 substrates were measured in plasma and the supernatants of bone marrow-derived macrophages (BMDM) using multiplex immunoassays and protein levels were analysed in cell lysates and cell surface proteins. Genome-wide expression and pathway analyses were

performed in livers, aortas, and BMDM of Adam17$^{ex/ex}$.Ldlr$^{-/-}$ and Adam17$^{wt/wt}$.Ldlr$^{-/-}$ mice. Functional experiments of atherogenesis were performed in BMDM, RAW264.7 and endothelial cells. Furthermore, TUNEL and Ki67 co-stainings were performed in the aortic roots of Adam17 deficient and wild-type mice.
Results: Atherosclerotic plaque size at the aortic root was significantly increased in Adam17$^{ex/ex}$.Ldlr$^{-/-}$ compared to Adam17$^{wt/wt}$.Ldlr$^{-/-}$ mice. Soluble TNF-α (sTNF-α), sTNFR1 and sTNFR2 were significantly reduced released in plasma and supernatants of BMDM of Adam17$^{ex/ex}$.Ldlr$^{-/-}$ mice. Consistently, we detected higher TNFR2 protein levels in whole cell lysate and on the cell surface of BMDM of Adam17$^{ex/ex}$.Ldlr$^{-/-}$ mice. Pathway analyses predicted effects of Adam17 deficiency on inflammation, apoptosis, adhesion and proliferation, which were validated in functional studies in BMDM, RAW264.7 and endothelial cells using RNAi. We observed increased proliferation and adhesion as well as decreased apoptosis in BMDM of Adam17$^{ex/ex}$.Ldlr$^{-/-}$ mice. These findings were confirmed in RAW264.7 cells after siRNA mediated knockdown of Adam17. Furthermore, significantly less apoptotic cells and higher numbers of proliferating macrophages were detected in lesions of Adam17-deficient mice corroborating the results from the in vitro studies.
Conclusion: Results of the current study establish ADAM17 as a protective factor of atherosclerosis and show that a constitutive upregulation of TNFR2 signaling promotes pro-atherogenic cellular functions.

Kognitionstörungen: Von der Grundlagenwissenschaft zur Diagnostik

FV08 – Talk Lachmann

Tau ohne T181 Phosphorylierung als neuer Biomarker der Alzheimererkrankung

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HINTERGRUND: Im Gegensatz zur diagnostischen Aussage von P181 in Cerebrospinalflüssigkeit (CSF) ist nichts bekannt über eine mögliche diagnostische Aussagekraft der Tau-Fraktion ohne Phosphorylierung an dieser Position.
ZIELSTELLUNG: Analytische und klinische Validierung des ersten Tests zur Messung der Konzentration an Tau ohne Phosphorylierung in CSF von Menschen.
METHODEN: Der Antikörper (1G2), der vorzugsweise an Position T181 des Tau bindet, wenn diese nicht phosphoryliert ist, wurde verwendet um einen Sandwich-ELISA zu etablieren, der in der Lage ist, dieses Tau ohne T181 Phosphorylierung in humanen CSF zu messen. Die Messmethode mittels dieses ELISA wurde analytisch und klinisch validiert.
ERGEBNISSE: Die Nachweisgrenze des Tests beträgt 25 pg/ml; die Koeffizienten der Variation (CVs) der optischen Dichtebestimmungen als Wiederholungsmessungen von Standardskurven betrugen 3,6 - 15,9 %. Die mediane Intratest-Ungenauigkeit von Doppelbestimmungen befand sich in einem Bereich von 11,2 -15,3 %. Die Taukonzentrationen ohne T181 Phosphorylierung sind stabil in CSF-Proben, die versandt wurden an verschiedene Labore unter Umgebungsbedingungen. Die Inter-Labor-Variantion betrug ungefähr 30%. Die CSF-Tau-Konzentration ohne T181-Phosphorylierung ware hochsignifikant erhöht in Patienten mit früher Alzheimererkrankung oder milden kongnitiven Störungen (Mild Cognitive Impairment) mit n=58 und 109.2 ± 32.0 pg/mL verglichen zu nicht-dementen Kontrollen (n=42, 62.1 ± 9.3 pg/mL, $p<0.001$). Die ROC-Analyse zeigte eine Sensitivität von 94,8 % und eine Spezifität von 97.6%.
SCHLUSSFOLGERUNG: Das erste Mal ist eine Test dargestellt, der eine diagnostisch nutzbare Messung von Tau-Konzentrationen ohne T181-Phosphorylierung im humanen CSF erlaubt.

Glycocalyx: Mediator und Marker der endothelialen Funktion

FV09 – Talk Shahzad

Stabilization of endogenous Nrf2 by minocycline protects against Nlrp3-inflammasome induced diabetic nephropathy

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Background: While a plethora of studies support a therapeutic benefit of Nrf2 activation and ROS inhibition in diabetic nephropathy, the Nrf2 activator bardoxolone failed in clinical studies in type 2 diabetic patients due to side effects. Hence, alternative approaches to target Nrf2 are required. Intriguingly, the tetracycline antibiotic minocycline, which has been in clinical use for decades and limits mitochondrial dysfunction and apoptosis, has been shown to convey anti-inflammatory effects in diabetic patients. As the mechanism underlying minocyclines nephroprotection remains unknown we speculated that minocycline reduces renal ROS generation and inflammation, potentially through a Nrf2 dependent mechanism.
Methods: The effect of minocycline on inflammasome activation and oxidative stress was studied in murine models of diabetic nephropathy, db/db mice and the STZ diabetes model. We assessed albuminuria, glomerular extracellular matrix accumulation as well as expression of Nrf2 and inflammasome regulators. Nrf2-ubiquitination was analyzed by immunoprecipitation.
Results: Here we show that minocycline protects against dNP in mouse models of type 1 and type 2 diabetes, while caspase-3,-6,-7,-8 and -10 inhibition is insufficient, indicating a function of minocycline independent of apoptosis inhibition. Minocycline stabilizes endogenous Nrf2 in kidneys of db/db mice, thus dampening ROS-induced inflammasome activation in the kidney. Indeed, minocycline exerts antioxidant effects *in vitro* and *in vivo*, reducing glomerular markers of oxidative stress. Minocycline reduces ubiquitination of the redox-sensitive transcription factor Nrf2 and increases its protein levels. Accordingly, minocycline mediated Nlrp3 inflammasome inhibition and amelioration of dNP are abolished in diabetic $Nrf2^{-/-}$ mice.
Conclusions: Taken together, we uncover a new function of minocycline, which stabilizes the redox-sensitive transcription factor Nrf2, thus protecting from dNP.

Immune modulation in cancer and its therapeutic potential

FV10 – Talk Busch

Characterization of myeloid-derived suppressor cells (MDSC) and the expression of immunoglobulins by tumor-associated macrophages (TAM) in glioblastomas

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Background:
Recent evidence indicates that myeloid-derived suppressor cells (MDSC) are markedly elevated in glioblastoma patients. Furthermore, subfractions of myeloid cells in blood and tumors have been reported to express variable immune receptors, i.e. T cell receptors or immunoglobulins.
Objective:
We investigated immunoglobulin expression in tumor-associated macrophages (TAM-Igs) and circulating monocytes from glioblastoma patients, particularly with respect to variability of their Ig-repertoires. Also, we analyzed MDSCs in the blood of glioblastoma patients.
Material and methods:
Peripheral blood and tumor tissue of glioblastoma patients were used to isolate MDSCs, monocytes and TAMs. MDSCs were identified and quantitated using FACS sorting. Monocytes and TAMs were purified to homogeneity using immunomagnetic microbeads. To evaluate the expression of Igs in the cell preparations, RNA isolation followed by PCRs was conducted using complement reverse and consensus forward primers for constant and variable region Ig gene sequences, respectively. DNA isolated from amplification-positive bands was used for TOPO-Cloning followed by Sanger sequencing of 20 independent clones/isolate. Analyses of CDR3 regions and the V-, D- and J-chain usage of the Igs were performed using IgBLAST and VBASE2.
Results:
First, our results showing highly elevated levels of MDSCs in the peripheral blood from glioblastoma patients were confirmed by independent recent reports. More importantly, we demonstrated the expression of variable Igs by circulating monocytes and TAMs in glioblastoma patients. Purity of TAM/monocyte preparations was confirmed by the absence of T- and B-cell-specific gene expression demonstrating that all analyzed Igs were due to specific expression by the purified TAM/monocytes. Furthermore, this work revealed that human monocytes and TAMs express Ig-repertoires in a highly restricted fashion as compared to normal B cells. Specifically, analysis of the antigen-binding CDR3 regions showed remarkably smaller repertoires in TAM compared to monocytes. Finally, the complexities of the TAM-IG in glioblastoma patients showed an inverse correlation to the brain tumor burden as determined by 3D volumetry in Cranial CT and MR images.

Conclusion:
We confirmed high levels of MDSCs in the peripheral blood of glioblastoma patients and showed for the first time the expression of Igs by circulating monocytes and TAMs. The Ig-repertoires in glioblastoma patients vary significantly between TAMs and circulating monocytes suggesting a biological immunosuppression function of the tumor. This is further corroborated by the observation of an inverse correlation between the tumor size and the complexity of the expressed Ig-repertoires as expressed by the corresponding TAMs/monocytes. Further studies are needed to establish the functions of MDSCs and myeloid cells expressing variable immunoreceptors for the exertion of immune surveillance and evasion by the host and the tumor, respectively.

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Zirkulierende mikroRNAs – diagnostisch relevant?

FV11 – Talk Haselmann

Results of the first external quality assessment (EQA) scheme for analysis of circulating tumor DNA (ctDNA)

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Background: ctDNA comprises the tumor-derived fraction of cell-free circulating nucleic acids (cfDNA) and is considered to have a high potential for future early diagnosis of relapse and therapy failure in the management of malignant diseases. External quality assessment (EQA) schemes are an important cornerstone of quality assurance in molecular genetic diagnostics. Accordingly, this pilot EQA aimed at addressing issues of analytical quality in this new area of laboratory diagnostics. Methods: The EQA scheme consisted of two panels comprising three samples dedicated to the analysis of BRAF status and of KRAS codons 12/13. Each sample contained 2ml DNA-free human K3 EDTA plasma spiked with 100ng/ml DNA previously isolated from tumor cell lines and fragmented by sonification. Laboratories were asked to use their routine procedures and protocols for ctDNA isolation and genotyping. A panel of assessors reviewed the final returns to assess the quality. Results: 42 laboratories from 10 European countries participated in this pilot EQA scheme. Delivery times of the samples to the participating laboratories varied by a wide margin as did the time to ctDNA isolation with a median time of 180 hours. There was no influence of the total time elapsed to analysis on the correctness of genotyping results. 28% of laboratories used automated ctDNA isolation procedures, while 72% isolated ctDNA manually. Manually performed extraction appeared to be more efficient, albeit with greater standard deviation. Regarding the quantification of the isolated ctDNA our results indicate that certain methods like PicoGreen or real-time PCR are more reliable for the exact quantification of ctDNA. Regarding the results for genotyping, in total 197 genotypes were determined with an overall error rate of 6%. Interestingly, the error rate depends on the method used for genotyping with the highest error rate noticed for Sanger sequencing at 23% followed by NGS and Pyrosequencing at 3.8% and 3.3% respectively. Conclusion: Our findings reveal a superiority of certain methods for exact quantification of isolated ctDNA as well as for the correct identification of sequence variations of ctDNA. The later one might be explained by the fact that ctDNA represents a minor fraction in circulating cell-free DNA and thereby adding importance to issues of analytical sensitivity and validity for diagnostic quality. With respect to quality assurance there is an urgent need for harmonisation of the different methods being used in this new diagnostic field, especially if taking into account the tremendous impact of genetic testing results on therapeutic decisions and therewith on an individual's life.

Kardiomyopathie

FV12 – Talk Häuser

Characterization of aberrant mRNA-Splicing in Pompe disease by NMD inhibition

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Background The precise identification and characterization of genetic variants in monogenic diseases has a wide influence on diagnosis and therapy. About 10 % of pathogenic variants are splicing variants. Due to the complex mechanism of splicing regulation it is difficult to predict

the effects of variants on mRNA splicing. Possible consequences are exon skipping, intron retention, generation of novel splice sites or the utilization of a cryptic splice site. Common consequences are a frame-shift and the generation of premature termination codon. This leads to RNA degradation via the nonsense mediated decay (NMD) pathway.

In a patient with the clinical symptoms of non-classical infantile Pompe disease and a confirmed acid alpha-glucosidase (GAA) deficiency, we detected two novel, exonic variants in the GAA gene. Both base pair exchanges suggested either an amino acid exchange or a splice defect as consequences. However, conventional investigation of the leucocyte mRNA of the patient and his parents was inconclusive. Degradation of the respective mutated RNA by NMD was suspected.

We developed an approach in order to characterize novel splicing mutations in a simple and non-invasive manner.

Material and method Isolated blood lymphocytes from patient and his parents were cultured in standard leucocyte medium supplemented with different concentrations of the NMD inhibitors ocadaic acid, anisomycin, and wortmannin for 24 h. Cells were harvested and RNA was isolated. The reverse transcribed cDNA was amplified in allele specific PCRs and qPCR assays.

Results Compared to the non-stimulated lymphocyte controls nonsense mediated RNA decay was inhibited by anisomycin. The consequences of aberrant RNA splicing were detectable: The maternal mutation results in exon skipping, the paternal mutation in intron retention. Furthermore NMD inhibition increases the amount of GAA-RNA in patient's lymphocytes as well as in the cells of his parents. The residual function of the resulting protein has to be investigated.

Discussion and conclusion RNA analysis in lymphocytes with and without NMD inhibition is a simple method for analysing splice defects in all monogenic disorders with expression of the disease causing gene in lymphocytes. A further advantage for the patient is the use of blood cells instead of fibroblasts, because a skin biopsy can be avoided and analysis times are reduced.

The exact characterization of pathogenic variants is an important aspect of diagnosis, prediction of disease severity and genetic counselling. In vitro NMD inhibition in lymphocytes of affected patients allows the characterization of splice defects. In the future successful inhibition of NMD in vitro might help to identify patients, who may profit from a therapeutic intervention with NMD inhibitors. Even expression of a partial protein with low or no activity reduces the risk for the patient to develop antibodies hampering enzyme/protein replacement therapy.

Kardiomyopathie

FV13 – Talk Kallweit

Long Non-Coding RNAs as Genetic Factors of Human Coronary Artery Disease

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Background: Coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide and is modulated by environmental and genetic factors. Genome-wide association studies (GWAS) have revealed various loci of CAD susceptibility, contributing to genetically determined CAD risk. In spite of these advances, the pathophysiology underlying the majority of these loci is largely unknown. Recent studies have revealed that aside of protein-coding RNAs, long non-coding and circular RNAs (circRNAs), characterized by non-canonical splicing events, are abundantly expressed across the genome.

Objectives: The aim of the present study was to systematically identify long non-coding and circRNAs at CAD susceptibility loci and to understand their potential role in the pathophysiology of this frequent disease.

Material and Methods: Genomic regions of interests were identified by systematically analyzing published GWAS loci of CAD susceptibility and related traits such as lipid levels, hypertension, diabetes mellitus, smoking and stroke. Haplotype structures of these loci were investigated to determine their exact physical size. Furthermore, annotated protein-coding, long non-coding and circRNAs were identified using next-generation sequencing and mapped to the identified loci. To this end, next generation sequencing (RNA-seq) was performed in RNA isolated from atherosclerosis-related tissues (peripheral blood mononuclear cell, endothelial cells, smooth muscle cells and fibroblasts), organs (liver and vessels) and cell-lines (MonoMac, THP-1, HEK-293, U-937, HepG2, CaCo2). CircRNAs were identified within the haplotype blocks in a subset of RNase R treated samples.

Results: A total number of 80 loci related to CAD and an additional 397 loci of related traits were identified with a p-value of $\leq 5 \times 10^{-8}$. Haplotype block margins of CAD-related loci were defined using genetic variation of the European population within the 1000 genomes data. In parallel, RNA-seq revealed protein-coding, long non-coding and circRNAs. A total of 84.000 backsplicing events, corresponding to putative circRNAs, were identified in RNase R treated samples with 2x 596 million reads. Currently, functional studies of mechanisms of CAD such as cell adhesion, proliferation and apoptosis are performed by modifying induced pluripotent stem cells with over-expression and knock-down approaches.

Conclusion: Here, we identified various protein-coding, long non-coding and circRNAs, which are expressed at risk loci of CAD. CircRNAs are a novel species of transcripts, possibly related to the pathophysiology of this disease. Systemic studies by modulating the expression of long non-coding and circRNAs might reveal novel functional pathways affecting CAD risk.

Pathogenese und Biomarker des Metabolisches Syndrom

FV14 – Talk Wütherich

Suitable control material for external quality assessment of whole blood glucose devices in patient near testing

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Background: Quality assurance of medical laboratory examinations is regulated by the "Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations – Rili-BAEK" (1). In Rili-BAEK the same quality criteria are applied regardless whether the measurement is carried out in a core laboratory or as patient near testing. According Rili-BAEK criteria, the permissible relative deviation for glucose in plasma and whole blood is 15% and the target value should be based on a reference method value for external quality assessment (EQA). The consensus value may be used instead if the population of the participants' results shows a considerable deviation to the reference method based target value (Rili-BAEK; part E1). Through this practice a significant bias between the reported results and the reference method value may not be identified. In such cases a main purpose of EQA schemes is failed, which is in patient near testing mainly caused by the lack of suitable control material. To meet the needs of EQA schemes suitable whole blood control materials have to:

1. remain stable over a period of up to five days (to allow postal delivery);
2. technically comply with all or at least the majority of the available testing devices for glucose used in patient near testing.

Objective: The aim of the present study was to evaluate the stability and applicability of two different whole blood stabilizers for the use in EQA schemes for glucose on patient near testing devices.
Methods: Whole blood was drawn by venous puncture from 20 healthy volunteers (approval from the ethical committee and informed consent obtained prior to sampling). Blood samples were filled into tubes containing two different stabilizing substances. Additionally, a lithium-heparin tube was drawn. Two stabilizing substances for whole blood were measured on five commercially available patient near testing instruments for plasma-referenced glucose. Immediately after venous puncture, drops of whole blood from the venous blood collection system were used for measurements on the glucometers. In addition, glucose concentration was obtained by the core laboratory method using lithium-heparin plasma. Stabilized whole blood samples were stored at room temperature over a period of seven days and measured on the investigated glucometers daily at the same hour. Differences of glucose concentration between the glucometers and changes over the study period were monitored. Imprecision was obtained by five repeat measurements.
Results: Both investigated materials remained stable for at least four days. The observed changes over time were within the imprecision of the respective method. The bias between glucometers comparing initial measurements on whole blood without stabilizers was up to 25 % at concentrations between 4 and 6 mmol/L.
Conclusion: The investigated stabilizers of glucose in whole blood show a good performance for at least four days and can be applied to a selection of commercially available devices.

References:

1 Revision of the "Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations – Rili-BAEK" (unauthorized translation). LaboratoriumsMedizin 2015; 39(1).

Pathogenese und Biomarker des Metabolisches Syndrom

FV15 – Talk Jaeger

Zirkulierende microRNAs miR-192 und miR-194 sind mit inzidentem Diabetes mellitus assoziiert

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Hintergrund: Diabetes mellitus Typ 2 (T2DM) wird oft erst diagnostiziert, wenn Patienten bereits Komplikationen entwickelt haben. Eine frühzeitige Prävention erfordert diagnostische Marker, welche Personen mit erhöhtem Diabetes-Risiko frühzeitig erkennen helfen. Die Komponenten des Metabolischen Syndroms (MetS) haben hierfür eine eingeschränkte Sensitivität und Spezifität, sodass nach neuen prognostischen Biomarkern für T2DM gesucht wird. Als solche neuartigen Biomarker kommen zirkulierende microRNAs in Frage.
Ziel: Identifikation zirkulierender microRNAs, welche mit der Entwicklung von T2DM assoziiert sind.

Studiendesign und Methoden: In einer prospektiven Screening-Studie wurde die Expression von 380 microRNAs in Seren von 9 Patienten *mit* inzidentem Diabetes und 9 Patienten *ohne* inzidenten Diabetes während 6 Jahren Nachbeobachtung verglichen. Die mit inzidentem Diabetes assoziierten microRNAs wurden mittels quantitativer real-time PCR in zwei Schritten validiert. Zunächst wurde in einer Querschnittsstudie (je 43 Patienten mit Euglykämie, gestörter Nüchternglukose und manifestem Diabetes) die Assoziation der zirkulierenden microRNAs mit den glykämischen Stadien untersucht. Danach wurde in einer erweiterten Stichprobe bei 35 Patienten *mit* inzidentem Diabetes und 178 Patienten *ohne* inzidenten Diabetes während sechsjähriger Nachbeobachtung das prognostische Potential der Kandidaten-microRNAs für die Entwicklung eines Diabetes untersucht und mit demjenigen etablierter Biomarker verglichen.
Resultate: Die Screening-Studie und anschliessende Bestätigungsanalyse mit qRT-PCR zeigten erhöhte Spiegel von miR-122, miR-192, miR-194 und miR-215 im Serum von Patienten mit inzidentem Diabetes. Die Validation in der Querschnittsstudie bestätigte signifikant erhöhte Serumkonzentrationen von miR-192, miR-194 und miR-215 in Patienten mit manifestem Diabetes. Eine multiple logistische Regressionsanalyse der Ergebnisse der Längsschnittstudie zeigte, dass miR-192 und miR-194 auch nach Korrektur für andere Einflussfaktoren signifikante Prädiktoren für die Diabetesentwicklung sind. C-Statistiken ergaben eine vergleichbar gute diagnostische Performance für inzidenten Diabetes von miR-192, miR-194 und HbA1c. Die beste prognostische Performance wurde durch die Kombination von 3 Parametern, nämlich miR-192, HbA1c und C-Peptid erreicht.
Schlussfolgerung: Diese Studie zeigt eine enge Assoziation von miR-192 und miR-194 mit der Entwicklung eines künftigen Diabetes. Die Kombination dieser microRNAs mit bereits etablierten Biomarkern könnte die Risikoabschätzung für die Diabetes-Entwicklung verbessern.

DGKL/DVTA: Update Biobanking

FV16 – Talk Nauck

Implementation of a workflow based LIMS within the DZHK for processing of specimen including biobanking

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Objectives
The German Centre for Cardiovascular Research (DZHK) was founded in 2012 as one of the six German Centres for Health Research, sponsored by the Federal Ministry of Education and Research (BMBF). The most important goal of the DZHK is to make research findings available as quickly as possible to all patients and to advance the diagnosis, treatment and prevention of cardiovascular disease. In view of this mission the DZHK initiated guideline relevant clinical studies, registers and cohorts involving all 17 clinical partner site as well as external, non-DZHK member sites and will expand these activities in future. To support these studies, a scientific infrastructure for clinical research has been implemented including a trusted third party (University Medicine Greifswald) and a commercially available IT-system for clinical data (SecuTrail®; University Medicine Göttingen). To complete this infrastructure, a workflow based laboratory information and management system (LIMS) has been implemented. One aim of the DZHK-LIMS is the support of the whole process, beginning with the collection of biosamples at all sites, the processing of the samples and the storage in local – meaning DZHK decentral - freezers.
Methods
This DZHK-LIMS will be managed as a central core at the University Medicine of Greifswald, and will be connected to all study sites via the internet. At the specific study sites there will be access to the DZHK-LIMS directly or – in future – via the local LIMS, when there are connected via interfaces.

The DZHK-LIMS has to fulfill several features to support the clinical studies within the DZHK. The workflows have to be defined to specific roles according to the tasks of a study nurse in the study center and a technician in the laboratory. The workflows themselves are divided into small tasks and each task ca be presented on one screen. A task has to be finished, before the next task can be opened. This represents a simple tool to achieve a high safety within all workflow processes, because no step can be skipped. Later on, the LIMS will be connected to analyses platforms, to collect and document measurement results of laboratory analyses, which will be performed in future.
Results and Discussion
The CentraXX system from Kairos full filled all criteria for the DZHK LIMS. The preparation of the functional specifications was done in view of the several pre-defined workflows. In January 2016 a series of workshops started with the members of three pilot sites at Greifswald, Hamburg and Berlin, and Kairos. The functional specifications were transferred into workflows, considering several practical issues to make the workflows most convenient and safe for the users. Within a time period of six months, the main workflows were defined and implemented in the productive system. The productive system is ready to use at the three pilot sites and is currently in the evaluation phase.

DGKL / DVTA: Update Biobanking

FV17 – Talk Zumbülte

Decay marker for quality control of serum-specimens

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Background
Nowadays, one of the main targets is the quality control (QC) of blood specimens, due to increasing installation of biobanks as example. Pre-analytical variations have principal impact on most routinely performed diagnostic assays. Especially mass spectrometry multiparametric proteomics analyses of blood samples are seriously affected by limited protein stability based on high intrinsic proteolytic activity of serum and plasma specimens. Still, QC-tools are not available yet and correspond to the 'QC-gap'.
Objective
To get valid information about blood samples quality, a monitoring routine is needed. Therefore we have established the monitoring of pre-analytical quality for serum and plasma samples by analysing the time dependent decay pattern of endo- and exogenous peptides with liquid chromatography-mass spectrometry (LC/MS).
Materials and Methods
Serum samples from cancer patients (late-stage gastrointestinal tumors, n=81) and healthy controls (n=12) were analysed for a set endogenous peptides and two exogenous reporter peptides (RP), which were added into the tubes before blood withdrawal, as well as decay RP-intermediates and RP-anchor. Proteolytic fragments were quantified by LC/MS at different pre-analytical time-points ranging from 1h to 24h.
Results
The obtained set of endogenous decay markers could be reduced from 62[1, 2] to now ten. In combination with the two exogenous RP and theirs intermediates, the endogenous peptides provide us an insight into the time dependent changes of serum peptide profiles. Furthermore, calculated ratios of short and intermediate fragments allow us to predict the average age of an unknown sample. Classification accuracy was high with values always above 0.89 for areas under receiver operating characteristic curves.
Discussion and conclusion
Based on the high intrinsic proteolytic activity of a blood specimen the peptides are continuously and time-dependent processed. This 'proteomic degradation clock' can be used to evaluate the pre-analytical quality of serum samples and might have impact on quality control procedures of bio-banking repositories in the future.

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Update mikrobiologische Diagnostik

FV18 – Talk Maneg

Comparison of two modern molecular biological systems (SeptiFast, Roche; Unyvero, Curetis) with conventional microbiological diagnostics in ICU patients with sepsis, septic shock or septic nosocomial pneumonia

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Background
In surgical ICU patients postoperative complications with serious infections and sepsis can occur. Besides primary sepsis, sepsis can also develop from nosocomial pneumonia, particularly in ventilated patients. In the context of sepsis diagnostics two CE-certified test systems have been established in addition to the classical microbiological methods. The SeptiFast test (Roche) is used for the detection of pathogens from whole blood. The Unyvero system (Curetis) enables rapid diagnosis of pathogens causing nosocomial pneumonia, including frequent resistance genes. Investigations on a combination of both methods with respect to key parameters such as detection rates, days on adequate treatment, sensitivity and specificity compared to conventional methods might represent a very interesting approach in establishing a more rapid molecular diagnosis especially for septic ICU patients.

Materials/methods
This is a prospective controlled single centre observational study including patients from a surgical ICU. Study evaluation was blinded in respect to the results of combined application of the commercially available test systems Unyvero and SeptiFast versus conventional microbiological diagnostics such as swab and blood culture (BC) on the ICU. Only patients fulfilling the current international sepsis criteria and suffering from clinically diagnosed septic nosocomial pneumonia, sepsis, or septic shock were included. A control group of patients with no signs of infection or sepsis from the same ICU was included.
Results
In this study 73 septic patients (m/f 37/36, mean age 68 years) were included. 24 out of 73 septic patients revealed positive blood cultures, with an average time to gram-stain report to the clinician in charge of 25 hours.

For the SeptiFast system sensitivity and specificity turned out 67 % and 87 %, respectively, in regard to BC-proven bloodstream infections. Furthermore 27 (37 %) of ICU patients with sepsis or septic shock showed significant benefit either because more sensitive detection of causative pathogens (11, 15 %) and/or a significant reduction in turnaround time (TAT), (15, 21 %).

In 29 septic patients with a supposed pneumonia bronchial secretions were analyzed by the Unyvero method (sensitivity: 93 %, specificity: 87 %). Of these patients 19 (66 %) turned out positive, 9 negative, and 1 invalid. In 14 (48 %) of the positive patients pathogens recovered by the Unyvero system could be verified by culture, additional pathogens could be identified in 7 patients, 5 (17%) patients showed negative results upon conventional culture.
Conclusion
Compared to the results of the routine microbiological diagnostic methods both molecular test systems turned out superior in respect to TAT, sensitivity, and specificity. Consequently, molecular pathogen detection should be regarded as a valuable tool to complement classical microbiological diagnostics in patients suffering from severe sepsis on the ICU.

Update mikrobiologische Diagnostik

FV19 – Talk Ghebremedhin

Comparison of the geno- and phenotypic detection methods for the carbapenem resistance in clinical Gram-negative isolates

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Background: The emergence and dissemination of carbapenemase-producing Gram-negative bacteria is a worldwide emerging public health threat. Moreover, the accurate detection of carbapenem-resistant Gram-negative bacteria is clinically relevant for better therapy options and infection control measures. This study aimed to compare the rapid biochemical method Carba NP test with the PCR and sequence analysis of carbapenemase genes in Gram-negative rods.
Methods: 116 carbapenem-resistant *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates were collected during 2-year period. PCR was performed to detect the carbapenemase genes and Carba NP assay for the phenotypic detection of carbapenemase activity.
Results: By PCR analysis KPC, IMP, VIM, NDM or OXA gene variants were detected in the bacterial isolates. The Carba NP assay detected the activity of all investigated KPC, NDM, IMP and VIM producing isolates. 24 of 47 *A. baumannii* isolates which were either positive for OXA-23 ($n=14$), OXA-72 ($n=3$), OXA-58 ($n=3$) OXA-51 ($n=1$), OXA-40 ($n=1$), OXA-66 ($n=1$), or OXA-164 ($n=1$) could not be detected appropriately by the phenotypic Carba NP assay. In addition, the Carba NP assay was not able to identify four *E. coli* isolates producing OXA-181 ($n=2$), OXA-48 ($n=1$) and OXA-232 ($n=1$) and one OXA-48-producing *C. freundii* isolates.
Conclusion: The biochemical-based assay Carba NP test is an easy and cost-effective technique and offers an alternative for the rapid detection of carbapenemase production in *Enterobacteriaceae* and *P. aeruginosa* isolates, but has limitation in the detection of OXA-type carbapenemases in *A. baumannii* isolates. However, the Carba NP assay enabled the detection of the enzyme activity of two or more carbapenemase types within the same carbapenem-resistant bacterial isolate.

Postersessions – DGKL

DGKL-Endocrinology

DGKL-P001

Role of p45-NF-E2 in regulating syncytiotrophoblast formation in Human Placenta

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Background: The transcription factor p45-NF-E2, known to regulate megakaryocyte maturation, has been associated with impaired placental vascularization and intrauterine growth restriction (IUGR) in mice. Trophoblast and placenta specific deficiency of p45 NF-E2 in mice is responsible for excess syncytiotrophoblast formation. Post-translational modifications of Gcm-1 have been shown to regulate placental cell-fusion.
Aim: To study the role of p45-NF-E2 in regulating syncytiotrophoblast formation and placental vascularization in human placenta and its translational relevance for placental dysfunction in humans. In addition, we aim to study the mechanistic association between p45 NF-E2 and Gcm-1 involved in regulating syncytiotrophoblast formation.
Methods: We evaluated Gcm-1 and p45-NF-E2 expression (immunoblotting and immunofluorescence) in human placentae from healthy controls and patients with pregnancy complications (IUGR). Furthermore, a human choriocarcinoma cell line, Bewo, was stimulated with 8-Br-cAMP and in-vitro syncytia formation was evaluated using E-cadherin staining and marker gene expression (Gcm-1, Syn-1, hCG-β). Expression of p45-NF-E2 under these experimental conditions and its functional role using p45-NF-E2 knock-down or overexpression in Bewo cells was evaluated. Furthermore, post-translational modifications (acetylation and sumoylation) of Gcm-1 and the mechanisms regulating these were studied under these conditions.
Results: The diseased placenta samples showed enhanced syncytiotrophoblast formation, reduced expression of p45-NF-E2, and enhanced Gcm-1 acetylation as compared to controls. In-vitro, 8-Br-cAMP treatment enhanced syncytia formation, Gcm-1 expression and acetylation and was paralleled with reduced p45-NF-E2 expression. p45-NF-E2 deficiency was sufficient to increase spontaneous syncytiotrophoblast formation and Gcm-1 acetylation. Forced expression of p45 NF-E2 was sufficient to repress Gcm-1 expression and Gcm-1 acetylation which failed to increase even after 8-Br-cAMP treatment in these cells. This acetylation of Gcm-1 was regulated through a reduced interaction between CBP and NF-E2, increased acetyl-transferase activity of CBP, and an increased interaction between CBP and Gcm-1. Further, the deficiency of p45-NF-E2 was found to enhance SENP mediated Gcm-1 desumoylation in Bewo cells and overexpression of p45-NF-E2 reduced Gcm-1 desumoylation. Human IUGR placentae also showed enhanced SENP mediated Gcm-1 desumoylation compared to control placentae.
Conclusion: We identify a novel function of p45-NF-E2 during human placental development. Reduced p45-NF-E2 expression alters placental protein acetylation and sumoylation and may be an early cause and a suitable marker of placental dysfunction.

DGKL-P002

Lysophosphatidylcholines Activate PPARd and Protect Skeletal Muscle Cells from Lipotoxicity

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Lysophosphatidylcholines (LPC) are about to become an important biomarker in the prognosis of type 2 diabetes. Metabolomics studies demonstrate a negative correlation of plasma LPCs with insulin resistance, obesity, and type 2 diabetes. The physiological function of LPCs on metabolic pathways is largely unknown. Here we investigated the effects of LPCs on human skeletal muscle cells differentiated to myotubes. First we validated the concentrations of LPC(16:0) and LPC(18:1) which do not induce cell stress parameters. Concentrations higher than 50 µM were needed to induce the release of intracellular creatine kinase and lactate dehydrogenase. Below this concentration neither expression of ER stress marker ATF3 nor of inflammatory markers IL6 or CXCL3 were affected. Transcriptome analysis of human myotubes treated with 10 µM LPC for 24 h revealed enrichment of up-regulated PPAR target transcripts, including ANGPTL4, PDK4, PLIN2 and CPT1α. The increase in both PDK4 and ANGPTL4 RNA expression was abolished in the presence of either PPARδ inhibitor GSK0660 and GSK3787. The induction of PDK4 by LPCs was blocked with siRNA against PPARD. Luciferase reporter gene assays demonstrated activation of the ligand binding domain of PPARδ by LPC(16:0) and LPC(18:1). To address the direct binding of LPCs to PPARδ, gel shift assays were performed using recombinant PPARδ and RXRα. Both LPC(16:0) and LPC(18:1) were able to enhance PPARδ DNA binding activity. Further results show that the LPC-mediated activation of PPARδ can reduce fatty acid-induced inflammation in skeletal muscle cells. Treatment of human myotubes with 10 µM LPC(16:0) and LPC(18:1) ameliorated palmitate-driven ER-stress analyzed as ATF3 RNA expression and XBP1 splicing as well as palmitate-mediated cytokine induction. The protective effect of LPC was prevented in the presence of GSK0660. Taking together, the results show that LPCs are PPARδ agonists and hence can contribute to PPARδ-dependent anti-inflammatory effects.

DGKL-P003

Circadian Rhythm of circulating microRNAs

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Background: MicroRNAs are a major research focus since several years, especially in the quest to identify novel biomarkers for diagnosis and progression of various diseases. MicroRNAs belong to the class of small, single stranded, non-coding RNAmolecules that are transcribed in the nucleus and further processed in the cytoplasm by enzymes such as Drosha and Dicer. Then, microRNAs are integrated in RNA-silencing complexes that modify gene expression through translational repression or degradation of mRNA. Attempts to generate mircroRNA profiles from either tissues/cells or serum/plasma that are indicative for certain diseases have yielded inconsistent results and no reproducible prediction/diagnosis profile has made it into clinical routine use yet. One problem consists in the limited understanding of the presence of certain microRNAs for instance in blood and their functional consequences. In addition, many factors such as age, gender, nutrition state and others have been shown to influence the presence of microRNAs in a given clinical setting that are often not taken in consideration.
Study question: The aim of the present study was to generate a physiological profile of circulating microRNAs in the blood of healthy subjects over the course of the day.
Methods: We recruited 4 healthy young, normal-weight, male adults to profile serum microRNAs during the day using repetitive blood draws (8 am, 12:45 pm, 5:15 pm) on two separate days within 2 weeks. MicroRNAs were analyzed using qPCR-based whole miRNome arrays after several pre-analytic procedures and including spikein controls and sample normalization ("commonly expressed sample mean").
Results: A total of 1066 microRNAs were tested for their presence in serum. Roughly 60% were not reliably detectable (Cq value >30). Those microRNAs that were highly expressed did not reveal a consistent circadian rhythm or pattern. Several microRNAs (miR-320, miR-24-3p und miR-1280) had no specific fluctuation during the day and were consistently detectable. Among the microRNAs with variable expression levels, miR-365-3p demonstrated an expression increase during the day (average fold change 1.96) suggesting some kind of circadian rhythm. MiR-637 displayed an expression maximum and miR-1207 a minimum at noon time.
Discussion: A limited number of microRNAs are reliably present in serum in meaningful concentrations in our study. Daily fluctuation only occurs reliably in a few microRNA entities implying the potential existence of a circadian rhythm that probably is confounded by food intake. The low number of subjects in our study did only allow to observe qualitative changes which is a limitation. In conclusion, studies with larger cohorts on the circadian behavior of circulating microRNAs with and without starvation should be conducted to shed light on the physiological function of circulating microRNAs in peripheral blood.

DGKL-P004

Biphenyl based stationary phases for improved selectivity in complex steroid assays

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Background: Steroid hormones and their corticoid precursors are important biomarkers to diagnose and monitor a broad spectrum of endocrine disorders. For a better understanding of these disorders, the assessment of steroid profiles is an important field in clinical research. Therefore, we developed an UHPLC-MS/MS assay that combines a simple and effective sample preparation with a powerful MS method quantifying a broad steroid panel (cortisol, cortisone, corticosterone, 11-deoxycortisol, 21-deoxycortisol, 17-OH-progesterone, 11-deoxycorticosterone, progesterone, aldosterone, testosterone, dehydroepiandrosterone and dehydroepiandrosterone sulfate).
Methods: After a manual protein precipitation step using zinc trifluoroacetate in methanol as a novel approach, the eluates were directly injected into the UHPLC-MS system. For chromatographic separation we investigated different stationary and mobile phases. In order to enable a reliable quantification the respective stable isotope-labelled counterparts of the targeted analytes were employed as internal standards. For detection we used a Xevo TQ-S mass spectrometer operating in the ESI positive mode. Multiple reaction monitoring was performed for all analytes.
Results: Excellent chromatographic separation of all isobaric compounds (corticosterone ↔ 11-deoxycortisol ↔ 21-deoxycortisol and 17-OH-progesterone ↔ 11-deoxycorticosterone) was achieved employing a Kinetex Biphenyl column (150 x 2.1 mm, 1.7 µm). In combination with an innovative mobile phase consisting of 0.2 mM ammonium fluoride and methanol we were able to establish a highly selective and sensitive UHPLC-MS/MS method for the quantification of twelve endogenous steroids in human serum.
Conclusions: We could demonstrate that Biphenyl columns are a powerful tool for comprehensive, MS based steroid assays including various isobaric substances.

DGKL-P005

Bezafibrat verbessert die Insulinsensitivität und die Metabolische Flexibilität in diabetischen STZ-Mäusen

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Background: Bezafibrate (BEZ), a pan activator of peroxisome proliferator-activated receptors (PPARs), has been generally used to treat hyperlipidemia for decades. Clinical trials with type 2 diabetes patients indicated that BEZ also has beneficial effects on glucose metabolism, although the underlying mechanisms of these effects remain elusive. Even less is known about a potential role for BEZ in treating type 1 diabetes. To study the effect of BEZ we used the streptozotocin (STZ)-treated mice, an insulin-deficient mouse model of type 1 diabetes.
Materials and methods: Glucose metabolism was studied using glucose, hemoglobin A1C, and insulin measurements as well as glucose and insulin tolerance tests. Insulin signaling was assessed by Western blot and gene expression by real-time PCR. Metabolic flexibility was assessed using indirect calorimetry. Mitochondrial mass and function were analyzed by transmission electron microscopy and oxygen consumption measurements, respectively. Plasma metabolites were measured using LC-MS/MS technique. Pancreatic islets were studied by immunofluorescence staining.
Results: Here we show that BEZ markedly improves hyperglycemia and glucose and insulin tolerance in STZ-treated mice. BEZ treatment of STZ mice significantly suppressed the hepatic expression of genes that are annotated in inflammatory processes, whereas the expression of PPAR and insulin target gene transcripts was increased. Furthermore, BEZ-treated mice also exhibited improved metabolic flexibility as well as an enhanced mitochondrial mass and function in the liver. Plasma acylcarnitine levels were decreased, whereas plasma lysophosphatidylcholine levels were increased upon BEZ treatment. Finally, we show that the number of pancreatic islets and the area of insulin positive cells tended to be higher in BEZ-treated mice.
Conclusions: Our data suggest that BEZ may improve impaired glucose metabolism by augmenting hepatic mitochondrial performance, suppressing hepatic inflammatory pathways, and improving insulin sensitivity and metabolic flexibility. Thus, BEZ treatment might also be useful for patients with impaired glucose tolerance or diabetes.

DGKL-P006

Serum chemerin and its association with metabolic phenotypes: results of a population-based study

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Background: Chemerin is an adipokine that has been identified to play a crucial role in adipocyte differentiation and the pathogenesis of obesity-related disorders. However, existing studies on chemerin levels often refer to small sample sizes of very selective study populations.
Objective: The aim of the present study was to investigate the associations of serum chemerin with different metabolic phenotypes in a well-characterized large population-based study in order to evaluate a possible role of chemerin as a biomarker of obesity-related diseases.
Material and Methods: The study sample comprised 4,149 subjects from the population-based Study of Health in Pomerania. Serum chemerin levels were determined using enzyme-linked immunosorbent assay technique. Multivariable linear regression models were used to assess the association of chemerin with the amount of visceral (VAT) and subcutaneous adipose tissue (SAT), blood pressure (BP) values as well as glucose and lipid profiles. Associations to manifest diseases including diabetes, dyslipidemia, and hypertension were analysed using logistic regression models.

Results: We observed independent positive associations between VAT as well as SAT and chemerin with a stronger relation found for VAT. After adjustment for waist circumference (WC) and a variety of other potential confounders increased chemerin levels were associated with a higher amount of glycated haemoglobin (HbA1c), an unfavourable lipid profile and increased systolic BP values. Logistic regression further revealed significant positive associations of chemerin with dyslipidemia [highest vs. lowest quartile: odds ratio (OR) 1.32 (95% confidence interval (CI) 1.05-1.65)] and hypertension [highest vs. lowest quartile: OR 1.31 (95% CI 1.02-1.69)]. The positive association of chemerin with prevalent diabetes disappeared after adjustment for WC [highest vs. lowest quartile: OR 1.16 (95% CI 0.76-1.75)].
Conclusion: The present study showed that chemerin levels are significantly related to measures of body fat as well as to metabolic syndrome related factors including HbA1c, several lipid marker, or BP in a population-based cohort. Interestingly, the majority of the detected associations persisted significant even after the adjustment for WC suggesting that the relation between chemerin and the analysed obesity-related parameters cannot be solely explained by an accumulation of adipose tissue. In the long run it might be possible that chemerin act as an important predictive marker for metabolic diseases.

DGKL-P007

Increase of HbA1c with age in the healthy adult population – results from the Study of Health in Pomerania

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Background: Determination of glycated hemoglobin A1c (HbA1c) is a key measure to examine long-term glycemia, particularly important in the diagnosis of diabetes as well as monitoring blood glucose levels in diabetic patients [1]. Additionally, HbA1c is used for routine screening to identify prediabetes. The rise of HbA1c with age in individuals without diabetes is known for some time [2,3]. However, the cut-off HbA1c values for diagnosis of diabetes as well as target Hba1c values for diabetic patients do not reflect this age-dependency potentially leading to overdiagnosis and overtreatment of patients.
Objective: To evaluate HbA1c levels in relation to age in a large healthy population, samples from the Study of Health in Pomerania (SHIP) have been examined.
Methods: 4287 individuals (2110 men / 2177 women) from the general population of Northeastern Germany as part of the cohort SHIP-0 have been included. HbA1c concentrations were determined by high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany). Descriptive analyses and linear regression models were performed using SAS 9.4.
Results: Subjects were between 20 and 81 years old with a median age of 50 years (Q1: 36; Q3: 63 years). The median HbA1c concentration was 34.4 mmol/mol (Q1: 30.1, Q3: 39.9 mmol/mol) in the whole population. We observed an increase in HbA1c concentration over 10-year age-groups: subjects < 30 years [median: 32.2 mmol/mol (Q1: 28.4; Q3: 35.5)] had 7.7 mmol/mol lower median HbA1c concentration compared to subjects> 70 years [median: 39.9 mmol/mol (Q1: 34.4; Q3: 45.9)]. Linear regression analysis adjusted for sex and body-mass-index confirmed this positive association between age and HbA1c [beta per year increase 0.21 (standard error 0.009)]. The exclusion of subjects taking medication, except thyroid therapy and sex hormones, did not change the results substantially.
Conclusion: In a population-based setting, an age-related increase in HbA1c concentration independent of medication was observed. These findings implicate that age is a factor which should be considered when using HbA1c values for diagnosis of diabetes mellitus.

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DGKL-P008

Clinically relevant discrepancies between immunoassay results for Ciclosporin A

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Background: Ciclosporin A (CsA) is an important drug in transplantation medicine. Therapeutic drug monitoring for CsA is essential because under or over exposure can result to therapeutic failure or to toxicity, respectively. For kidney transplantation (KTx) patients the

CsA therapeutic range is 100-150 ng/mL in the the stable phase after KTx (1). Even though LC-MS/MS is considered the gold-standard for quantification most participants in proficiency testing use automated immunoassay (2). Discrepancies of CsA results were noted between two laboratories, which used two different immunoassays from the same manufacturer: one reported results below the therapeutic range and one above. This caused confusion of the clinician regarding dose adjustment. The CsA concentrations determined by the two assays were different by a factor of almost two. Both systems had valid internal quality controls and had passed proficiency testing on a regular basis.

Objective: Method comparison to elucidate possible systematic differences between two CsA immunoassays and a LC-MS/MS procedure.

Methods: Triggered by the observed discrepancies in one patient method comparison measurements with spare whole blood samples from 30 patients were conducted. Both immunoassays were compared to CsA concentrations measured by LC-MS/MS. The CsA measurements were performed on the Dimension Vista and the ADVIA Centaur XPT (both Siemens Healthcare Diagnostic, Eschborn, Germany), and a validated LC-MS/MS (3). The immunoassay results were obtained on the same day whereas the LC-MS/MS samples were collected over a period of 14 days and analysed within two days after shipment from Greifswald to Stuttgart.

Results: Regression analysis of the two immunoassays revealed a slope of 0.568 and a correlation of r^2=0.994 with the results from the Dimension Vista higher than those from the ADVIA Centaur XPT. Between the Dimension Vista and ADVIA Centaur XPT results a systematic difference of approximately 90 % was observed. Both immunoassays correlated well with LC-MS/MS (r^2=0.979 and r^2=0.978). The slope of the regression line between CsA concentration obtained by Dimension Vista and LC-MS/MS was 1.248 whereas the slope between ADVIA Centaur XPT and LC-MS/MS was 0.704.

Discussion and Conclusion: Clinical decisions for dose adjustments of CsA are based on whole blood concentrations. Results obtained with the investigated immunoassays may be above or below the therapeutic range in the same sample depending on the assay used. We conclude that proficiency testing may not be sufficient from a clinical perspective possible reasons being wide passing limits or inappropriate control materials.

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DGKL-P009

Adiponectin and mortality in smokers and non-smokers of the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

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Introduction: Cardiovascular diseases (CVD) are an important cause of morbidity and mortality worldwide. Decreased concentrations of adiponectin have been reported in smokers. Aim of our study was to analyze the effect of cigarette smoking on the concentration of adiponectin and potassium in active smokers (AS) and life-time non-smokers (NS) of the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study as well as their use for risk prediction.

Materials and methods: Smoking status was assessed by a questionnaire and measurement of plasma cotinine concentration. Adiponectin serum concentrations were measured by ELISA. Adiponectin was binned into tertiles separately for AS and NS and Cox regression was used to assess the effect on mortality.

Results: 777 LURIC patients were AS and 1178 NS. Within 10 years (median) of follow-up 221 AS and 302 NS died. In unadjusted analyses AS had lower concentrations of adiponectin. However, after adjustment for age and gender there was no significant difference in adiponectin concentration anymore. In a Cox regression model adjusted for age and gender adiponectin was significantly associated with mortality only in AS with a HR (95% CI) of 1.60 (1.14-2.24) comparing the 3rd with the 1st tertile, but not in NS. In a model additionally adjusted for the risk factors diabetes mellitus, hypertension, coronary artery disease, body mass index, LDL-cholesterol and HDL-cholesterol adiponectin was significantly associated with mortality with HR of 1.83 (1.28-2.62) and 1.56 (1.15-2.11) for AS and NS, respectively.

Conclusions: Increased adiponectin is a strong and independent predictor of mortality both in NS and AS and its determination could be used to identify individuals at increased risk.

DGKL-P010

Sex steroids, HPA-axis and heart rate respond to an acute psychosocial stress depending on the subjective assessment in healthy young men

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Background: In this study, we used the Trier Social Stress Test (TSST) and a Placebo-TSST in healthy young men to investigate the endocrine and subjective stress response. Objectives were to determine 1) the response patterns of endocrine hormones and heart rate during a psychosocial stressor and 2) the covariance between endocrine- and cardiac response and subjective stress measures.
Methods: Twenty-nine healthy young, non-smoking men participated in the TSST, while 30 age- and BMI-matched males completed the Placebo-TSST. Throughout the procedure, cortisol, cortisone, alpha-amylase, and CBG were measured in saliva samples. In addition, total cortisol, cortisone, corticosteroid binding globulin (CBG), copeptin, testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP), estradiol, progesterone, dehydroepiandrosterone sulfate (DHEAS), and aldosterone were determined in serum. Free cortisol in serum was calculated using Coolen's equation. Adrenocorticotropic hormone (ACTH) was measured with a plasma sample. State Anxiety Inventory (STAI) was collected as a subjective marker. Heart rate was acquired through electrocardiography (ECG).
Results: Heart rate and all hormones of the HPA-axis showed significant time x group interaction effects (all $p < 0.001$), which was most pronounced in cortisol response in serum and saliva. Kinetic profiles of HPA-axis hormones showed sequential peak response from ACTH to cortisone. Among sex steroids, progesterone and androstenedione showed significant increase in TSST-group. The percentage increase of STAI correlated significantly with that of heart rate ($r=0.531$, $p<0.0001$) and that of cortisol in serum and in saliva ($r>0.45$, $p<0.001$). The response of sex steroids correlated well with that of the heart rate and the HPA-axis.
Conclusion: Subjective measures (STAI) showed the most significant correlation with the hormone response of the HPA-axis and the heart rate. The change in the sex steroid levels at an acute psychosocial stress might imply the partial secretion of the reproductive hormones from the adrenal glands and their neuroprotective function against glucocorticoids at an acute stress.

DGKL-P011

The renin-angiotensin-aldosterone system in smokers and non-smokers of the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

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Introduction: High concentrations of renin and aldosterone are risk factors for cardiovascular diseases (CVD) which are the leading cause of morbidity and mortality worldwide. Enhanced activation of the renin-angiotensin-aldosterone system (RAAS) by cigarette smoking has been reported. Aim of our study was to analyse the effect of cigarette smoking on parameters of the RAAS in active smokers (AS) and life-time non-smokers (NS) of the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study as well as the utility of RAAS parameter for risk prediction.
Materials and methods: We determined the concentration of aldosterone, renin, angiotensin-I and angiotensin-II in participants of the LURIC study. Smoking status was assessed by a questionnaire and measurement of plasma cotinine concentration. Parameters were log transformed before entering analyses, where appropriate. We used a multivariate Cox regression analysis to assess the effect of parameters on mortality.
Results: From 3316 LURIC participants 777 were AS and 1178 NS. Within a median observation period of 10 years 221 (28.4%) AS and 302 (25.6%) NS died. After adjustment for age, gender and the use of anti-hypertensive medication only angiotensin-I was significantly different in AS compared to NS with an estimated marginal mean (95% CI) of 1607 (1541-1673) ng/L and 1719 (1667-1772) ng/L, respectively. For both NS and AS renin and angiotensin-II were directly associated with mortality in the multivariate Cox regression analysis. Angiotensin-I was only associated with an increased risk for mortality in NS (HR (95% CI) of 0.69 (0.53-0.89)).
Conclusions: Increased renin and angiotensin-II are independent predictors of mortality in AS and NS while angiotensin-I was associated with reduced risk of death in NS only.

DGKL-P012

Suitability of the VACUETTE® FC Mix blood collection tube for long-term stability testing of glucose

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Background: Due to prolonged exposure of blood collection tubes to transport times of partly up to 48h, the aim of the study was to demonstrate the comparison of glucose concentration in specimens centrifuged directly after blood collection to specimens stored in whole blood at room temperatures as well as the long term stability of initial glucose concentration in specimens centrifuged directly after blood collection. The recommended additive for effectively stabilizing the glucose concentration is the composition of citrate, EDTA and sodium fluoride in order to prevent glycolysis. The study was designed to evaluate tubes containing that recommended additive composition indicating a superior performance to another anticoagulant sodium fluoride/potassium EDTA.
Materials and Method: Fifteen healthy volunteers were recruited and venous blood drawn into 4 blood collection tubes (VENOSAFE™ FC, Terumo; VACUETTE® FX NaF/KEDTA and two samples VACUETTE® FC Mix, Greiner Bio-One) of which three different glucose tubes to be centrifuged directly after blood collection and the second sample of VACUETTE® FC Mix tube to be centrifuged after 48h storage in whole blood. All samples were inverted according to the manufacturers' recommendations. Directly after blood collection, the plasma was measured immediately after centrifugation in order to obtain initial values of 30 min for comparison evaluations of all other time points by using the Glucose Hexokinase method on a Beckman Coulter Analyzer AU640. The glucose concentration has been determined after 24h, 48h, 72h and 96h after re-centrifugation prior to measurement in order to show the long-term stability.
Results: The stability of glucose concentration up to a 96h time period has been shown. Even the specimens which were centrifuged 48h after blood collection and kept for further stability testing at room temperature, demonstrated a stable glucose concentration up to 96h. The glycolysis results in a 7.7% lower initial glucose concentration in currently commercial tubes containing just sodium fluoride and EDTA. The deviations of single glucose concentration determinations were found to be within analytical tolerances.
Conclusion: On the basis of these results, the VACUETTE® FC Mix tube is a blood collection tube suitable for reliable determination of blood glucose being one of the most frequently measured analytes in the laboratory and also of utmost importance in diabetes diagnosis, monitoring and therapy. It is found to be useful in improving the stability of glucose concentration up to 96h primarily in case of long transport distances up to 48h transport time without having to use a secondary tube, particularly due to further increased centralization of laboratories.

DGKL-P013

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

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Background: Reliable detection of Gestational Diabetes Mellitus (GDM) is required to prevent maternal and fetal complications. With transport times of up to 48h, the aim of the study was to demonstrate long term stability of initial glucose concentration in specimens centrifuged directly after collection and compared to whole blood specimens stored at room temperatures. Recent guidelines from the German Diabetes Association recommend use of tubes with an additive composed of citrate, EDTA and sodium fluoride, as in VACUETTE® FC Mix tubes, to effectively stabilize glucose levels for the diagnosis of GDM due to the incomplete inhibition of glycolysis by sodium fluoride alone.
Materials and Method: The current study was conducted at ISALA Hospital (Zwolle, Netherlands) using VENOSAFE™ FC Mixture® versus VACUETTE FC Mix blood collection tubes. Altogether, 43 pregnant donors who were healthy (n=19) or diagnosed with gestational diabetes by 75g-Oral Glucose Tolerance Test (n=24) were recruited. Informed consent was given by all donors and the study was approved by EC Netherlands. Venous blood was drawn from each donor into four tubes (two tubes each tube type). One tube of each type was centrifuged directly after blood collection according to manufacturer recommendations and the second one after whole blood storage for 48h at room temperature. Following collection, plasma was measured immediately after centrifugation to obtain initial values (fasting) and after 48h for evaluation of glucose stability using the Hexokinase method on a COBAS 8000 (Roche Diagnostics, Mannheim, repeatability VC 1%, total precision VC 1.7%). Statistical evaluation was done by STATISTICA 12.
Results: Evaluation of all clinical results for glucose concentration and any deviations was done on the basis of maximal allowed deviation for a single value (for glucose 11%) according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of both tubes for performance testing did not reveal any clinically nor statistically significant deviations ($p<0.05$). The values of both tubes resulted in an initial highest deviation of 5.5%, and 6.4% after 48h (both healthy). Comparable highest deviations for initial values in relation to 48h values were obtained for VENOSAFE and VACUETTE tubes with 5.4% (healthy) and 6.6% (GDM), respectively. The storage of whole blood specimens for 48h showed no significant deviation (10.5%, healthy).

Conclusion: Based on these results, the VACUETTE FC Mix blood collection tube is suitable for reliable determination of blood glucose, one of the most frequently measured laboratory analytes and of primary importance in diagnosis, monitoring and therapy of GDM. The stability of glucose concentration in whole blood specimens drawn in the VACUETTE FC Mix tube and stored up to a 48h at room temperature has been shown. Use of this tube will improve the stability of glucose with extended transport times up to 48h, which is more common with centralization of laboratory testing, negate the need for sample aliquotting and allow for good obstetric practice.

DGKL-Hematology & Hemostasis

DGKL-P014

NMD as a modifying mechanism in Haemophilia A: Exploitation for diagnostics and therapy

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Haemophilia A is a common inherited coagulation disorder resulting from the lack of the blood clotting Factor VIII (F8). In response to current replacement therapies, 30% of patients with a severe manifestation of the disease produce specific inhibitors, tremendously aggravating treatment. Nonsense Mediated Decay (NMD) represents a cellular translation dependent surveillance mechanism with modulating effects reported in various genetic diseases and cancer. mRNA transcripts containing mutation induced premature termination codons result in truncated proteins with potentially deleterious effects and are thus degraded by the NMD machinery. Whereas the role of NMD in β-Thalassaemia has been clearly defined, such a role in Haemophilia A is less well understood.

Here we set out to thoroughly explore the role of NMD in the outcome of systematically screened, patient-derived mutations in the F8 gene. To achieve this, we are generating F8 expression constructs with and without these mutations for use in cell culture transfection experiments assaying NMD-eliciting activity. NMD dependence of specific mutations is then determined by monitoring for increases in mRNA levels following NMD inhibition by siRNA depletion of Upf1 (the master regulator of NMD) as well as pharmacological treatments. Truncated F8 proteins rescued from NMD are then further analysed for functionality with the ultimate aim of determining the potential of NMD inhibition for novel therapeutic perspectives in the future.

DGKL-P015

Optimizing specificity and reproducibility of a biomimetic SPR-biosensor for acquired anti-factor VIII

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Introduction:
Bleeding disorders can be caused by many different reasons, one of which is the presence of anti-factor VIII (FVIII) antibodies. The diagnosis uses a complex step by step approach and is followed by a sophisticated and expensive treatment. The most common diagnostic method for detecting malfunctions of the clotting system caused by antibodies is the Bethesda Assay. Its major problems are its lack of standardization and low sensitivity in the lower measuring range. A high sensitivity method for detecting FVIII antibodies in human plasma could improve and accelerate the diagnosis and thus the time to therapy from hemophilia patients with FVIII antibodies.
Motivation:
A high sensitivity SPR biosensor detecting FVIII auto- and alloantibodies and discriminating their inhibitory properties was established previously [1]. The high sensitivity of the SPR-System requires stringent antibody purification while maintaining high antibody activity for optimal results. The aim of this project is to further improve sensitivity, specificity and reproducibility of the assay. To this end, a generic workflow for the complete removal of all contaminants while maintaining maximum antibody activity will be investigated.
Material und Methods:
FVIII antibodies were detected with Surface plasmon resonance on a Biacore System (Biacore X and X100). HC1500 Chips from Xantec with immobilized full-length FVIII Kogenate from Bayer were used. For isolation of immunoglobulins from plasma, different methods were tested so far: Protein A/G columns, Protein L, Protein A magnetic beads and columns with immobilized anti-human-immunoglobulin-antibodies. The purification efficiency was assessed by subsequent determination of total protein content and immunoglobulin concentration. Quality and purity of the preparation was assessed by SDS-Page.
Results:
Isolation of immunoglobulins using agarose columns with immobilized anti-human-immunoglobulin-antibodies is a promising approach due to its high specificity. Although the yield is still low compared to protein A/G, the sensitive SPR method can detect the purified

low-concentrated antibodies. A combination of different affinity purification methods will be assessed to further enhance purity and yield of the antibody preparations.

Outlook:

Our method for antibody extraction from plasma that produces samples of high purity and yield as well as good reproducibility and ease of use paves the way to an application of the SPR biosensor in patient care. This SPR biosensor grants the possibility to assess both presence and activity of antibodies in one measurement. Thus, enhanced characterization of FVIII antibodies could lead to a more specific treatment for the individual patient.

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DGKL-P016

Hereditary spherocytosis - evaluation of a diagnostic algorithm based on erythrocytic and reticulocytic parameters

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Hereditary Spherocytosis is the most frequent form of haemolytic anaemia in mid and northern Europe. The prevalence in the German population is estimated at about 1:2000 to 1:2500.

In 2011, Mullier et al. (1) published a 2-step diagnostic tool based on erythrocytic and reticulocytic parameters. The algorithm includes reticulocytes, immature reticulocytes fraction, hypochromic erythrocytes and microcytic erythrocytes. Combining standard complete blood count parameters with the two specific reticulocytic markers, they identified Hereditary Spherocytosis with a positive-predictive value of 75% and a negative-predictive value of 100%.

We evaluated 295 blood samples containing 50 Hereditary Spherocytosis-confirmed cases. EDTA blood samples were analysed on a Sysmex XE5000 instrument.

For the pretest we are able to report a sensitivity of 100% and specificity of 91% combined with a positive-predictive value of 70% and a negative-predictive Value of 100%.

The differentiation-step reveals a sensitivity of 94% and specificity of 100% combined with a positive-predictive value of 100% and a negative-predictive Value of 99%.

Taken together, the data confirm the algorithm published by Mullier et al. and integrated into ICSH 2015 guidelines (2) as an appropriate routine-tool to assess Hereditary Spherocytosis.

We are currently preparing to introduce the algorithm into the daily routine by configuring the associated parameters in the LIS.

Cases with positive results will then be assessed with the acknowledging assays, namely Acidified Glycerol Lysis Time (AGLT) and Eosin-5-Maleimid-binding-Assay (EMA).

1) Mullier et al., Additional erythrocytic and reticulocytic parameters helpful for diagnosis of hereditary spherocytosis: results of a multicentre study. Ann Hematol, 2011, 90, 759-768
2) King et al., ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. Int J Lab Hematol, 2015, 37, 304-325

DGKL-P017

Magnesium sulphate as alternative anti-coagulant compared to EDTA and Citrate: Differences in spontaneous and agonist-induced platelet activation and aggregation

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INTRODUCTION
Although introduced as in vitro anticoagulant in 1882 by J. Bizzozerro to avoid platelet clumps (1) magnesium sulphate fell into obliviation when EDTA was introduced in the hematology laboratory in the 1950s. It was rediscovered when it was shown to be effective to differentiate EDTA-induced pseudo-thrombocytopenia (2, 3).
OBJECTIVES, MATERIAL AND METHODS
In our present study we compared the effect of in vitro anticoagulants (EDTA, citrate and MgSO4) on the spontaneous and agonist- induced expression of platelet activation markers (CD62; CD63) and their effects on platelet aggregation as measured by aggregometry according to Born and impedance aggregometry (MultiplateR).
RESULTS
In contrast to EDTA-anticoagulated platelets, showing a very different spontaneous CD62 and CD63 expression, citrate and magnesium sulphate anticoagulation was associated with low basal activation marker expression. Upon agonist stimulation the extent of platelet activation was similarly high, independent from the kind of anticoagulation.

The agonist-stimulated aggregation of EDTA and MgSO4 anticoagulated platelets was low (maximal aggregation: 0 to 30 %), whereas in citrate anticoagulated blood, platelets showed approximately 80 to 90 % aggregation. Using impedance aggregometry the effect of different agonists is expressed as "area under the curve" (AUC). The stimulation of citrate or MgSO4 anticoagulated platelets with different agonist resulted in an AUC ranging from 7,50 to 82,50 and from 2,6 to 64,6, respectively.
CONCLUSIONS
Our study shows that the agonist-induced expression of CD62 and CD63, universally interpreted as parameter of platelet activation is not linked to the aggregation capacity of differently anticoagulated platelets.

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DGKL-P018

Laboratory pitfalls in concomitant use of direct oral anticoagulants and heparins

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Background: The three direct oral anticoagulants (DOACs) dabigatran, apixaban and rivaroxaban are well-established and widely used in clinical practice. Heparin bridging for patients requiring perioperative interruption of DOACs is still under discussion and only rare data are available.
Objectives: We show the influence of concomitantly used DOACs and heparins on laboratory assays for the first time.
Methods: Healthy donors and patients receiving DOACs were investigated for spiking experiments with heparins and DOACs. The laboratory measurement of heparins, DOACs and other coagulation parameters was performed with routine methods on the ACL TOP 700. Additionally, the DOACs were quantified with ultra-performance liquid chromatography/ electrospray ionization-tandem mass spectrometry.
Results: Dabigatran shows no influence in the applications for low molecular weight heparin (LMWH), unfractionated heparin (UFH), rivaroxaban and apixaban, whereas all FXa-inhibiting drugs affected all anti-Xa assays in their own specific way. Samples of patients with a therapeutic intake of DOACs spiked with UFH and LMWH showed the pharmacokinetic profiles expected, but increased pharmacodynamic effects.
Conclusion: Direct thrombin and FXa inhibitors exhibit distinct effects on assay results when used concomitantly with heparins. These interactions must be considered in the interpretation of assay results under bridging therapy, especially as overlapping therapy of DOACs using heparins is not recommended for these drugs.

DGKL-P019

Effect of the preventative Allopurinol therapy within the framework of the peripheral blood stem cell donation

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Introduction: The aim of the clinical trial is the examination of the therapeutic benefit of a preventative therapy using the uricostatic „Allopurinol" in allogenic stem cell donors with elevated uric acid concentrations.
Methods: The stem cell donor collective consists of 72 probands, who were divided into 2 main groups according to their uric acid values of the pre-examination. Group 1 (30 donors) includes all donors with increased uric acid concentrations and therefore receives „Allopurinol" collateral to a stem cell mobilization by G-CSF administration. Group 2 (42 donors), however, contains all donors with normal uric acid concentra-tions.
Results: The mean uric acid concentration of group 1 decreased from 7.01 mg/dl (pre-examination) to 5.03 mg/dl (follow-up examination), whereas in group 2 this value rose from 5.16 mg/dl to 6.15 mg/dl. This rise results from the increased proliferation of leucocytes and progenitor cells caused by the growth factor G-CSF. This leads to an augmented protein degradation and finally to a gain of uric acid serum concentration. In order to confirm the preventative benefit of „Allopurinol", the hypothetical uric acid increase of group 1 without the Allopurinol therapy was calculated. As a result, the mean uric acid value of group 1 would increase from 7.01 mg/dl to 8.13mg/dl (hypothetical value).
Conclusion: These results give an indication about the positive effect of „Allopurinol" pertaining to the preventative therapy within the framework of the peripheral stem cell donation.

DGKL-P020

The retrospective analysis of the effect of patient-specific parameters on the required quantity of apheresis cycles and the CD34+ yield of autologous stem cell donors

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Introduction: In order to conduct a stem cell apheresis prior to a respective therapy, stem cells have to be mobilized from the bone marrow into the peripheral blood. In this paper we elaborated patient-specific as well as diagnosis-independent parameters, which have an influence on a successful stem cell mobilization.
Methods: Retrospective data were obtained from 302 apheresis cycles of 177 patients in the years 2009 to 2011. Correlation of potentially predictive factors (sex, age, BMI and CD34+ initial value) and quantity of apheresis cycles and CD34+ outcome was statistically analyzed using SPSS20.
Results: Using a demand-normalized analysis, there is no statistically significant correlation between age, BMI or sex and number of apheresis cycles needed. The p values were found to be 0.44, 0.53 and 0.86, respectively. Regarding the initial CD34+ value the p value of 0.017 displays a statistical significance in correlation to the applied apheresis cycles. As an example, donors showing < 15 CD34+ cells/µl needed in the mean 3.6 apheresis cycles, donors showing > 100 CD34+ cells/µl needed in the mean 1.1 apheresis cycles.
Conclusion: The initial CD34+ value could be identified as significant predictive factor for successful apheresis. The consequence for practical applications is the use of a drug-modified mobilization in case of patients with a low CD34+ initial value.

DGKL-P021

Evaluation of the influence of platelet function on the bleeding risk in patients with acquired aortic valve stenosis

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Background
Aortic valve stenosis due to degenerative calcification in elderly people is often accompanied by an acquired von Willebrand syndrome type 2A. These patients have a risk of increased bleeding during and after surgery. However, bleeding events can only be observed in a minority of patients. Therefore, we examined the platelet function regarding its influence on the bleeding risk.

Patients & Methods
We enrolled 77 consecutive patients with degenerative aortic valve stenosis who underwent valve replacement in our institute. For analyzing the platelet function we used PFA-200 (col/ADP and col/EPI) and light transmission aggregometry (LTA) with ten different agonists. Furthermore, we determined the level of von Willebrand factor (VWF) antigen and VWF activity, as well as blood loss, transfusions and echocardiographic data.
Results
We observed a reduced platelet response in 75% of the patients. The signaling pathways affected most frequently were the ADP, epinephrine, PAF and thrombin pathways. Our LTA measurements revealed a relationship between platelet function and VWF activity. Patients with decreased VWF activity had an improved platelet function compared to patients with VWF activity in the normal range. Nevertheless, platelet function was not associated with the severity of stenosis.
Conclusions
An impairment in platelet function seems to be quite common in patients with degenerative aortic valve stenosis. The results of our study might indicate a compensatory effect of platelet function in the case of a low VWF activity level, which could explain moderate blood loss despite the high bleeding risk in this clinical picture.

DGKL-P022

The international-normalized-ratio (INR) as predictor of mortality in patients referred for coronary angiography – The Ludwigshafen Risk and Cardiovascular Health Study

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Background: The international-normalized-ratio (INR) is typically used to monitor patients on warfarin or related oral anticoagulant therapy. A high INR indicates a higher risk of bleeding, while a low INR suggests a higher risk of thrombosis. To the best of our knowledge there are no reports about INR and mortality in CAD patients not on oral anticoagulant therapy. Therefore, the aim of our study was to investigate the association of the INR with all-cause and cardiovascular mortality in a cohort of patients scheduled for coronary angiography.
Methods: The LUdwigshafen RIsk and Cardiovascular Health (LURIC) study is an ongoing prospective study of 3316 patients of German ancestry who had an indication for coronary angiography. For 2583 (77.9%) coronary artery disease (CAD) was confirmed. For the present study we excluded patients on coumarin therapy (n=222) and patients with an INR more than 5 standard deviations (SD) away from the mean (n=30) leaving 3064 patients for analysis. Associations of the INR with mortality were investigated using Cox proportional hazards regression with adjustment for cardiovascular risk factors and medication.
Results: The INR ranged between 0.70 and 1.79 with a median of 1.04. There was no significant difference between CAD positive and CAD negative patients (1.05±0.09 and 1.04±0.09). During a median follow-up of 9.9 years (8.8-10.7), 884 patients (28.9%) died, 547 patients (17.9%) from cardiovascular causes. After adjustment for cardiovascular risk factors the INR was associated with all-cause mortality in all patients and the CAD negative group with HR (95% CI) of 1.14(1.07-1.21) and 1.16(1.09-1.23) per 1-SD increase, respectively. Cardiovascular mortality increased with higher INR with HR of 1.23(1.01-1.51) and 1.21(1.13-1.30) per 1-SD increase for CAD negative and CAD positive patients, respectively. Hazard ratio plots indicate a rising mortality risk in CAD patients approximately from an INR of 1.05 upwards.
Conclusion: In the LURIC study, the INR was positively associated with all-cause and cardiovascular mortality in patients with prevalent CAD not on oral anticoagulant therapy. Our results might indicate that not only a high thrombogenic potential but also a higher bleeding potential poses a risk for patients with CAD and warrant further research.

DGKL-Immunology, Autoimmunity, Allergy

DGKL-P023

Alpha-Melanocyte stimulating hormone potentially inhibits basophil activation, indicating a novel function of this neuropeptide in airway allergy

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Background: It is well established that basophils play an important role in the initiation and control of airway allergy with potent effector functions during allergic responses. Using a mice model we could show that the neuropeptide alpha-melanocyte stimulating hormone

(a-MSH) inhibits allergic airway inflammation. Thus, we wanted to assess, whether basophils functionally respond to a-MSH in patients with upper airway allergy.
Methods: Human basophils of patients with allergic rhinitis to grass pollen only and nonatopic controls were purified with magnetic beads with a purity of 98-100%. Expression of the Melanocortin-Receptor 1 (MC1R) was analysed by FACS, RT-PCR and Western immunoblotting. Activation of basophils was studied by calcium 2+ mobilization assay and CD63 and CD203c surface expression using the basophil activation test and release of cytokines with ELISA.
Results: Human peripheral blood basophils expressed MC1Rs at protein and mRNA level. The MC1R was functionally active in isolated basophils as shown by α-MSH-mediated intracellular increase of calcium^{2+}. Further, α-MSH suppressed anti-IgE or grass pollen induced basophil activation assessed with CD63 surface expression in basophils of normal individuals and patients with allergic rhinitis ($p<0.001$). The effect of α-MSH on basophil activation was MC1R-mediated as shown by blockade with a peptide analogue of agouti signaling protein. α-MSH also inhibited anti-IgE and grass pollen induced upregulation of the clinically relevant activation marker CD203c. Moreover, α-MSH triggers an anti-inflammatory activation pattern showing by induces the release of the anti-inflammatory cytokine IL-10 and α-MSH significantly inhibited anti-IgE, fMLP or PMA induced release of IL-4, IL-6 and IL-13 ($p<0.05$-0.01).
Conclusion: Our data show that a-MSH inhibits the allergic immune response in human basophils which classifies α-MSH as a promising and novel treatment strategy for patients with allergic rhinitis.

DGKL-P024

A novel assay platform for the detection of antiphospholipid antibodies differentiating patients with antiphospholipid syndrome from patients with infections and asymptomatic carriers

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Background: Antiphospholipid antibodies (aPL) can be detected in asymptomatic carriers and infectious disease patients apart from patients with antiphospholipid syndrome (APS) by recommended classification enzyme-linked immunosorbent assays (ELISAs). In contrast, APS-specific aPL appear to recognize β2 glycoprotein I (β2GPI)-domain 1 (D1)-related epitopes. A novel assay platform which employs a hydrophobic membrane as solid phase in a line immunoassay (LIA) reaction environment enabling the preferential presentation of D1 epitopes was developed. We tested whether this new multiplex LIA can differentiate aPL in patients with APS from those in asymptomatic aPL+ carriers or infectious disease controls (IDC).
Methods: Sixty-one APS patients (56 primary, 22/56 with obstetric events only, and 5 secondary), 146 controls including 24 aPL+ asymptomatic carriers, 73 IDC, and 49 healthy subjects were analyzed in multiplex LIA employing a novel reaction environment based on a hydrophobic solid phase coated with cardiolipin (CL), phosphatic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidyl¬glycerol, phosphatidyl¬inositol, phosphatidylserine, β2GPI, prothrombin, and annexin V. aPL detected by LIA were compared with aCL and aβ2GPI by ELISA and Lupus Anticoagulant activity. Human monoclonal antibodies (humoAbs) against human β2GPI (HCAL, EY2C9, MBB2) or phospholipids alone and β2GPI-domain specific APS patient sera (9 with aD1-positive and aD4/5-negative aPL reactivity; 5 with aD1-negative and aD4/5-positive aPL reactivity) were used to assess aPL reactivity in LIA.
Results: Comparison of LIA with the aPL-classification assays demonstrated good agreement for IgG/IgM to (ß2GPI) aß2GPI and CL (aCL), yet there was a significant difference for aCL IgG testing (McNemar's test, $p > 0.05$). In contrast to ELISA, aCL IgG by LIA were significantly lower in aPL+ carriers and IDC samples and aß2GPI IgG by LIA in aPL+ carriers than in APS patients. The humoAb MBB2 against D1 recognized β2GPI bound to LIA-matrix and in anionic PL-complexes. Absorption with CL micelles abolished the reactivity of a phospholipid-specific humoAb but did not affect the binding of aβ2GPI humoAbs in particular MBB2. In contrast to the 5 exclusively D4/5-reactive sera, all 9 exclusively aD1-positive sera demonstrated aPL binding in LIA. Altogether, these findings indicate a preferential presentation of APS-specific epitopes on D1 in LIA resulting in disease-specific aPL detection by LIA. Interaction of ß2GPI of the patient sera with the immobilized phospholipids leads to a conformational change of the ß2GPI molecule and to the formation of a ß2GPI layer preventing the binding of D4/5-reactive aPL.
Conclusions: Despite the good agreement in detecting aPL by LIA and ELISA in APS, only LIA appears to differentiate APS from asymptomatic carriers likely through the preferential exposure of D1.

DGKL-P025

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

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

DGKL-P026

Platelet membrane proteins integrated into nanodiscs for the detection of ITP-related antibodies

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Introduction
In patients with severe thrombocytopenia excessive bleeding episodes can occur. In immune thrombocytopenia (ITP), antibodies are produced either against autologous platelets or against structures on allogenous platelets after pregnancy or after platelet transfusion. Currently, diagnosis of ITP is a process of exclusion. In most cases, an autoimmune disorder can merely be assumed after other causes of a low platelet count have been excluded. Although tests for patient anti-platelet antibodies are available, they have low sensitivities and a negative result does not necessarily rule out ITP.
Motivation
With the novel nanodisc (ND) technology, a sensitive and specific test for anti-platelet antibodies can be developed. NDs are self-assembled bilayers stabilized by synthetic membrane scaffold proteins, into which membrane proteins can be integrated in a native and functional form. Our aim is the detection of patient anti-platelet antibodies using NDs including platelet membrane proteins immobilized onto biosensor surfaces. The reconstitution of membrane proteins in NDs allows for correct protein folding and a native lipid environment, thus increasing diagnostic performance of future tests.
Methods
Platelets were isolated from the whole blood of voluntary donors as a starting material for ND assembly. As common protocols for ND preparation are costly in terms of labor and time, including membrane isolation and long incubation steps, they are not easily applicable in a diagnostic context. For this reason, different simplified generation protocols were designed and tested. Assembly conditions of NDs were varied, and the resulting NDs examined by size exclusion chromatography (SEC), western blot (WB) and surface plasmon resonance spectroscopy (SPR).
Results
The generation of NDs from human platelets could be demonstrated by SEC and the size of those NDs corresponds to previously published results. The integration of different platelet membrane proteins reported triggering ITP was verified by WB. A novel generation protocol for platelet nanodiscs could be implemented that takes only about two thirds of previously published methods and uses only common laboratory equipment. NDs prepared by this protocol showed similar characteristics than those prepared according to standard methods and had the same or even higher amount of integrated protein. First preliminary results using NDs in SPR clearly showed that the detection of anti-platelet antibodies is possible.
Conclusion
Our novel protocol allows a fast and direct generation of NDs from platelets and the confirmation of integrated platelet membrane proteins. Based on those promising first results, the next steps will be the examination of different sensor surfaces and immobilization techniques in order to find the best assay format for detection of anti-platelet antibodies from patient samples.

DGKL-P027

ANA-Screening by indirect immunofluorescence testing: Do we miss something?

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Background:
The diagnosis of systemic autoimmune disorders can be challenging due to the huge number of different autoantibody specificities with clinical importance and the diversity of symptoms in patients with suspected systemic autoimmune disease.

Laboratory testing for autoantibodies in serum or plasma is therefore of great value.

The indirect immunofluorescence test (iIFT) on HEp-2 cells is the most frequently used screening procedure. Because of its high negative predictive value this test is used to limit the number of additional tests that have to be ordered on samples; a necessity for effective diagnostics in a field with over a 100 different diseases and more than 160 auto-antigens described. Common practice is to stop further testing if an inflammatory rheumatic disease is suspected and evaluation of ANA screening is negative.

Aim:
Based on routine laboratory analysis of patient samples subjected to ANA screening we evaluated what we could potentially miss if we follow common practice to advice against further testing in case of a negative ANA screening result. The analysis included a mixed patient group with either suspected or unlikely presence of systemic antibodies related to inflammatory and rheumatic diseases.

Material and Methods:
1219 serum samples with negative ANA iIFT readings were subjected to further ANA **specificities** determination using Line Blot and ELISA based methods.

Results: Of those 1219 serum samples 15 were reported positive for LineBlot/ ELISA. About 60% (9/15) of these samples belonged to patients with known autoimmune diseases under therapy or strong clinical suspect of autoimmune disease. In all but two cases, these autoantibodies were directed against SSA antigen. In 40% (6/15) of the samples, repeated testing after several weeks/ months did not confirm persistence of autoantibodies.

Discussion:
Our results indicate that decision to stop testing if the evaluation of the iIFT is negative is reliable in the majority of cases.

Considering that even a positive ANA screening test still requires positive clinical findings for proper diagnosis of autoimmune diseases and since a negative result does not rule out autoimmune diseases the most important finding is not whether we have discrepancies between iIFT and specificity testing but the non-selective indication that has led to ANA testing (e.g. condition following an accident) in some cases. In these cases, follow up was negative several weeks or months after the initial discrepancy.

Conclusion:
ANA iIFT testing to screen for presence of systemic autoantibodies gives satisfactory results provided that clinical aspects are taken into account. The results strongly support the necessity for a clear indication for ordering ANA screening and ANA specifities testing to make the best of this laboratory diagnostic tool.

DGKL-P028

Development of a microarray for the label-free detection of antiphospholipid antibodies

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Background
The antiphospholipid syndrome (APS) is an autoimmune disease with a prevalence of 1-6 % in a normal population. Deep vein thrombosis, repeated miscarriages and myocardial infarction are just some symptoms that can occur during the course of APS. Main antibody targets are cardiolipin and β_2-glycoprotein I (β_2-GPI). Other APS relevant antigens include prothrombin, phosphatidylserine, annexins A5 and A2, protein C and S and tissue plasminogen activator. Due to the heterogeneity in terms of clinical manifestations but also antibody spectrum a definite diagnose of APS is often not straight forward.

Aim
This work describes the establishment of a label-free, optical biosensor to monitor multiple antigen-antibody interactions within a single measurement using “polarized imaging reflectometric interference spectroscopy” (pi-RIfS). It combines several APS relevant antigenic structures, namely β_2-GPI, prothrombin, cardiolipin and a β_2-GPI/cardioliopin complex.

Materials and Methods

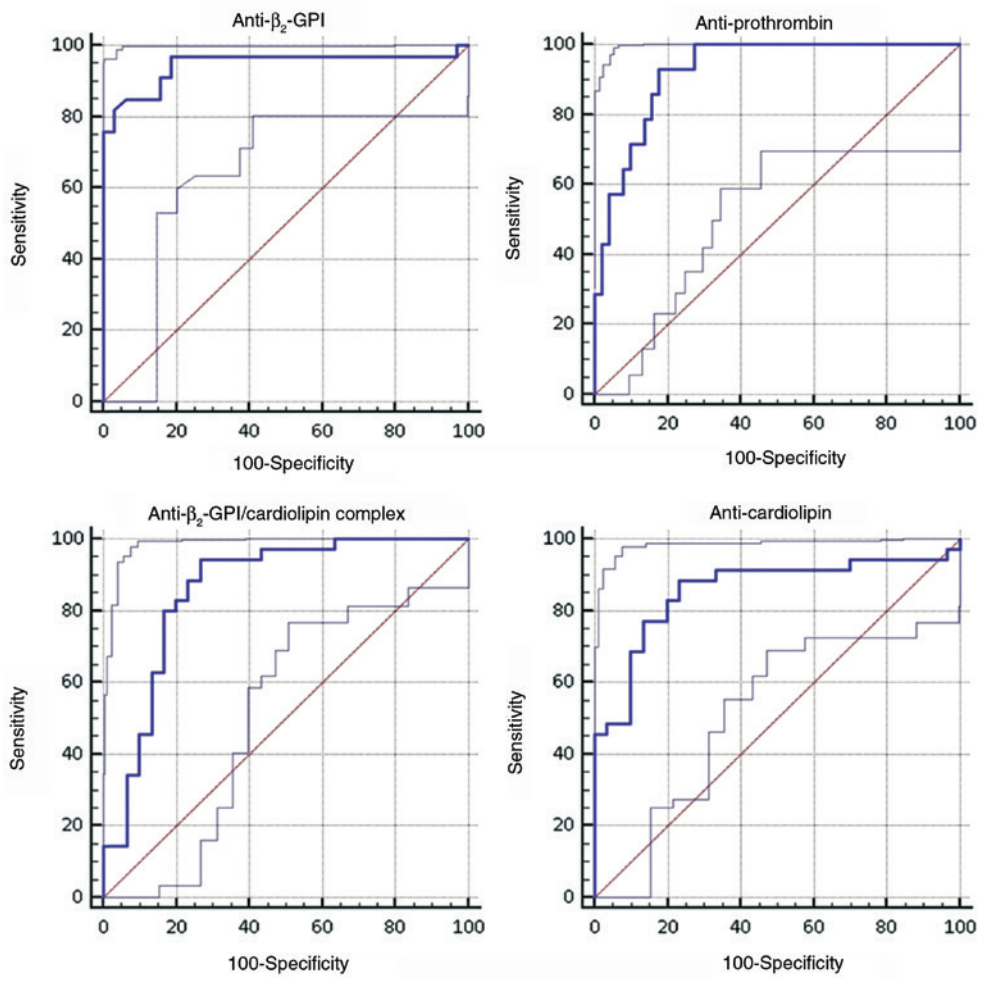
Transducers were modified with di-amino-poly(ethylene)glycol, amino dextran and 11-aminoundecyltrimethoxysilane (11-AUTMS). Antigens were coupled either directly or via biotin-streptavidin interaction. APS patients, SLE patients and healthy controls were measured with the microarray as well as with ELISA systems. Sensitivity, specificity and AUC were calculated with ROC analysis.

Results and Discussion

Covalent immobilization via 11-AUTMS resulted in a sensitive biosensor with attenuated unspecific binding and a clear differentiation of patient and control sera. The detection of four different antibodies could be achieved within one single measurement. For the detection of anti-β_2-GPI and anti-prothrombin the pi-RIfS microarray gave excellent results. Sensitivity was calculated to be 81.82 and 92.86 %, respectively and specificity was 96.87 and 82.35 %. The test for anti-cardiolipin and anti-β_2-GPI/cardiolipin-complex shows lower sensitivity (88.57 and 94.29 %) as well as specificity (76.67 and 73.33 %). Reason might be the hydrophobicity of the cardiolipin which causes stronger background signals than a solely proteinogenic surface coating. Comparing the pi-RIfS data with the respective ELISA assays, it can be concluded that both systems are in good agreement. Especially the detection of anti-β_2-GPI seems to be as valid as the ELISA with a confidence interval (CI) of 0.860-0.987.

Conclusion

The parallel detection of multiple parameters is becoming more and more important, not only in the field of clinical diagnostics. Thereby, real-time assays play a central role among a great variety of readout systems, as they may provide kinetic data for analyte binding. For APS diagnostics, the introduction of further antigens into routine measurements could provide helpful information about the state of the disease in every investigated individual and an identification and characterization of subpopulations of patients.



DGKL-P029

New markers for celiac disease: anti-neo-epitope human and microbial transglutaminases

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Objectives: Microbial transglutaminase (mTg) and human tissue Tg (tTg) complexed to gliadin peptides present neo-epitopes. Antibodies against these complexes are called tTg neo-epitope and mTg neo-epitope. Reliability of antibodies against the non-complexed and complexed forms of both transglutaminases to reflect intestinal damage and to diagnose pediatric Celiac Disease (PCD) was compared.
Methods: 95 PCD patients, 99 normal children (NC) and 79 normal adults (NA) were tested using the following ELISAs detecting IgA, IgG or both IgA+IgG combined: *AESKULISA®* tTg (tTg, RUO), *AESKULISA®* tTg New Generation (tTg neo-epitope (tTg-neo)), *AESKULISA®* mTg (RUO) and *AESKULISA®* mTg neo-epitope (mTg-neo, RUO). Marsh criteria were used for the degree of intestinal injury.
Results: All anti-mTg-neo and anti-tTg-neo levels were higher ($p<0.001$) compared to the single antigens. tTg-neo IgA and IgG+IgA were higher than mTg-neo IgA and IgA+IgG ($p<0.0001$). The antibody activities reflecting best the increased intestinal damage were: mTg-neo IgA> mTg-neo IgA+IgG > tTg-neo IgG ≥ mTg-neo IgG> tTg-neo IgA> tTg-neo IgA+IgG. Taken together, mTg-neo IgG and tTg-neo IgA & IgA+IgG correlated best with intestinal pathology ($r=0.5633$, $r=0.6165$& $r= 0.6492$; $p<0.0001$, $p<0.0001$ & $p<0.0001$ respectively).
Conclusion: The complexed forms of both transglutaminases exhibited a higher OD activity and better reflected intestinal damage in PCD, compared to the non-complexed forms. mTg is immunogenic in children with CD and by complexing to gliadin its immunogenicity and pathology reflection is enhanced.

DGKL-P030

Activity-Based Assays for the Quantification of Serine Proteases of Leucocytes and Mast Cells

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Objective: GASPIDs (granule associated serine proteases of immune defense) form a family of serine proteases intimately involved in the function of the immune system as well as the development of inflammatory disorders. Better characterized GASPIDs include neutrophil elastase and cathepsin G (involved in the antimicrobial defense, but also in the pathophysiology of lung diseases such as COPD and emphysema), proteinase 3 (e.g. target of anti-neutrophil antibodies in Wegeners vasculitis), mast cell tryptases and chymases (which promote allergic inflammation), and lymphocyte granzymes (which contribute to the induction of the caspase cascade in apoptosis). All of these enzymes are expressed as zymogens, activated by the cysteine protease cathepsin C, and stored fully catalytically active in cytoplasmic granules. To quantify these GASPIDs, which form a complex proteolytic network specific for each immune cell, we developed activity-based assays for its key members.
Methods: Various peptide substrates with chromogenic and fluorogenic leaving groups (e.g. AMC, pNA, SBzl) were compared with respect to their ability to detect and quantify the enzymatic activity of isolated GASPIDs. Neutrophil progenitor cell lines (U937 and HL-60) and mast cell lines (HMC-1 and LUVA) were utilized in order to evaluate whether the assays are capable of quantifying GASPIDs in differently prepared crude lysates. Isolated enzymes, selective inhibitors, and immunoblots were used to validate measurements. Practicability of these methods in terms of handling, timing, and effort was also taken into consideration.
Results and Discussion: Considerable optimization of the enzyme assays and methods to prepare crude lysates was required to achieve sufficient specificity, selectivity, and reproducibility. The adjusted assays have proven to be suitable to quantify several proteases in one sample derived from the cell culture in a single microtiter plate well. Thus, this approach appears to be adequate to assess the proteolytic network of leucocytes and mast cells and to examine the effects of modulators of the immune system.
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DGKL-P031

CD63 and CD203c expression during specific immunotherapy (SIT) for wasp venom allergy using Basophile Activation Test (BAT)

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Background : SIT is an established therapy for wasp venom allergy. The aim of our work is to investigate the progression of surface antigen CD63 and CD203c expression during SIT using BAT. The study was approved by the institutional ethical review board.
Methods:
We included 71 patients in our study (61 wasp, 10 honey bee; 19 aborted).

Here we report on 40 adult patients with SIT against wasp venom in course of 3 year with.

Blood samples were collected before, 3 days (3d), 2 weeks (2w) and 6 months (6m) after SIT start (further blood samples will be repeatedly collected every 6 months until 3 years). For all samples we determined CD63 and CD203c expression using BAT after stimulation with various wasp venom concentrations. We evaluated the relative proportion of activated basophile granulocytes at 57µg/l venom concentration (a2) and the calculated concentration c50 to stimulate 50% of total activatable basophile granulocytes.
Results:
Venom stimulation in BAT shows:

CD63 expression (and inversely c50), (n=36), decreased in 29 and increased in 2 patients, while it was constant in 5 cases; Median changes to baseline at 3y were a2=-83% ($p< 0.01$) and c50=668% ($p=0.02$).

CD203c expression (and inversely c50), (n=40), decreased in 29, increased in 6 and did not change in 5 patients. Median changes to baseline at 3y were a2=-82% ($p< 0.01$) and c50=1293% ($p<0.01$).
Conclusion:
Individual progression of CD63 and CD203c in BAT do not show a uniform behavior during the investigated time, but over 80% of the patients show a decreased expression of the surface proteins. Statistically significance can be demonstrated for CD63 and CD203c expression after 3 years. Further work is required to gain inside the correlation with sting challenge.

DGKL-P032

Comparison between the performance of the HELMED® Blot Module and the HELIA® using the AESKUBLOTS® ANA-17 Pro

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Introduction: Immunoblotting is a common method for efficient profile testing of autoimmune and infectious diseases. Automation offers higher throughput testing, therefore AESKU.SYSTEMS developed two solutions to facilitate automated immunoblot testing.
Objective: To compare the performance of the HELMED® Blot Module that is a fully automated Blot processor, and the HELIA®, an automated analyzer for line immunoassays.
Methods: 39 routine samples were tested on the *AESKUBLOTS*® ANA-17 Pro (AESKU.DIAGNOSTICS) utilizing in parallel the HELMED® Blot Module and the HELIA® system (both AESKU.SYSTEMS, Wendelsheim). By performing samples with the HELMED® Blot Module the *AESKUBLOTS*® were analyzed by the AESKU.SCAN® software.
Results: 28 samples were found to be positive for one or more parameters. 2 samples showed equivocal results and 9 were completely negative for all ANA antigens. Overall agreement (concordance correlation coefficient) between the HELMED® Blot Module and the HELIA® system was 0.9476 (95% CI: 0.9216 to 0.9652). Notably, all discordant samples were characterized by very borderline signal. Comparing the level of immunoreactivity of the different coated antigens and sample diversity the Pearson precision (ρ) was 0.9718 (95% CI: 0.9547 to 0.9825; $p<0.0001$).
Conclusions: The HELMED® Blot Module and the HELIA® system are able to identify the ANA positive samples with the same level of band intensity of the coated antigens. Both approaches are able to reduce inter-laboratory variability and time required to perform ANA testing, especially in high throughput laboratories.

DGKL-P033

Elevated MMP-3 levels in early RA patients sera with Borrelia Antibodies

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Aim: To investigate the influence of an infectious disease on Rheumatoid Arthritis (RA), patients from the ADAPTHERA study cohort were screened for antibodies to Borrelia antigens.
Methods: 204 sera from early RA patients with disease duration < 6 months were screened using the following ELISAs (AESKU.DIAGNOSTICS): AESKULISA® Rf-AGM, AESKULISA® CCP-IgG and -IgM, AESKULISA® Borrelia-IgG and –IgM, AESKUBLOT® Borrelia-IgG and -IgM and AESKULISA® DF MMP-3. Statistical analysis was performed using MedCalc V.15.6.1
Results: 10.8% of the patients tested at their 1st visit were positive for Borrelia-specific antibodies. 18/204 patients were positive for IgG or IgM, or both, and 4/204 patients had equivocal results.

5/22 Borrelia positive patients were CCP positive but negative for Rf-AGM. Interestingly, 10/13 patients that were negative for RA parameters had high MMP-3 levels, while only 1/9 of CCP positive patients showed elevated MMP-3 titres.

Measurements were continued in 3 serologically RA negative up to visit 4, only one patient showed CCP positivity. IgM antibody titres for Borrelia antigens remained highly positive and correlated with coexisting high MMP-3 levels. 2/3 patients remained positive in Borrelia Western blot for IgG and negative in RA parameters. 1/3 patients remained positive in CCP and showed negative Western blot results in Borrelia.

Interestingly, classical RA parameters remained negative in most patients which showed high MMP-3 and positive Borrelia outcomes throughout the investigated time period.

Co-measurement of MMP-3 and correlation with further serological markers may assist in improved differential diagnosis and helps to eliminate unnecessary patient therapies, thus saving time, money and improving overall patient care.

DGKL-P034

RELIABILITY OF THE HELIOS® SYSTEM FOR THE EVALUATION OF ANA SCREENING TEST BY INDIRECT IMMUNOFLUORESCENCE (IIF)

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BACKGROUND According to a global statement, IIF technique should be considered as gold standard in anti-nuclear antibodies (ANA) testing. During the last years the biomedical industries developed different automated systems of autoantibody IIF microscope reading and pattern recognition in order to address the high number of IIF method challenges. Aim of the study was to evaluate the reliability of the fully-automated HELIOS® system (AESKU.SYSTEMS, Wendelsheim, Germany) for detection of ANA comparing its diagnostic performances with the traditional visual interpretation of IIF by laboratory experts.
METHODS
A total number of 403 samples with suspected autoimmune diseases were collected for ANA testing. The conventional ANA-IIF analysis was performed by using the HEp-2000 slides from immunoconcept, processed on the instrument PHD (Bio-Rad laboratories) and read in a LED fluorescence microscope. In parallel, the ANA-IIF was carried out by using the *AESKUSLIDES*® ANA HEp-2 (AESKU.DIAGNOSTICS, Wendelsheim, Germany) processed on the fully-automated HELIOS® processor and reader.
RESULT Comparing the HELIOS® system with the routine method a good concordance of 92.1% was found regarding the positive and negative results. 22 samples that were ANA positive in routine and HELIOS® negative, were classified as weak positive (low titer 1/80) and follow-up tests (ENA and dsDNA) were negative. The 10 samples positive on HELIOS® and negative in routine include borderline results.
CONCLUSIONS Our results study showed that the HELIOS® system has proved that it can be used for a cost-effective and accurate ANA screening in a diagnostic laboratory practice.

DGKL-P035

IMMUNOFLUORESCENCE STUDIES FOR THE SPECIFIC DIAGNOSTICS OF MYASTHENIA GRAVIS BY IFA IN THE AUTOMATED HELIOS®

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Objective: Myasthenia gravis is an autoimmune disease that is characterized by muscle weakness and fatigue. It is associated with antibodies directed against e.g. the acetylcholine receptor (AchR) and muscle-specific kinase (MusK) in the postsynaptic membrane at the neuromuscular junction. AchR antibodies (AchRAb) are highly specific and present in 80-90 % of patients, whereas autoantibodies against MusK

(MusKAb) are found in 30-40 % of seronegative patients. Immunofluorescence studies were performed to underline the specificity of AchRAbs while comparing with other autoimmunological syndromes by verifying the performance of the *AESKUSLIDES*_AChR® from AESKU.DIAGNOSTICS in the automated systems for indirect immunofluorescence HELIOS® (AESKU.SYSTEMS).
Methods: In total 80 sera were tested, comprising the following samples: 20 AchRAb, 10 ANA, 10 ENA, 10 SLE, 10 MusK and 20 blood donors. All were tested on the automated system HELIOS®.
Results: Our data show that 19 out of 20 AchRAb sera showed AchR autoantibody binding with *AESKUSLIDES*_AChR®. Interestingly, all MusK positive sera and healthy blood donors were negative. ANA, ENA and SLE sera showed different fluorescent patterns.
Conclusions: *AESKUSLIDES*_AChR® showed a very good distinction of AchRAbs among MusK, ANA, ENA, SLE and healthy blood donors' sera. Thereby, differences were expressed in patterns belonging to the cytoplasm, nuclear and plasma membrane.

DGKL-P036

MULTICENTER STUDY OF THE ANTINUCLEAR ANTIBODY VALIDATION (ANA) BY IFA IN THE AUTOMATED HELIOS® AND ANTIBODIES ANTI nDNA, LKS AND ANCA IN THE HELMED®

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Objective: To verify the performance of the *AESKUSLIDES*® ANA HEp-2, *AESKUSLIDES*® ANCA, *AESKUSLIDES*® nDNA and *AESKUSLIDES*® rLKS from AESKU.Diagnostics in the automated systems for indirect immunofluorescence HELMED® and HELIOS® (AESKU.Systems).
Methods: In total 436 samples were tested at three different reference laboratories in Colombia, comprising the following samples: 131 DNA, 177 ANA, 64 ANCA and 64 LKS. All were tested on the automated systems HELMED® (DNA, ANCA and LKS) and HELIOS® (ANA). In addition, reading of ANA slides was performed by HELIOS® and manually by microscope. The results were compared to the daily-routine-method used in which samples were run on the PHD system with Bio-Rad reagents.
Results: ANA samples had a correlation between 91.9% and 98.3%. A comparative titer discrepancy of 1 dilution was observed in 1.7% to 3.6% of the samples. Pattern differences were focused on non-observation of patterns on both sides and were found in 7.1% to 18.6% of the samples. 1.8% to 5.1% of the differences were expressed in patterns belonging to the cytoplasm.
A concordance of 100% was obtained for DNA and for ANCA, for LKS it was 90.0% to 96.2%.
Following Kappa indexes were calculated: ANA 0.84-0.96, DNA 1.0, ANCA 1.0, and LKS 0.90-0.94. Interpretation of the kappa index is very good for all tests.
Conclusions: Performance of HELIOS® and HELMED® showed a very good correlation to the standard method in all laboratories for all tests. Thereby, both systems were able to simplify the daily routine workflow regarding their process speed, precision, reliability and efficiency.

DGKL-P037

High Sensitive Detection of Anti-DFS70/LEDGF by Radioimmunoprecipitation Assay - Diagnostic Implications

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Autoantibodies against the chromosome associated protein DFS70/LEDGF (dense fine speckles, lens epithelium derived growth factor) are increasingly being regarded as biomarkers excluding systemic autoimmune rheumatic diseases (SARD). Their presence, presumed because of their immunofluorescence (IF) pattern seen in IF-ANA can be confirmed by specific tests (ELISA, CIA, Western blot), an approach likely prone to the underestimation of their prevalence. Hence we studied the prevalence of anti-DFS70 by means of high sensitive radioimmunoprecipitation assays (RIPA).
Methods: Left-over sera submitted for routine ANA-IIFT (1100) or for basic clinical chemistry tests (350), stored at -18 °C for three months, were assayed in one session each by RIPA, ELISA and IIFT. Antigens used in RIPA were ^{35}S-methionine labelled full-length (FL-) DFS70 and its C-terminal fragment (CT-DFS70, aa 323 - 530) generated by *in vitro* transcription/translation of cDNAs identical with the canonical sequence. Antibody bound radioactivity precipitated by protein A sepharose was measured by liquid scintillation counting. Antibody concentrations (Ab-ratio) were calculated (Frey et al. 1998) based on 30 controls (confidence level 99.0 %). The RIPA specificity was proved by absorption of positive sera with affinity-purified CT-DFS70 expressed in *E. coli*. For ELISA the anti-DFS70-ELISA (Euroimmun) was used. Positive sera were

subjected to IF-ANA on HEp-2 cells (INOVA) with chessboard titrated conjugates (Jackson Laboratories), to FARR-assay and ENA-testing. For RIPA an Ab-ratio of > 15.0, for ELISA a cut off of > 1.0 and for IIFT a titer of 1: ≥ 80 were regarded as positive. The routine IF-ANA results were not communicated.

Results: The prevalence of anti-DFS70 measured by RIPA was 10 % (n = 110) in sera submitted for routine ANA-screening and 12 % (n = 42) in sera received for basic clinical chemistry tests, figures which were considerably higher than the prevalence of 5.0 % and 2.6 %, resp. found by ELISA. In both series antibodies reacting with the CT-DFS70 (80.5 % and 76.7 %, resp.) prevailed the anti-FL-DFS70 (70.9 % and 58.1 %, resp.). RIPA specificity was confirmed in all sera by absorption (mean absorption rate 68 %) with CT-DFS70. The majority of sera harbouring high Ab-ratios (> 90) reacted with both antigens, significantly different to sera showing low Ab-ratios (< 45), which preferentially were monospecific for either CT-DFS70 or FL-DFS70. There was a significant correlation of RIPA reactivity and ANA titer inasmuch as in low titer sera (1: < 160) the Ab-ratio averaged Ab-ratios of about 40, increasing to rations of > 90 in high-titer sera (1: 320 – 1:1280) exhibiting a speckled IF pattern. No correlations existed between ANA-titer and cut off values measured by ELISA. Low anti-DFS70 concentrations (Ab-ratio < 35) were also seen in 13 sera negative for ANA by IIFT (titer 1:< 80). Anti-dsDNA was present in 14 (12.7 %) of RIPA positive sera, the concentration of which was inversely correlated to anti-DFS70 Ab-ratios. SARD markers like anti-Ro, -SSA. -U1snRNP, -Ribosomes were found in 7 of the anti-dsDNA positive sera and in 7 more RIPA positives, resulting in an overall presence of SARD markers in 20.9 % of the sera submitted for ANA-screening.

Conclusion: The armamentarium for detection of anti-DFS70 commonly used in routine ANA-screening omits a considerable number of anti-DFS70 positive sera, the significance of these antibodies in the context of SARD has to be revisited in prospective studies.

DGKL-Inflammation

DGKL-P038

In vivo measurement of microglial activity in a mouse model of chronic kidney disease

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Cognitive impairment and dementia are strikingly increased in chronic kidney disease (CKD) patients of all stages compared to the general population. The induction or enhancement of neuro-inflammation may be involved in impairing the cerebral function in these patients. Using a mouse model of CKD, we indeed found neuronal dysfunction coinciding with microgliosis within the murine brain. In microglia, we observed activation of the inflammasome.

We supposed microglial behavior to be altered after inflammasome activation. Therefore, we established *intravital* microscopy in transgenic mice, which express EGFP under the CXC3R1 (fractalkine receptor) promoter to label microglia. Using this model, we aimed to investigate CKD-related alterations of microglial behavior due to inflammation *in vivo*.

We found significant increase of microglial phagocytic cups in uremic mice when compared to sham-operated control mice, indicating increased phagocytic activity coinciding with CKD. Moreover, in CKD mice we observed significant increase of process motility of ramified microglia with respect to process retraction as well as extension to local cell destruction suggesting an increased potential of microglial activation.

These results provide for the first time insights into CKD-driven alterations of microglia- activity coinciding with inflammasome activation. This observation further underpin our hypothesis that neuro-inflammation plays a crucial role in CKD-related impairment of brain function, implicating novel therapeutic intervention strategies.

DGKL-P039

The atypical inhibitor of NF-?B, I?B?, contributes to the anti-inflammatory response via regulation of interleukin-10 expression in macrophages

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IκBζ belongs to the family of atypical inhibitor of NF-κB proteins and induces the transcription of diverse pro-inflammatory cytokines, such as IL-6, IL-12 and CCL2 in macrophages. Contrary to expectations IκBζ-deficient ($Nfkbiz^{-/-}$) mice develop a pro-inflammatory phenotype. Intrigued by these contradictory findings we performed gene expression analyzes of peritoneal and bone marrow-derived macrophages from IκBζ-deficient and wild-type mice in order to find new IκBζ target genes which can explain the pro-inflammatory phenotype of $Nfkbiz^{-/-}$ mice.

As one of the genes dysregulated in IκBζ-deficient macrophages we identified interleukin 10 (IL-10). In the presence of pro-inflammatory agents such as lipopolysaccharide or peptidoglycan IκBζ-deficient macrophages expression of IL-10 mRNA and protein is diminished. In contrast to previously described pro-inflammatory target genes of IκBζ the regulation of IL-10 expression is independent of both the macrophage subtype and the status of macrophage polarization. Confirming these results ectopic expression of IκBζ in the macrophage cell line RAW264.7 efficiently induces transcription of Il10. Chromatin immunoprecipitation experiments revealed IκBζ-mediated trimethylation of histone H3 at lysine K4 at the proximal region of the Il10 promoter. Consequently, IκBζ induces IL-10 expression by acting as a transcriptional activator. In summary, our findings show for the first time the IκBζ-dependent expression of an anti-inflammatory cytokine, IL-10, that is crucial in regulating immune responses. The newly identified function of IκBζ might open up new approaches for the treatment of chronic inflammatory diseases.

DGKL-P040

Regulation of long term TNF incubation-dependent constitutive signalling in primary human monocytes by A20 and adjuvant proteins

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Objective:
Controlled termination of inflammation is the base for the exact regulation of inflammatory events. Dysregulation of this process may result in severe diseases such as acute/chronic inflammatory diseases or immunparalysis. Due to the complexity of inflammation-related signalling, the regulation of the involved mechanisms is still not completely understood.
Methods:
Using primary human monocytes and the monocytic cell line THP-1, constitutive signalling was monitored *via* IL-8 mRNA expression or IκBα degradation as a read-out. Long term TNF incubation-associated mechanisms were further analysed using siRNA, western blot, kinase inhibitors, and FACS analysis.
Results:
Following long term TNF incubation for 12-48 h, a phase termed as phase III of TNF stimulation, a slightly induced level of intracellular signalling was observed (represented by increased IL-8 mRNA and reduced IκBα protein levels). Specific inhibition of TAK1 and IKKβ restored IκBα levels during TNF long term incubation indicating an involvement of NF-κB-associated events. The microarray-based expression analysis of NF-κB depended genes revealed a strong induction of TNFAIP3 (A20) and TNIP1 (ABIN1), but also IL-8 and IκBα mRNA under these conditions. An initial reduction of the A20 protein level, suggesting a MALT1-dependent cleavage, was found in phase I (i.e., following TNF incubation up to 2 h), followed by a strong increase of A20 protein and mRNA levels in phase III. Knockdown of A20 during long term incubation (48 h) showed an increase of IL-8 mRNA expression and IKKβ phosphorylation in combination with a decreased RIP1 degradation. Equivalent effects were obtained following knockdown of the adjuvant proteins ABIN1 and CYLD, which were also induced in phase III.
Conclusions:
Following TNF long term incubation for 48 h, constitutive signalling in human monocytes was observed. Mechanistically, this signalling is strongly controlled by A20-dependent restriction of IKKβ phosphorylation in combination with the degradation of RIP1. A20 adjuvant proteins such as CYLD also appear to be involved in the regulation of constitutive signalling. Taken together, our results revealed a new type of TNF-induced signalling and its regulation, potentially playing an important role in the resolution of inflammation.

DGKL-P041

Effects of Elastase Inhibitors on Neutrophil Granulocytes and Precursors

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Objectives: Neutrophil granulocytes play a key role in the innate immune defense but also in the development of inflammatory disorders. Human neutrophil elastase (HNE), a serine protease selectively expressed by neutrophils, takes part in the killing of bacteria, that have been phagocytosed or trapped in neutrophil extracellular traps. Excessive HNE release, however, or its uncontrolled extracellular activity (e.g. due to the consumption of inhibitors such as alpha-1-PI) contributes to the development of inflammatory and degenerative disorders, most prominently chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS) and rheumatoid arthritis. In order to control excessive HNE actions, a multitude of synthetic inhibitors have been designed over the past decades and both small-molecule and

naturally occurring inhibitors have been investigated in phase III clinical trials. However, the effects of HNE inhibitors on neutrophil granulocytes and their precursors have not been studied systematically.
Methods: An *in vitro* cell culture model was established utilizing human neutrophil precursor cell lines and isolated neutrophils. HNE activity was quantified by fluorescent peptide substrate-based assays, that were validated using isolated HNE, selective protease inhibitors and immunoblot. Enzyme kinetic measurements were performed to determine the affinity and reactivity (K_i, k_{obs}/I) of inhibitors towards HNE.
Results and conclusion: HNE inhibitors differed largely in their ability to inhibit intracellular elastase compared to the isolated protease. The reduced potency on intracellular HNE likely reflects the stability of inhibitors and their ability to penetrate neutrophil granules. The information gained by comparing results from the cell culture assay with enzyme-kinetic data is essential to understand the effects of HNE inhibitors *in vivo* and may foster the design of targeted inhibitors for therapeutic use.

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DGKL-P042

Ärzte ohne Grenzen e.V. - Wer wir sind, was wir tun

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ÄRZTE OHNE GRENZEN ist eine private, internationale, medizinische Hilfsorganisation. Die Organisation hilft Menschen, die durch (Bürger-) Kriege oder Naturkatastrophen in Not geraten. ÄRZTE OHNE GRENZEN gewährt diese Hilfe allen Opfern, ungeachtet ihrer ethnischen Herkunft, politischen oder religiösen Überzeugung. Im Namen der universellen medizinischen Ethik und des Rechts auf humanitäre Hilfe arbeitet ÄRZTE OHNE GRENZEN neutral und unparteiisch und fordert ungehinderte Freiheit bei der Ausübung ihrer Tätigkeit. Darüber hinaus engagiert sich die Organisation als Sprachrohr für Menschen in Not. Die Mitarbeiter beziehen öffentlich Stellung, wenn sie selbst Zeugen von massiven Menschenrechtsverletzungen oder schweren Verstößen gegen das humanitäre Völkerrecht werden.

Jährlich arbeiten etwa 2.000 internationale und rund 20.500 nationale Mitarbeiter von ÄRZTE OHNE GRENZEN in mehr als 65 Ländern. Ihre Kompetenz und ihre Einsatzbereitschaft für Menschen in Not wurden 1999 mit dem Friedensnobelpreis geehrt.

Die Aktivitäten sind dabei sehr vielfältig: Wiederaufbau und Inbetriebnahme von Krankenhäusern oder Gesundheitszentren, mobile Kliniken zur Versorgung von ländlichen Gebieten, Impfprogramme, medizinische Versorgung in Flüchtlingslagern, psychologische Betreuung, Aufbau von Ernährungszentren, Wasser- und Sanitärprojekte sowie Gesundheitsversorgung von besonders gefährdeten Gruppen (z.B. Straßenkinder, Slumbewohner). Wir arbeiten in allen Projekten mit nationalem Personal zusammen und legen Wert auf die Fort- und Weiterbildung der einheimischen Mitarbeiter. Ärzte ohne Grenzen e.V. arbeitet in Kriegs- und Krisengebieten, die unter anderem von verschiedenen Epidemien betroffen sein können. Epidemiologen geben den medizinischen Mitarbeitern eines Projekts Hilfestellung im Projektaufbau, Projektverlauf und bei der Evaluierung verschiedener Programme. Zudem unterstützen sie den Aufbau von Frühwarnsystemen in potentiellen Epidemiegebieten, deren ausgewertete Daten eine Epidemie frühzeitig erkennen lassen und so nötige Maßnahmen schnell eingeleitet werden können.

DGKL- Infectious Diseases

DGKL-P043

Early and Rapid Detection of Chlamydia trachomatis Serovars L1-L3 (Lymphogranuloma venereum) by Realtime PCR

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Background and objectives. Lymphogranuloma venereum (LGV) is a rare sexually transmitted disease which is caused by *Chlamydia trachomatis* serovars L1-L3. The disease is endemic in parts of Africa, South-East Asia, Latin America and the Caribbean. Since 2003 the disease has been increasingly reported also in developed countries, mostly affecting homosexual men (Men who have sex with men, MSM) of which most are also coinfected with syphilis, HIV and HCV (Serovar L2b).

In 2007 the Robert Koch-Institute stated in a nationally published bulletin (HIV & more 2/2007) a fivefold increase of confirmed LGV infections per month in Germany between 2004 and 2007.

The diagnosis is difficult to establish on clinical grounds alone. Early and accurate diagnosis and treatment are essential not only to cure the infection and prevent further damage to tissues but also to prevent further severe infections with possibly irreversible sequels. In contrast to *C. trachomatis* infections caused by serovars A-K which can be treated with doxycycline over a period of one week, infections caused by serovars L1-L3 require a prolonged treatment with doxycycline for three weeks.

We established a realtime PCR based on published primers and probe for L1-L3 that can easily be implemented in every day's diagnostics.

Materials and methods. Over a period of 23 months we tested 511 specimens (urogenital and anal swabs) for the presence of *C. trachomatis* using a commercial PCR system (GeneXpert, Cepheid). Samples derived from 323 male patients from the age of 17 to 78 years (median 38 years) who report to our HIV polyclinic at regular intervals. As a second step all *C. trachomatis* positive samples were extracted using a semi-automatic nucleic acid extraction platform (Maxwell16, Promega). Specific DNA of *C. trachomatis* serovars L1-L3 was later on amplified in a RotorGene6000 realtime cycler. The runtime of the PCR was approximately 2 hours. As positive control DNA of *C. trachomatis* serovars L1-L3 purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig) was used (DSM10192).
Results. 60 samples (11.7%) were tested positive for the presence of *C. trachomatis* and 450 (88.1%) were tested negative. The result of one sample (0.2%) was invalid.

Five samples (8.3%) were tested positive for the presence of *C. trachomatis* serovars L1-L3, 54 (90%) were tested negative and one sample (1.7%) was invalid.
Conclusions. Our results demonstrate that even though rare, establishing an in-house realtime PCR for the early detection of *C. trachomatis* serovars L1-L3 on-site is reasonable in order to start early and appropriate treatment in such a high-risk patient collective.

DGKL-P044

Comparison of the carbapenemase gene detection methods Check-Direct CPE and the in-house RT-PCR in clinical isolates

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Background: Carbapenemase-producing Gram-negative bacterial strains are increasing globally and turn out to be a challenge in health care facilities. Rapid diagnostic tests for the accurate detection of carbapenem-resistant bacteria are clinically relevant for better therapy options and infection control measures. This study aimed to establish and evaluate a multiplex real-time PCR assay for the simultaneous detection of carbapenemase gene variants in Gram-negative rods. Furthermore, a comparison of the in-house real-time PCR and the Check-Direct CPE assay was performed.
Methods: 114 carbapenem-resistant *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates were collected during a 2-year period. The Check-Direct CPE assay and the in-house RT-PCR were performed to detect the carbapenemase genes.
Results: The analysed bacterial isolates were PCR positive either for KPC, VIM, NDM or OXA variants. The Check-Direct assay as well as the in-house RT-PCR detected the carbapenemase genes of all investigated bla_{KPC}, bla_{NDM}, and bla_{VIM} isolates. The in-house RT-PCR detected 53 of 67 (79.0 %) and the Check-direct assay 30 (44.8 %) bla_{OXA} gene variants.
Conclusion: The identification of bla_{OXA-23} variant remains as one of the challenging issues due to the predominance of this OXA type. The in-house established RT-PCR assay is a cost-effective and alternative assay for the rapid detection of carbapenemase genes in Gram-negative bacteria. The assay identified all KPC, NDM and VIM isolates correctly from solid media as well as directly from positive blood culture vials, but has limitation in the detection of the OXA-type carbapenemases $bla_{OXA-40, -66, -72}$ and $_{-164}$. However, as the comparison revealed the commercial Check-Direct CPE kit has limitation for the detection of oxacillinases (OXA) as well. Moreover, this assay was not able to detect $bla_{OXA-40, -58, -66}$ and $_{-164}$.

DGKL-P045

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

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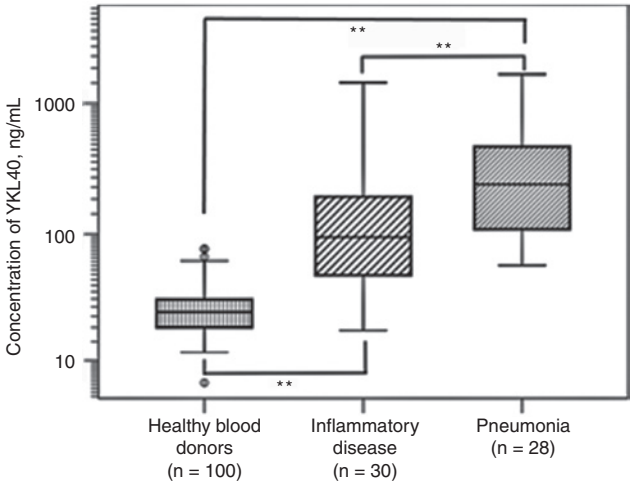
Background
Pulmonary inflammatory diseases such as pneumonia and COPD are among the leading causes of death in industrialized countries. New and more specific inflammation markers could aid diagnosis and monitoring of these diseases. Chitinase-3-like 1 (Chi3l1 or YKL40) is a 40 kDa-sized chitin- and heparin-binding lectin with a proposed role in inflammatory disease. It is secreted by macrophages and epithelial cells directly in the inflamed tissue. Some findings indicate it may have special relevance in pulmonary disease.
Subject
Estimating the diagnostic relevance of YKL40 compared CRP concentrations in sera from patients suffering acute pneumonia.

Methods

Measurement of CRP (Cobas) and YKL40 (R&D Systems) serum concentrations in three collectives: a) Healthy blood donors (n=100); b) Patients with non-pulmonary inflammatory disease (n=30); c) Patients with radiologically confirmed pneumonia (n=28). The study was approved by the local ethics committee, and all patients gave informed consent.



The inflammatory and pneumonia collectives did not differ significantly in gender (37 % female vs. 43 % female), age (66 ± 13.4 a vs. 70 ± 12.7 a; p = 0.488) or CRP serum concentration (11.6 ± 11.4 mg/dL vs. 13.2 ± 8.5 mg/dL; p = 0.86). The collectives, however, differ significantly with respect to the YKL40 Serum concentrations (213 ± 426 vs. 405 ± 301 ng/mL; p < 0,005). Patients with inflammatory disease had approximately 10 times elevated YKL40 compared to healthy controls (27.8 vs. 213 ng/mL), but pneumonia patients had the highest YKL40 serum values (405 ng/mL). YKL40, but not CRP can distinguish between other inflammatory disease and pneumonia (AUC = 0.73 vs. 0.51). This makes it a promising marker for POC pneumonia diagnostics [1]

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DGKL-P046

Unyvero, a Multiplex PCR Assay for Respiratory Specimens: A Comparison with Culture

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Background and objectives. Hospital acquired pneumonia is a frequent and severe complication in intensive care unit patients with a mortality rate between 25 to 50%. Early and appropriate antibiotic treatment improves prognosis. However, conventional microbiology is time-consuming. At best, culture plus susceptibility results are available 48h after the sample was taken if the pathogen can be cultured at all. The Unyvero Application (Curetis AG, Holzgerlingen) is a semi-automated microbiological analysis system that combines sample preparation and qualitative pathogen detection in a disposable cartridge. The system can currently detect 20 pathogens and 17 antibiotic resistance markers in eight parallel multiplex end-point PCR reactions followed by hybridization onto an array. Time-to-result is below five hours with minimal hands-on-time. The aim of the study was to evaluate the performance of this new assay and to assess the agreement between the Unyvero Application and culture.

Materials and methods. Over a period of seven months we have analyzed 146 respiratory specimens of 116 critically ill patients (61% male, 39% female) from the age of 3 months to 85 years (median= 61.5 years). For the Unyvero Application all specimens were processed according to the recommendations of the manufacturer.

Results. In general, the results of 142 of 146 cartridges could be evaluated. Four cartridges did not give any valid results due to technical failure. 52 specimens (36.6%) were positive by the Unyvero Application and 90 specimens (63.4%) were negative. In contrast 32 specimens (22.5%) were positive by culture and 110 specimens (77.5%) were negative. Sensitivity of the assay compared to culture was 91%, the negative predictive value was 97%. Detection rate of the assay was significantly higher than detection rate of culture (79 pathogens by Unyvero vs. 38 pathogens by culture), especially for pathogens that are sensitive to environmental influences (e.g. Streptococcus pneumoniae, Haemophilus influenzae). Time-to-result of the assay ranged from 5 hours to 94 hours (median 7.25 hours).

Conclusion. The Unyvero Application is a useful diagnostic tool for the early detection of pathogens in respiratory specimens from critically ill patients

DGKL-P047

Phagocytosis of Streptococcus gallolyticus subsp. gallolyticus by monocyte-derived macrophages

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Introduction: *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG) is an inhabitant of the human gastrointestinal tract and a facultative pathogen. SGG has been identified as pathogen of infective endocarditis in about 20 % of streptococcal-induced cases. It is assumed that SGG translocates through colonic malignancies in the bloodstream and is then able to induce an infective endocarditis. For a better understanding of pathogen-host interactions, the phagocytosis of SGG by monocyte-derived macrophages (MDM) from individuals was compared to the phagocytosis by PMA-treated THP-1 cells.
Methods: To acquire MDMs, cells from buffy coats of two human blood donors were separated by density centrifugation with Biocoll (Merck). Monocytes were isolated from these peripheral blood mononuclear cells (PBMC) with the Pan Monocyte Isolation Kit (Miltenyi Biotec). Further, the monocytes were stimulated with macrophage-colony stimulating factor (Miltenyi Biotec) in RPMI1640 (Gibco) for 8 days. The purity of the monocyte cell fraction and the differentiation of the monocytes to macrophages were analyzed by flow cytometry. The monocyte cell line THP-1 was differentiated in RPMI1640 supplemented with PMA (Sigma) into macrophages. In both cell culture models, two SGG isolates were added (MOI=5) and phagocytic uptake was enabled for 30 min. Cells were washed and incubated in medium with antibiotics for at least 20 min. Cells were lysed (0 h; 24 h) and vital bacteria were quantified by plating assay. Furthermore, after 24 h the IL-6 concentration in the supernatant was determined by ELISA (Thermo Fisher).
Results: In THP-1 macrophages 70 % of the initial added inoculum of the SGG isolate BAA-2069 was phagocytized whereas the uptake of the isolate UCN 34 was only 43 %. After 24 h incubation 1.5–4.5 % viable bacterial cells were found in PMA-treated macrophages. In contrast, only 44 % of the isolate BAA-2069 and 25 % of the isolate UCN 34 were phagocytized through MDMs. No viable bacterial cells were detected after 24 h of incubation in this macrophage model. After stimulation with SGG isolates high IL6 concentrations were measured in the supernatant of MDMs (BAA-2069: 150 pg/ml; UCN 34: 183 pg/ml). In the supernatant of THP-1 macrophages the IL6 concentration was considerably lower. The stimulation by BAA-2069 and UCN 34 led only to a slight increase of IL6 secretion (37 pg/ml and 41 pg/ml, respectively).
Conclusion: Comparable strain-dependent differences in phagocytic uptake were shown independent from the cell culture model used. However, the overall uptake is higher in THP-1 macrophages. In contrast, the inflammatory response in MDMs is considerably higher compared to PMA-treated macrophages.

DGKL-P048

Screening of patients for Methicillin-resistant Staphylococcus aureus (MRSA) in a Hospital in Saxony - Germany

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Background: MRSA is especially of importance in hospitals and nursing homes. Patients carrying open wounds, invasive devices or bearing a weakened immune systems are at greater risk of nosocomial infections. The screening upon hospital admission prevents the cohabitation of MRSA carriers with non-carriers.
Objective: Determining the ratio of MRSA-positive patients upon admission to hospital.
Material and Methods: We conducted a retrospective study among patients admitted to the two hospitals of Oberlausitz-Kliniken gGmbH (Saxony, Germany) with specific comorbidity risk factors such as patients with a known history of MRSA, patient transfers from other health centers, patients with chronic skin lesions, patients from foreign hospitals and patients undergoing dialysis. Data for age, sex, inpatient unit and comorbidity risk factors were collected. Moistened swabs (COPAN Transystems) were used to collect material from patients anterior nares, throat, inguinal and other positions. The swabs were inoculated directly onto BBL chromagar MRSA II (BD), Agar CNA (BD) and BH Infusion (Oxoid). Identification of MRSA-isolates was done on the basis of colony characteristics, identification and antibiogram by MicroScan WalkAway®System (Siemens). Samples without growth after 48 hours were considered negative.
Results: A total number of 845 patients at high risk of MRSA-colonization was studied of which 69 patients were MRSA-positive. The mean age of patients was 71,7 years. MRSA-positive patients were assigned to the units: internal medicine (66,7%), surgery (30,5%), pediatrics (1,4%) and intensive care unit (1,4%). 39,2% of the patient had chronic skin lesions, 36,2% were patients from external centers, 15.7% were dialysis-patients and 8,7% were patients with a known history of MRSA.

Four out of 69 MRSA-positive patients were classified as nosocomial MRSA cases (nosocomial in this setting means, samples were collected later than day 3 of hospital stay). The mean age of patients was 73,5 years. 50% of these cases were detected in internal medicine and 50% in surgery unit.

Conclusions: The percentage of MRSA-positive patients among patients with a high risk was 7,7% and the proportion of nosocomial MRSA cases in MRSA-positive patients was 5,8%. This indicates that 94,2% of MRSA-positive patients were positive upon admission. Consequently, the control of nosocomial infections is in responsibility of a multidisciplinary team including medical units, laboratory and microbiologists, hygiene and infection control. Thus, a proactive surveillance during admission to hospital should be considered in patients of high risk groups for MRSA colonization.

DGKL- Cardiovascular Diseases

DGKL-P049

Activated protein C protects against accelerated atherosclerosis in diabetes by restricting p66shc expression in macrophages

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Objective
Atherosclerosis is two- to four-fold increased in diabetic patients. The mechanisms of accelerated atherosclerosis in diabetes remain poorly defined. As plasma levels of activated protein C (aPC), a protease known for its cytoprotective effects, are reduced in diabetes we hypothesized that aPC protects against diabetes induced atherosclerosis.
Methods
Eight weeks old control ($ApoE^{-/-}$) mice on a pro-atherogenic background were made diabetic (DM) or fed high fat diet (HFD) for 20 weeks. Supplementary *in vitro* studies with macrophages were conducted.
Results
Diabetic $ApoE^{-/-}$ mcie displayed smaller but less stable plaques with more macrophages and less smooth muscle cells (SMC; immunohistochemical analyses) compared to HFD $ApoE^{-/-}$ mice. Expression of $p66^{Shc}$ and CD36 was increased in macrophages, but not in SMCs isolated by laser dissection from plaques of DM $ApoE^{-/-}$ mice as compared to HFD $ApoE^{-/-}$ mice. *In vitro* glucose induces $p66^{Shc}$ and CD36 expression in macrophages. Glucose induced $p66^{Shc}$ expression remains high even if normoglycemia is restored, suggesting epigenetic control of $p66^{shc}$. Treatment with aPC normalized $p66^{Shc}$ expression and strongly induces expression of DNMT-1. Furthermore in macrophages glucose induced Proinflammatory cytokines (IL-6, TNF-alpha) expression and oxLDL uptake remains high even if normoglycemia is restored which is again reversed by aPC. Expression of $p66^{shc}$, CD36, and DNMT1 is increased in plaques of DM $ApoE^{-/-}$ mice compared to $ApoE^{-/-}$ HFD mice (immunohistochemical analyses). Bone marrow transplantation from $p66^{shc-/-}$ into ApoE-/- revealed complete protection from glucose induced atherosclerosis.
Conclusion
These data shows that aPC epigenetically reverses glucose induced sustained expression of $p66^{Shc}$, IL-6, TNF-alpha and oxLDL uptake in macropages, identifying a novel mechanism underlying aPC dependent vascular protection. aPC based therapies may be a useful therapeutic adjunct for the treatment of atherosclerosis in diabetic patients.

DGKL-P050

Evaluation of the impact of physical activity on the 99th percentile in two high sensitivity cardiac troponin assays

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<u>*Background*</u>: Determination of cardiac troponin (cTn) in the blood of patients is one central means in the diagnosis of acute myocardial infarction (AMI)[1]. In view of potential underdiagnosis of AMI in women, sex-specific upper reference limits (URL) for decision are suggested[2]. In a previous evaluation of three contemporary sensitive cTnI assays in the same reference population, a sex-specific URL only in one assay was found[3]. The detailed analysis revealed significant impact of physical activity and creatine kinase (CK) activity, as surrogate marker for it, on

measured cTnI levels. For this contemporary cTn assay adjustment for CK activity abolished sex-differences in the URL, implicating cross-reactivity with a factor related to physical activity.
Objective: Evaluation of assay performance of two high sensitivity (hs) cTn assays in one large reference population and examination of the influence of physical activity on the URL.
Methods: The DONOR SHIP cohort comprised 2593 healthy blood donors recruited in 2005 at the University Medicine Greifswald. CK activity has been determined before on the Dimension RxL. cTnT and cTnI were determined by means of the hs-cTnT assay on cobas e411 (Roche) and the ARCHITECT *STAT* hs-cTnI assay (Abbott), respectively. All participants with valid measurements of CK activity, cTnT and cTnI were included. Statistical analysis was performed by quantile regression.
Results: Analysis of the hs-cTnT assay included 2206 individuals. The 99th percentile was estimated at 11 ng/L (95%-confidence interval [CI]: 9.4-12.6 ng/L). Age had no influence, but sex was significantly affecting cTnT levels resulting in sex-specific URL of 14 ng/L for men and 7.6 ng/L for women (p=0.019). CK activity was positively associated with cTnT in both sexes (p<0.001 each). Adjustment for CK activity eliminated sex-differences for the URL resulting in an adjusted URL of 9.4 ng/L (95%-Ci: 8.4-10.3 ng/L). 2286 individuals were included in the analysis of the hs-cTnI assay resulting in a URL of 687 ng/L (95%-CI: 539-834 ng/L). No significant sex- or age-effects were detected.
Discussion: Though applied to the same reference population both hs-cTn assays differed considerably. Only for the hs-cTnT assay sex-differences and association with CK activity were apparent. However, adjustment for CK activity diminished the influence of gender. These findings implicate for the hs-cTnT assay cross-reactivity with a factor increased upon physical activity. The suspected candidate would be skeletal troponin (sTn)[4]. Alignment of the amino acid sequences of cTnT and sTnT shows high homology between the proteins especially in regions including epitopes for capture and detection antibodies utilized by the hs-cTnT assay with 100% and 91% homology, respectively. Epitopes for antibodies employed in the hs-cTnI assay are located in regions with <25% homology between cTnI and sTnI. Thus cross-reactivity with sTnT might introduce relevant bias to the hs-cTnT assay.

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DGKL-P051

Characterization of CD248 (TEM1) during diabetic nephropathy

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Diabetic Nephropathy (dNP) is the most common cause of chronic kidney disease worldwide and a growing public health challenge. Although mesangial cells (MC) are suggested to play a pivotal role during the initiation and progress of dNP, molecular structures targeting the MC for therapeutic interventions remain unknown and the intercellular interactions between MC and other glomerular cells remain poorly understood. To characterize the role of the mesangial specific transmembrane glycoprotein TEM1 during dNP we investigated expression of TEM1 *in vivo* in diabetic mice and *in vitro* in glucose stimulated murine mesangial cells. We found that TEM1 expression is upregulated in glomeruli of diabetic mice as well as in glucose stimulated mesangial cells.

To investigate whether the modulation of TEM1 is of functional relevance for development of dNP, we analyzed renal function and mesangial expansion in non-diabetic and diabetic wild-type and TEM1$^{-/-}$ mice after 26 weeks of streptozotocin induced diabetes. Blood, urine and kidney samples were obtained from diabetic animals and age-matched non-diabetic control littermates. Blood glucose levels were comparable in diabetic wild-type and TEM1$^{-/-}$ mice. Notably, albuminuria was attenuated in TEM1$^{-/-}$ mice, establishing that renal function is preserved when TEM1 is inactivated. Furthermore, loss of TEM1 protected diabetic mice from glomerular mesangial expansion, as determined in periodic acid-Schiff stained kidney sections. Loss of podocytes was not apparent in diabetic TEM1$^{-/-}$ mice, suggesting a novel interaction between MC and podocytes, which is dependent on TEM1. Regarding analyses of kidney tissue, we found that the hyperglycemia mediated TGF-β1 increase, the diabetes induced ER stress response, and diabetes mediated mTOR activation are attenuated in

TEM1$^{-/-}$ mice.

The underlying mechanism, however, remains unknown. We therefore specifically knocked out TEM1 in mesangial cells *via* CRISPR/Cas9n and compared the influence of high glucose treatment on signaling pathways that involve mTOR and ER stress response as well as on cell migration and mitochondrial dysfunction. New data of the ongoing experiments will be presented.

DGKL-P052

Phosphoproteome Analysis of Mouse Hearts from Endothelial-specific Knockout of Protein Tyrosine Phosphatase-1B

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Cardiovascular dysfunction (CVD) is a major cause of death worldwide. Cardiac hypertrophy, atherosclerosis, fibrosis, and reduced angiogenesis after myocardial infraction are among the important contributing factors of CVDs. Preserving the functional cardiac vascular network or stimulation of angiogenesis after myocardial dysfunction are promising approaches to improve the cardiac function. Angiogenic growth factors such as FGF, PDGF, and VEGF signal via receptor tyrosine kinases (RTKs). Tyrosine phosphorylation is required for the activation of RTKs, while protein tyrosine phosphatases (PTPs) are the negative regulators of RTK activation. A major PTP expressed in endothelial cells is Protein Tyrosine Phosphatase-1B (PTP1B), whose overexpression results in the inhibition of VEGFR2 phosphorylation, while endothelial-specific deletion of PTP1B improves the VEGF signal transduction. Genome-wide expression analysis has confirmed the enhanced expression of PTP1B in model systems of high cardiac afterload. Long term application of PTP1B inhibitor or shRNA mediated inhibition in mice showed to improve angiogenesis, cardiac remodelling and endothelial cell function, making PTP1B as a promising new target for treating CVDs. PTP1B inhibition or deletion protects mice from hypertrophy, however PTP1B is expressed in numerous tissues and complete deletion or inhibition can adversely affect other cell types and their mechanisms. To overcome this problem, endothelial cell-specific PTP1B KO (End-PTP1B-KO) mouse was generated and characterized. End-PTP1B-KO mice induced with cardiac hypertrophy showed reduced oxidative stress, cardiac hypoxia, fibrosis, and enhanced angiogenesis which results in improved survival. Enhanced phosphorylation of VEGFR2 signalling was also observed in cardiac hypertrophy induced End-PTP1B-KO mice which resulted in increased activation of downstream angiogenic mediators such as eNOS or ERK1/2. In this regard, it is interesting to identify novel proteins/pathways whose phosphorylation levels increase in hypertrophy induced End-PTP1B-KO hearts to device efficient therapies for CVDs. Towards this goal, heart tissue lysates derived from hypertrophy induced (7-days TAC, 20-weeks TAC and Control-SHAM) End-PTP1B-KO and WT mice are enriched for phosphorylated peptides by titanium dioxide followed by phosphoproteome analysis. The phosphoproteome comparison and subsequent characterization of differentially expressed novel PTP1B targets is in progression and will be presented. This study might help to better understand the function of PTP1B and to identify targets for novel therapeutics of CVDs.

DGKL-P053

Long Non-Coding RNA ANRIL in Stem Cell Differentiation

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Background: Genome wide association studies (GWAS) have led to the identification of the chromosome 9p21 locus, which is the strongest genetic factor of human atherosclerosis. 9p21 does not encode protein-coding genes but contains a long non-coding RNA antisense noncoding RNA in the INK4 locus (ANRIL). In previous work, we have identified linear and circular ANRIL isoforms, which are associated with the genotype at 9p21 and promote pro- and anti-atherogenic cell function, respectively. The different ANRIL isoforms share in part the same exons, and to date, it is not understood, which RNA motifs are important for the different ANRIL functions.
Objectives: The current study aims to investigate the expression of ANRIL isoforms in tissues and cells, which are relevant in atherogenesis, and to perform systematic deletions of ANRIL exons in induced pluripotent stem cells (iPSCs). After differentiation, the effects on cell function, cell morphology and mechanisms of atherosclerosis using functional high through-put assays will be studied.
Material and Methods: Isoform-specific quantitative PCRs were established and RNA from different human tissues (e.g. heart, vascular wall, liver, gut, adipose tissue, skeletal muscle, and fetal tissues; Clontech) and primary cells (smooth muscle cells (SMC), endothelial cells (EC), monocytes, fibroblasts) was reverse transcribed using random hexamer primers. Induced pluripotent stem cells (iPSCs) were cultured and transfected with CRISPR/Cas9 and guide RNAs, targeting different ANRIL exons. High through-put melting curve assays were established to detect cell clones with deletions. Cellular proliferation was determined using CellTiter-Glo Luminescent Cell Viability Assay (Promega). Apoptosis was induced by staurosporine and quantified by Caspase-Glo-3/7 Assay (Promega).

Results: Systematic expression profiling revealed that ANRIL is not expressed in fetal tissues and iPSCs but upregulated during cell differentiation. In adult tissues, we observed high ANRIL expression in the vascular wall, SMC and macrophages, which play an important role in atherogenesis. CRISPR/Cas9 technology was used to establish iPSC models with deletions of different ANRIL exons. Functional studies of proliferation and apoptosis in iPSC revealed no significant differences between the knock-out cell lines and control cell lines. Currently, the iPSCs cell lines are differentiated into SMC and macrophage and will be used to study the effects of ANRIL deletions on mechanisms of atherosclerosis in differentiated cells.
Conclusion: Using systematic expression profiling, we have identified cell types, which express high levels of ANRIL and are relevant in atherogenesis. Currently, the effect of the deletion of ANRIL exons on cellular functions is studied in iPSC-derived SMC and macrophages.

DGKL-P054

MIR-27B function uncovers a pro-hypertrophic non-canonical mitochondrial ATP synthesis pathway

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Despite its central role in driving cell growth, cellular ATP levels are paradoxically decreased in human and mouse hypertrophic cardiomyopathy in spite of augmented glycolysis. To understand this disconnect between low ATP levels and the capacity for pathologic cardiac growth, we screened a genetic model of constitutive myocardial glycolysis and pathologic hypertrophy for miRNAs regulators of mitochondrial function. In doing so, we have uncovered a stress-dependent Hif1α pathway regulating expression of miR-27b and its consequent repression of Atp5a1, a core component of Complex V. As a result, mitochondrial ADP levels are increased and re-channelled towards Mthfd1l, a rate-limiting enzyme in purine biosynthesis. Consistent with these findings, in vivo inactivation of miR-27b attenuates stress-induced hypertrophy, while cardiac-specific miR-27b expression promotes pathologic cardiac growth. Critically, activation of this pathway is correlates with cardiac pathology in humans. These results challenge the dogma of ATP as a necessary driver of cell growth and unveil a novel mechanism directly connecting stress-induced mitochondrial dysfunction with pathologic growth in cardiomyopathy.

DGKL-P056

Differential effects of novel anticoagulants: fXa versus fIIa inhibition on coagulation and inflammation

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Background and aims: Thrombin is the key protease in regard to thrombus formation; the same is not true in regard to protease dependent signaling. Hence, we postulate that inhibition of either fXa or fIIa may have comparable effects in regard to coagulation, but convey different effects in regard to inflammation and receptor dependent regulation of cellular effects.
Methods: WT mice were either treated with low and high dose of dabigatran or Rivaroxaban for 1 week and then were analyzed for tail bleeding assay or for arterial injury induced thrombosis formation and LAD ligation (ischemic/reperfusion) induced myocardial infarction.
Results : Rivaroxaban and dabigatran have comparable dose-dependent effects in regard to bleeding and thrombosis in in vivo models. However, while fIIa inhibition abrogates the anti-inflammatory effect of fIIa already at low dosages, e.g. at dosages at which bleeding time is already prolonged, this is not the case when using the fXa inhibitor Rivaroxaban. Although Infarcted heart areas were similar in both groups, fxa inhibition abolished proinflammatory cytokines, IL-6, TNF-alpha and macrophages infiltration in infarcted heart tissue. In addition we observed higher levels of blood plasma endogenous activated protein C (aPC) in fXa inhibition group, as compared to in fIIa inhibition group. Western Blot analysis revealed NF-Kβ levels significantly less in fxa inhibited group as compared to fIIa inhibition.
Conclusion: Taken together, these results strongly support that inhibition of fIIa and fXa have similar profiles in regard to the regulation of hemostasis, but differ in their ability to modulate the inflammatory response, with fXa inhibition being superior

DGKL-P057

Omega-6 Fatty Acids and Survival with Cardiovascular Risk: A complex picture? – The Ludwigshafen Risk and Cardiovascular Health Study

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Aims: Omega-6 polyunsaturated fatty acids (omega-6 PUFA) are recommended in European cardiovascular prevention guidelines. However, individual fatty acids have distinct biological characteristics and there have been conflicting reports about the association of omega-6 PUFA with cardiovascular risk.
Methods and results: omega-6 PUFA concentrations were measured in erythrocytes at baseline in a total of 3259 patients participating in the Ludwigshafen Risk and Cardiovascular Health Study (LURIC), using the HS-Omega-3 Index method. Associations of omega-6 PUFA with cardiovascular mortality were analyzed by Cox proportional hazards regression with adjustment for conventional risk factors separately for men and women.

During a median follow-up of 10.0 years, 975 patients (29.9%) died, 614 patients (18.8%) from cardiovascular causes. g-linolenic acid (GLA) was inversely associated with cardiovascular mortality in models adjusted for conventional cardiovascular risk factors with a HR of 0.86(0.78-0.95) per 1-SD increase. Adrenic acid (ADA) and docosapentaenoic acid n-6 (DPA) were both associated with an increased risk with HR of 1.13(1.04-1.22) and 1.11(1.03-1.21), respectively, while arachidonic acid was not. Gender stratified analyses revealed inverse associations of GLA for men and linoleic acid (LA) for women as well as direct associations of ADA in men and DPA in women.
Conclusions: We observed different and gender-specific effects for individual omega-6 PUFA. While LA and GLA were associated with a reduced risk, there was a direct association with risk for the longer chain n-6 PUFA ADA and DPA. These differences do not support global recommendations for polyunsaturated fatty acids, but rather investigating health effects of individual omega-6 PUFA in detail.

DGKL-P058

Identification and Characterization of Acetylated Non-Histone Proteins in Atherosclerosis Models Rieke Welzbacher, Sidra Shahid, Marlena Pantakani, Lutz Binder, Abdul R Asif and Krishna Pantakani

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Atherosclerosis is a chronic inflammatory vascular disease characterized by the development of atheromatous plaque, which triggers luminal narrowing and upon rupture can lead to myocardial infarction or ischemic stroke. Epigenetic processes such as DNA methylation, histone post-translational modifications, and miRNAs plays a crucial role in the progression of atherosclerosis by modulating endothelial cells, vascular smooth muscle cells, and macrophages. Recent studies have identified the increased expression of two histone deacetylases (HDACs) namely HDAC3 and HDAC9 in atherosclerosis and shown that their inhibition leads to reduction/halt in the progression of atherogenesis. However, non-histone proteins such as NF-kB are also targets of both histone acetyltransferases (HATs) and HDACs. Therefore, it is important to identify non-histone proteins, whose acetylation levels are altered by HDAC3 or -9 inhibitions in both endothelial cells and in macrophages to elucidate their functional relevance in reduction and/or halting the plaque formation. Towards this goal, we are using endothelial cells (HUVECs) and macrophages as *in vitro* cell culture model system and induced the inflammatory phenotype followed by HDAC3 inhibition. Subsequently, total protein lysates were prepared and subjected to enrichment of acetylated non-histone proteins by size-fractionation followed by immunoprecipitation using pan anti-acetyl antibodies. The immunoprecipitated samples are being analyzed by proteomic approach. The results of this analysis will be presented in scope of this study. This approach will allow us to identify novel acetylated non-histone proteins from atherosclerotic models. Subsequent characterization of identified targets might enable us with devising treatment options for atherosclerosis.

DGKL-Metobolomics and Lipidomics

DGKL-P059

Robust and practical strategy for lipidomics of tissue-derived mitochondria

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Background: Mitochondrial phospholipid content and composition have been shown to affect electron transport chain activity and respiratory function. However, little is known on the relationship between mitochondrial function and mitochondrial lipid composition, particularly in tissue samples. One reason could be 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.
Objectives: Development and optimization of a robust and practical strategy for the high-resolution analysis of lipid profiles from pure mitochondria of different tissues.
Methods: Three different isolation methods (differential centrifugation (DC), DC followed by ultracentrifugation (UC), magnetic bead-assisted method (MACS)) were compared and optimized using mouse skeletal muscle and liver tissue to define the mitochondrial extraction procedure yielding the purest mitochondria. Mitochondrial enrichment as well as contaminations by other organelles (endoplasmic reticulum (ER), nuclei, Golgi apparatus) were investigated by western blot analysis. Using non-targeted UHPLC-MS lipidomics, we compared the lipidome of muscle and liver mitochondria from mice.
Results: Applying our optimized strategy for the sensitive, comprehensive and reproducible investigation of the highly purified mitochondrial lipidome, we detected a total of 458 lipid species in mouse liver mitochondria and 464 lipid species in skeletal muscle mitochondria, both including 36 cardiolipins in as little as 100 µg (by protein) of mitochondria. The lipid patterns of skeletal muscle and hepatic mitochondria separated into distinct clusters in a PCA plot, indicating tissue-specific differences in the mitochondrial lipid pattern. The amount of cardiolipins and phosphatidylethanolamines, but not of phosphatidylcholines, was higher in muscle than in liver mitochondria.
Conclusions: We established and optimized a reliable and comprehensive method to obtain lipid profiles from pure tissue-derived mitochondria. We will integrate this tool into a systems biological analysis of mitochondria covering not only lipidomics but also proteomics and functional analyses such as respirometric and oxidative stress assays. The association of specific mitochondrial lipid alterations to mitochondrial functioning will allow the detection and determination of the role of mitochondrial adaptions in health and disease.

DGKL-P060

Comprehensive characterization of a human thyrotoxicosis model – Towards the identification of novel biomarkers of hyperthyroidism

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Introduction Thyroid hormones (TH) are essential for normal development, cell growth and the function of virtually all tissues. Their bioactivity is closely controlled by thyrotropin (TSH) as part of a negative feedback loop. Thus, measurements of TSH and free TH are generally regarded as the gold standard in diagnosis of thyroid function. Currently, there are only few biomarkers available to capture peripheral, organ-specific TH effects. To screen for novel, exclusively TH-dependent effects on peripheral tissues we used an untargeted metabolome and proteome approach in an experimental model of thyrotoxicosis in humans.
Methods Sixteen healthy young males were treated with 250 µg levothyroxine/day for eight weeks. Plasma was sampled before, after four and eight weeks of treatment, respectively, as well as four and eight weeks thereafter. Metabolite and protein levels were determined by mass spectrometry. Robust analyses were performed using mixed-effect linear regression models in a subsampling setting, with serum free thyroxine (FT_4) as exposure and metabolite/protein levels as outcome. To compile a molecular signature discriminating between thyrotoxicosis and euthyroidism, a random forest was trained and validated in a two-stage cross-validation procedure.

Results The levels of 65 metabolites and 63 proteins were significantly associated with serum FT_4, where the majority of these associations were positive. L-T_4 induced alterations comprised a number of clinical relevant physiological entities like energy and lipid metabolism, induction of oxidative stress, the coagulation cascade, the complement system or apolipoproteins. The results further raised concern about the implication of thyroid diseases in the judgment of kidney function or even cardiovascular complications, exemplified by increased ADMA concentrations under L-T_4. It has to be emphasized that all these molecular alterations were unrecognized by the volunteers as they did not results in manifest clinical symptoms of thyrotoxicosis. A subset of fifteen metabolites/proteins allowed a robust and good prediction of TH status (AUC=0.86) without prior information on TSH or free TH.
Conclusion Our results emphasize the power of untargeted OMICS approaches to detect novel pathways of TH action. Furthermore, beyond TSH and FT4, we demonstrated the potential of such analyses to identify new molecular signatures for diagnosis and treatment of thyroid disorders.

DGKL-P061

The origin of acyl carnitines in human plasma during fasting and exercise

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Acylcarnitines are currently in the focus of research as potential biomarkers in many disciplines of medical sciences. However, the origin of plasma acylcarnitines is not clarified. In two studies we investigated arterial-venous differences to elucidate the distinct contribution of liver and skeletal muscle to the dynamic changes of plasma acylcarnitines in fasting and exercising humans.

In the first study, after an overnight fast young healthy males (n=9) participated in an knee-extensor exercise study for 2 h with one fixed (=resting) and one exercising leg (incl. 3 muscle biopsies per leg) and plasma was collected from both femoral veins and femoral artery. In the second study, the participants performed 2 h cycling exercise (n=10) while plasma was collected from the brachial artery of the non-dominant arm and the liver vein via the right femoral vein to investigate acylcarnitine fluxes over the hepato-splanchnic bed. Hepatic and skeletal muscle arterial-to-venous plasma differences, as well as skeletal muscle acylcarnitine concentrations were investigated by UHPLC-MS.

The liver released pronounced amounts of C2- and C3-carnitine after an overnight fast. Exercise caused a systemic increase of most acylcarnitines in plasma, which declined very rapid to pre-exercise values in the recovery phase. In the exercising muscle tissue levels of most acylcarnitines increased. In contrast to that, levels of free carnitine decreased in the tissue of the exercising muscle and returned back to baseline levels after the end of the exercise bout. Almost no changes were detected in the tissue of the non-exercising leg. The exercising leg released mainly medium chain-carnitines into circulation, while even an uptake of long chain acylcarnitines was observed. The liver released continuously short chain, namely C2- and C3-carnitine, during exercise.

In summary, the liver is a major contributor to systemic C2- and C3-carnitine levels during fasting and exercise. The contracting muscle contributes to the increases in plasma medium-chain acylcarnitines during exercise, while the systemic increase of long-chain acylcarnitines during exercise is neither caused by skeletal muscle nor by the liver.

DGKL-P062

Targeted and global pharmacometabolomics in everolimus-based immunosuppression: Association of co-medication and lysophosphatidylcholines with drug response

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Large inter-individual variability in everolimus (ERL) dose response and a narrow therapeutic window complicate its use in immunosuppressive therapy after heart transplantation. Identification of new predictive biomarkers of ERL response and elucidation of the mechanisms

underlying this variability are of utmost clinical importance. Semi-targeted and non-targeted metabolic profiling was performed using high-resolution mass spectrometry in plasma samples of 41 heart transplant patients to identify metabolites associated with ERL dose response. Targeted quantification of ERL and its major metabolites was performed by LC-MS/MS. Both, non- and semi-targeted metabolic profiling revealed that decreased plasma lysophosphatidylcholines (lysoPCs) as well as decreased low-density lipoprotein (LDL) levels were associated with decreased ERL dose requirement. Furthermore, an altered ratio of 46-hydroxy and 24-hydroxy ERL observed in 15% of the patients have been described being independent from *CYP3A5*3* or co-medication, although an association of therapy with the mycophenolate formulation Myfortic® and the genetic variant *CYP3A5*1* with an increased ERL dose requirement was observed. In conclusion, the pharmacometabolomics study approach identified differences in ERL metabolism and novel metabolites associated with ERL dose requirement. Especially the role of lysoPCs as potential biomarkers for cardiac allograft vasculopathy related inflammatory processes, will require further investigation in order to understand their role in altered ERL dose requirement. Furthermore, investigations to elucidate the clinical relevance of the reported drug-drug interactions and differences in ERL metabolite patterns are needed.

DGKL-P063

Serum, standard plasma or platelet-free plasma - are there any preferences for metabolomics-driven biomarker studies?

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Background: Frozen plasma and serum samples stored in biobanks are frequently used in large scale biomarker studies. In the literature both are recommended sample materials of choice for metabolomics studies. However, serum metabolite pattern are affected by the clotting process and the needed exposure to room temperature, and plasma prepared by standard conditions still contains a considerable number of platelets. Whether these remaining platelets affect the results of metabolomics biomarker studies is unknown.
Methods: Applying an UHPLC-mass spectrometry-driven metabolomics approach we compared serum, standard EDTA-plasma (centrifugation: 4000g, 10 min, 4°C) and platelet-free plasma (prepared by three different centrifugation conditions) from the same ten volunteers.
Results: Subsequent to a centrifugation step we detected a mean cell count of 27,000 platelets/µl in standard plasma, i.e. one-tenth compared to blood. A comparison of the metabolite pattern in standard plasma and serum by principal component analysis revealed no similarity. In serum >50 % of the metabolites were significantly different from plasma. On the other hand the pattern of the platelet-free plasma and standard plasma corresponded well.
Conclusions: The coagulation process lead to significant differences in a considerable number of metabolites between plasma and serum greatly. The pattern in common and platelet-free plasma is almost identical. Thus standard plasma as stored in biobanks is still suitable for clinical metabolomics biomarker studies.

DGKL-P064

Evaluation of an in-tube Whole-blood Activation Assay for Multiparametric Analysis of Arachidonic Acid Metabolism

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Background: Eicosanoids are arachidonic acid (AA)-derived lipid mediators, which play an important role in inflammation. In previous work, we have shown that the eicosanoid response in an established whole-blood activation model of patients with suspected coronary artery disease is a marker for the severity of the disease.
Objectives: The previously established whole-blood activation model required cell culture dish incubation of whole blood. It was the aim of the current study to develop an in-tube activation system, allowing the application of this assay in clinical routine settings.
Material and Methods: Heparinized whole blood was incubated with or without LPS (100 ng/mL) for 4 and 24 hours. Samples were incubated at 37 °C with or without cell culture medium using cell culture dishes or heparinized blood collection tubes. Furthermore, blood collection tubes were incubated at 37°C and at room temperature to investigate whether the activation is dependent on temperature. RNA was isolated and the mRNA expression of key enzymes of AA metabolism (cyclooxygenase (COX) 1 and 2, prostaglandin E synthase (PGES), prostaglandin F synthase (PGFS), thromboxane synthase (TXS), 5-lipoxygenase (5-LOX), 5-LOX activating protein (FLAP) and 12-lipoxygenase (12-LOX)) were analyzed by established RT-qPCR assays. Corresponding metabolites (AA, prostaglandins and hydroxy eicosatetraenoic acids (HETEs)) were analysed in supernatants using an UHPLC-MS/MS based strategy.

Results: We observed no significant differences at the gene expression or mediator levels of investigated eicosanoid pathways when comparing the cell culture and in-tube assays after LPS activation. Addition of cell culture medium was not necessary to induce a reproducible eicosanoid release. However, we observed a temperature-dependency of the eicosanoid response, where incubation at room temperature led to significantly lower release of eicosanoids than when tubes were incubated at 37 °C.
Conclusion: We were able to establish an easy-to-use and reproducible in-tube whole-blood activation model for investigating the individual eicosanoid response. Since this assay does not require cell culture dish incubation, it can be used in clinical routine settings.

DGKL-P065

Using internal standards for quantitative LC-MS/MS Lipidomics – one size fits all?

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Objectives
Several different lipid species have emerged as potential biomarkers for diagnosis, prognosis and risk stratification in various cancer types recently. Different lipidomic approaches exist, but obtaining reliable and reproducible quantitative data from human plasma is often hampered by significant matrix effects. Despite being mayor human plasma lipids, neutral lipid species like cholesterolester (CE) and triacylglycerols (TAG) are often neglected. Therefore we developed and validated a LC-MS/MS lipidomic approach that includes neutral lipid species and fits the standards of clinical chemical testing.
Methods
128 lipid species from 8 lipid classes ((lyso-)phosphatidylcholines, ethanolamines and -serines, sphingolipids and ceramides, CE and TAG) were measured on a BRUKER micrOTOF-Q2 using one internal standard for each lipid class. Inter- and intraessay CV% was determined for each lipid class. To estimate accuracy, human serum samples were spiked with different lipids.
Results
To give sufficient yields of neutral lipids a second extraction step with chloroform should be added to the usually used chloroform/methanol mixtures. For HPLC separation of lipid classes' a third hydrophobic solvent system was introduced. Intraessay-variance was between 1.5 und 7.2%, interessay-variance was between 1.0 und 7.8%, except for PS showing a CV% of 22%. When measuring accuracy, a systematic error was observed, depending on the fatty acid acyl structure of respective lipids. When using non-natural saturated lipids as group standard, unsaturated lipid species are generally measured too low. Respective systematic errors may be at least partially compensated by introducing individual correction factors.
Conclusion
Whilst precision is generally sufficient, accuracy suffers from systematic errors when a single internal group standard is used. Introducing respective correction factors may overcome this problem.

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DGKL-P066

Influence of sampling techniques on human plasma and serum metabolome stability during the long-term sample storage

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Targeted metabolomics has become a valuable tool in disease research. Blood is the most preferred sampling matrix in pre-clinical research and diagnosis as it is well established and reflects the circulating metabolome of the whole organism. Large metabolomics cohort studies mostly rely on blood samples from blood banks. Current blood collection protocols utilize different sample treatments in order to isolate blood serum and plasma for the final metabolite extraction. In this study the long term storage stability, up to 12 months, for EDTA, heparin and citrate plasma and serum was investigated to fill in the gaps in the knowledge of human metabolome changes during the long-term sample storage. The blood from a healthy donor was drawn and processed according to methodology-specific protocols at the same time point for all techniques. To evaluate the storage stability sample aliquots were stored at -80 °C for a time period of up to twelve months. Quantification of

more than 180 endogenous metabolites including amino acids, biogenic amines, acylcarnitines, glycerophospholipids, sphingomyelins and hexose was performed employing the AbsoluteIDQ® p180 Kit (Biocrates Life Sciences AG). As expected, the number of detected metabolites as well as concentration levels differed between investigated sample types. Surprisingly, the obtained results revealed that metabolite stability was influenced not only by the storage conditions but also by the employed sampling technique. For all tested samples types the changes in metabolite levels were observed as early as after 4 week of storage. The obtained results highlighted the influence of the sampling technique on metabolite stability in human blood samples. Therefore, it is of utmost importance to consider not only the sample storage time but also sample type in case samples from blood banks are employed for metabolomics study.

Molecular Diagnostics

DGKL-P067

Further characterization of variants resulting from next generation sequencing by LOH analysis in tumor tissue

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Background.
Loss of heterozygosity (LOH) is a common genetic event in cancer development, characterized by a heterozygous germline mutation and a second, apparently homozygous variant in tumor DNA, due to the somatic loss of the wild type allele. This "second hit" mechanism is able to completely inactivate tumor suppressor genes, therefore leading to tumorigenesis.
Objectives.
A total of 488 samples were subjected to hybridization-based targeted resequencing of 16 paraganglioma (PGL), phaeochromocytoma (Phaeo) or medullary thyroid cancer (MTC) susceptibility genes. PGL's and Phaeo's are rare tumors of the autonomic nervous system with up to 40% being of genetic origin. Three syndromic conditions "Von Hippel–Lindau" (vhl), "multiple endocrine neoplasia type 2" (men2) and "neurofibromatosis type 1" (nf1) are by now established as additional causes of hereditary PGL/Phaeo.
Methods.
Genomic DNA was isolated from whole blood leukocytes and 300bp paired- end sequenced on an Illumina MiSeq using v2 chemistry. Bioinformatic analysis comprising whole genome alignment, variant calling and variant filtering with a cut-off set to 1% minor allele frequency was performed for exons, splice-sites and untranslated regions of the 16 candidate genes. In case of NGS-results revealing known mutations, respectively most likely pathogenic variants genomic DNA isolated by microdissection from formalin- fixed tumor tissue was analyzed for LOH by pyrosequencing-based allele quantification in order to further characterize, whether the detected variant may be responsible for the patients phenotype.
Results.
In case of 9 variants affecting 7 different genes, patients leukocytes and patients tumor tissue were tested for LOH in comparison to control leukocytes. LOH could be demonstrated based on allele frequencies (AFmut and AFwt) in case of mutations in the tumor suppressor genes NF1:c.5270T>A,p.Val1757Asp (AF patients tumor tissue: AFmut 95%, AFwt 5%) and TMEM127:c.265_268delCTGT (AF patients tumor tissue: AFmut 85%, AFwt 15%). Both patients were affected by multiple phaeochromocytoma. No LOH could have been shown for the following variants: VHL:c.-73C>T; MAX:c.172-6230C>T; KIF1B:c.2708C>T,p.Thr903Met; KIF1B:c.2842G>T,p.Ala948Ser; NF1:c.7474C>T,p.Gln2492X; SDHAF2:c.496C>T,p.Arg166Cys and SDHA:c.1A>G,p.Met1Val.
Conclusion.
As LOH may also appear in a non-hereditary, sporadic context where the first hit is a somatic event followed by a second somatic hit, we aim to sequence all, up to now, germline-negative PGL-,Phaeo- and MTC-patients (105 samples in total) based on tumor DNA for mutations in the 16 candidate genes. Complementing sequencing results by further analyses, e.g. immunohistochemistry, LOH-analysis, pedigree analysis, is often essential in order to assess a variants relevance correctly.

DGKL-P068

A novel strumpellin mutation and potential pitfalls in the molecular diagnosis of hereditary spastic paraplegia type SPG8

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Background:
The hereditary spastic paraplegias (HSPs) are progressive gait disorders which are caused by degeneration of cortico-spinal tract axons. With > 70 spastic paraplegia gene loci (SPGs), they are genetically highly heterogeneous. SPG8 is a dominant HSP caused by mutations in KIAA0196 gene. A total of eight variants, most of them altering single amino acids in the encoded strumpellin protein, have thus far been found in HSP patients. Various features of these alterations as well as the associated clinical parameters varied widely, thus hampering the definition of diagnostic criteria.
Methods:
The proband and his mother were examined by an experienced neurologist. Blood samples were collected, DNA extracted and copy number screening for the two most frequent HSP genes (atlastin, spastin) was carried out with the multiplex ligation-dependent probe amplification (MLPA). Sanger sequencing for HSP genes (atlastin, spastin) and KIAA0196 targeted all exons was performed. PolyPhen2 and SIFT predictions as well as Grantham scores were obtained for the presumably benign variants and for all variants reported in association with SPG8.
Results:
In the MLPA screen we did not identify aberrations. We thus turned to Sanger sequencing of less prevalent HSP genes and thereby identified the novel heterozygous variant c.1859T>C (p.V620A) in KIAA0196. When comparing all eight HSP-associated variants to the major variation databases entries, we found that the PolyPhen2 score completely failed to indicate pathogenicity. Similarly inconclusive observations resulted from the application of the SIFT and the Grantham scores.
Conclusion:
We noticed that strumpellin seems to harbor an unusually high number of very rare variants. Identification of such variants in patients requires cautious interpretation. Based on comparing all nine HSP-associated KIAA0196 alterations and current entries in variation databases we emphasize a potential for pitfalls in molecular diagnosis of SPG8. This is especially true for small families, complex phenotypes, non-dominant pedigrees, unusual protein positions, and/or multi-gene diagnostic settings, but probably also for very low pathogenicity scores.

DGKL-P069

Improved management of hepatitis D: Standardized quantification of HDV RNA

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Background: HDV is a small, defective RNA virus that can infect only individuals who have HBV; worldwide more than 15 million people are co-infected. HDV increases the severity of chronic HBV infection, frequently leading to cirrhosis, hepatic decompensation or hepatocellular carcinoma. The prevalence of HDV is declining in some endemic areas but increasing in northern and central Europe because of immigration and vaccine fatigue for hepatitis B. Interferon-alpha is currently the only available treatment option leading to suppression of HDV RNA in 25 - 30 % of patients. However, pegylated interferon therapy is associated with sometimes severe side effects and only a minority of patients is eligible for treatment. Novel alternative treatment options are currently in early clinical trials.
Objectives: HDV RNA quantification is a crucial tool to diagnose, treat and manage HDV infections. The majority of nucleic assay amplification tests for viral load monitoring of HDV RNA are developed in house based on real-time PCR using internal standards of different origin. This issue impedes comparability and was greatly improved with the establishment of the 1st WHO standard for HDV RNA [1]. A novel CE-certified and referenced real-time PCR assay, the RoboGene® HDV RNA Quantification Kit 2.0, has been compared to an earlier published in-house method [2].
Materials and methods: Evaluation data is represented, achieved according to the common technical specifications for in vitro diagnostic medical devices. Furthermore, samples of 15 HDV IgG pos. (genotype 1) and 5 HDV IgG neg. patients were analysed. The study included a longitudinal perspective. Samples were collected before, 12 and 48 weeks following treatment with pegylated interferon alpha.
Results: Besides optimal specificity, the assay shows linearity over 5 to $1 \cdot 10^9$ copies per run and allows a limit of detection down to 10 IU/ml. For diagnostic evaluation results from the kit were consistent with those from the applied in-house method before starting point of therapeutic treatment. Following 12 weeks 20 % of the patient samples were determined as negative by the kit, but 33% using the in-house method. 48 weeks following starting point of treatment 33% and 40% were negatively tested using the kit and in-house, respectively.
Conclusions: Hepatitis D represents a major and life-threating health burden in certain areas of the world. The assay can be reliably used to confirm HDV infection and allows highly sensitive monitoring of therapy efficacy. Future studies include the investigation of the performance in different HDV genotypes. Confirmation of hepatitis infection, as well as standardized assessment of antiviral treatment effectiveness may improve patient management in the future.

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DGKL-P070

A comparison of the differentiation of normal human dermal fibroblasts and PXE fibroblasts into myofibroblasts

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Background: Pseudoxanthoma elasticum (PXE) is a rare autosomal recessive disease, which is characterised by a progressive calcification and fragmentation of the elastic fibres. During the progression of the disease, an accumulation of extracellular matrix (ECM) molecules, such as proteoglycans, takes place. Fibroblasts play an essential role with regard to calcification and remodelling of the ECM. The cytokine transforming growth factor beta 1 (TGF-β1) and a loss of the cell-cell contact induces the differentiation of fibroblasts into myofibroblasts, which have an impact on the wound healing process and the remodelling of the ECM as well. Amongst others, myofibroblasts show an increased alpha smooth muscle actin- (α-SMA), collagen- and xylosyltransferase (XT) expression. Since little is known about the relation between the wound healing process and the remodelling of the ECM in PXE, we compared the differentiation of normal human dermal- and PXE fibroblasts into myofibroblasts.
Methods: Dermal fibroblasts of healthy control and PXE patients (each n=2) were treated with the profibrotic cytokine TGF-β1 to induce the differentiation into myofibroblasts. 48 hours after the treatment, cells were harvested, total RNA was isolated and gene expression analysis was determined by quantitative RT-PCR. For the wound healing assay, an artificial gap was created on a confluent cell monolayer. The migration of the fibroblasts towards this scratch was observed hourly by live cell monitoring. To compare the contractility of healthy- and PXE fibroblasts, cells were seeded on a collagen type 1 gel. After adhesion, cells were treated with TGF-β1 and the gel was photographed at different points in time. To determine the contractility, the gel area was quantified using ImageJ. The XT activity in cell culture supernatants was measured in a radiochemical assay. The α-SMA expression was analyzed by immunofluorescence.
Results: After treatment with TGF-β1, elevated mRNA expression levels, and therefore stronger inductive effects, of the genes *TGFb1*, *XYLT1*, *ACTA2* and *ELN* were observed in the control cells. As opposed to this, *XYLT2*, *DCN*, *Col1A1* and *MMP12* mRNA expression levels were elevated in PXE-fibroblasts. The wound healing assay revealed that the PXE cells migrate much faster than the control fibroblasts. Conversely, the PXE fibroblasts showed a decreased contractility compared to the control cell line. After treatment of the cells with TGF-β1, the XT-activity was elevated in both, control- and PXE fibroblasts, to the same extent. The α-SMA protein expression was decreased in PXE fibroblasts.
Conclusion: Due to possible pathological changes, PXE fibroblasts show, in comparison to control fibroblasts, an abnormal wound healing process as well as an altered differentiation into myofibroblasts. These are important findings in regard to reveal the pathomechanism of PXE.

DGKL-P071

Comparison of various patient sample matrices using the Quidel AnDiaTec® CMV real time PCR Kit and the R-gen CMV Kit

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Background: We, at the university clinic St. Poelten, are Lower Austria's largest hospital laboratory, ISO certified since 2010 and providing around 40% of all laboratory tests performed in Lower Austria's hospitals.
Objectives: Our aim is to maintain highest diagnostic standards and thus, we regularly compare newly available products against assays we currently have established for routine diagnosis. Quantitative Cytomegalovirus (CMV) real time PCR analysis is one of our high throughput testing parameters and a correct CMV DNA result is utmost important for patients' health. Therefore, we evaluated the new commercially available Quidel AnDiaTec® CMV real time PCR Kit against the in our laboratory so far best performing real time PCR kit which was the bioMerieux CMV R-gene® kit.

Materials and methods: Serum, plasma, whole blood and urine samples from 8 patients with previous positive CMV test results were used in the evaluation study. The samples were tested undiluted and in 3 serial dilutions down to the detection limit of the two assays in order to compare their analytical sensitivity. A total of 128 samples were generated and all samples were extracted using the automated MagnaPure LC 2.0 system (Roche Diagnostics). After extraction the eluates were directly used for real time PCR analysis in both assays in a LightCycler 480 instrument (Roche Diagnostics).
Results: Three of the urine samples were negative in both assays and thus, excluded from the calculation. All other samples were positive in at least one real time PCR assay. With the R-gene® kit 72 positive and 44 negative results were obtained. The AnDiaTec® test gave 85 positive and 31 negative results. The R-gene® assay detected 8 positives that were negative in the AnDiaTec® kit whereas AnDiaTec® detected 21 positives that were negative in the R-gene® PCR.
Conclusions: Our study results show that the AnDiaTec® test performed better than the R-gene® assay with a significance of 0,068. We therefore can recommend the use of this new commercially available CE-marked product for quantitative CMV testing, if it applies to your laboratory infrastructure and the national IVD regulations of your country.

DGKL - Epigenetics

DGKL-P072

Expresssion und Regulation der Typ-V Phospholipase A2 in Leukämie-, Prostata- und Mammakarzinom-Zelllinien

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Hintergrund: Sekretorische Phospholipasen A_2 ($sPLA_2$) spielen eine wesentliche Rolle in der Entzündung und Tumorgenese. Unterschiedliche Mechanismen der epigenetischen Regulation sind an der Expression der Typ IIA, III, and X $sPLA_2$s in Tumorzellen beteiligt.
Fragestellung: In der vorliegenden Arbeit wurde der bisher nicht untersuchten Frage nachgegangen, ob die Expression der Typ-V $sPLA_2$ (GV-PLA_2) ebenfalls durch epigenetische Mechanismen reguliert wird.
Material und Methoden: Die Expression der GV-PLA_2 in Leukämie-, Prostata- und Mammakarzinom-Zelllinien im Vergleich zu normalen Zellen wurde mit RT-qPCT analysiert. Die Bestimmung des Methylierungsgrades des GV-PLA_2-Gens erfolgte durch Sequenzierung Bisulfit-modifizierter genomischer DNA und methylierungsspezifischer *High Resolution Melting* (MS-HRM)-Technik. Der Effekt rekombinanter GV-PLA_2 auf das Zellwachstum wurde im Koloniewachstums-Assay ermittelt.
Ergebnisse: Die Konzentrationen GV-PLA_2-spezifischer mRNA waren in U937- und Jurkat-Leukämiezellen sowie in Blutleukozyten von Leukämiepatienten im Vergleich zu Leukozyten gesunder Probanden signifikant erniedrigt. Ähnlich verhielt es sich in DU-145- und PC-3-Prostata- und CAL-51 und MCF-7-Mammakarzinomzelllinien im Vergleich zu normalen Epithelialzellen der Prostata und Mamma. Die Bestimmung des Methylierungsgrades zeigte zellspezifisch erhöhte Werte in Tumorzelllinien und der Effekt von 5-Aza-2′-Deoxycytidin als DNA-Methyltransferase-Inhibitor und Trichostatin A als Histon-Deacetylase-Inhibitor auf die PLA2R1-Reexpression unterstrichen die Bedeutung der DNA-Methylierung und Histonmodifizierung auf die Regulation der GV-PLA_2-Expression. Die statistische Auswertung nach Spearman unterstrich eine signifikant negative Korrelation zwischen dem Promoter-Methylierungsgrad und der zellulären Expression der GV-PLA_2 ($r=- 0.697$; $p=0.01$). Im Koloniewachstums-Assay konnte schließlich eine zelltyp-abhängige tumorsuppressive Wirkung der GV-PLA_2 nachgewiesen werden, indem das Koloniewachstum von MCF-7, HepG2-Hepatomzellen und PC-3-Zellen nach Inkubation mit dem rekombinanten PLA_2-Isoenzym signifikant erniedrigt war.
Schlussfolgerung: Die Ergebnisse zeigten, dass epigenetische Mechanismen wie DNA-Methylierung und Histonmodifizierung an der verminderten Expression der GV-PLA_2 in Tumorzellen beteiligt sind, die tumorsuppressive Eigenschaften aufwies.

DGKL-P073

Altered patterns of H3 post-translational modification in cells with acquired resistance to topoisomerase inhibitors

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Background:
Epigenetic remodeling of chromatin structure is associated with alterations of gene expression and profound changes in proliferation and differential cellular states. Transmission of epigenetic states is achieved by DNA methylation and extensive post-translational modifications of histone proteins that help to orchestrate DNA packing.

Histone proteins are post-translationally modified at both terminal ends at lysine (methylation, acetylation) and serine (phosphorylation) residues. Histone H3 modifications such as H3K4 tri-methylation and H3K9 methylation are well known regulators for transcriptional activity and chromatin condensation.

For the majority of histone proteins numoerous variants differing in function and chromosomal deposition are described, e.g. the histone H3.3 deposition is associated with active DNA synthesis and DNA damage repair.

Aim:

Resistance to topoisomerase inhibitors is linked to changes of chromatin structure and cell cycle regulation. Resistant cells are not affected by DNA damage caused by these drugs, still their chromatin organization is altered and cell cycle slowed down. We aim at identifying post-translational modifications mediating these changes that can be used as marker for increasing chemoresistance to topoisomerases.

Material and Methods:

Two colon cancer cell lines (HT29, HCT116) were compared before and after selection for topoisomerase resistance and characterized regarding PTMs on histone proteins using mass spectrometry. Additional assessment of DNA damage and cell cycle duration was performed.

Results:

Among the differences of histone PTMs found when comparing cell lines before and after selection those concerning histone H3 were the most consistent ones. We identified reduction of H3K27 methylation and H3K36 and H3K37 methylation/acetylation, respectively. Tri-methylation of H3K27 also appears to be reduced in resistant cell lines.

Discussion:

Resistance to topoisomerase inhibitors provokes substantial changes in chromatin condensation and PTM patterns. Some histone marks associated with transcriptional regulation are switched from inactive to active states while other marks being more important for dsDNA breaks and DNA damage repair are now present in the majority of H3 proteins of resistant cell lines.

Conclusion:

Cells being resistant to topoisomerase inhibitors are subjected to profound changes in the regulation of chromatin structure. PTMs found in resistant cell lines suggest a constantly increased repair activity contributing to the resistance to topoisomerase inhibitors and the observed reduction in DNA damage suffered upon constant exposure to genotoxic drugs.

DGKL - Oncology and liquid profiling

DGKL-P074

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

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Background: For gynecological cancers only few data are available regarding the clinical performance of tumor marker assays based on LOCI™-technology.

Aim: In our study we investigated the diagnostic power of these markers in patients suffering from diverse gynecological cancers. Comparisons with appropriate control groups were performed.

Patients and Materials: CEA, CA 19-9, CA 15-3, CA 125 and AFP were analysed in sera of 336 individuals among them 177 female patients with gynecological cancers (73 breast, 22 cervical, 16 endometrial, 17 vulva and 49 ovarian cancers), 26 patients with precancerous gynecological diseases (11 vulva, 5 cervix and 10 breast), 109 patients with benign gynecological diseases and 24 healthy controls on the Dimension™ Vista 1500 analyser (Siemens Healthcare Diagnostics, Eschborn, Germany). We calculated the discriminative power between groups by Wilcoxon tests, areas under the curves (AUCs) in receiver operating characteristics (ROC) curves and sensitivities at a fixed specificity of 95%.

Results: Discrimination between cancers and healthy controls were observed for many markers, but only established tumor-associated markers discriminated between cancers and respective benign controls as well as between precancerous diseases and healthy controls.

In ovarian cancer CA 125 and CA 15-3 were significantly elevated in malignant as compared with benign diseases, with CA 125 being the best marker (AUC 0.86 and 77.6% sensitivity at 95% specificity). In breast cancer CEA and CA 15-3 were significantly higher in malignant than in benign diseases with CEA achieving the best AUC (0.65) and a 31.5% sensitivity at 95% specificity. None of the investigated markers was found to discriminate between benign and malignant cervical, endometrial and vulva diseases. In addition, CEA and AFP were able to distinguish precancerous breast and vulva diseases from healthy controls.

Conclusion: We demonstrated the high diagnostic performance of well-known cancer biomarkers for gynecological cancers using LOCI™-technology.

DGKL-P075

Genetic variation in ENOSF1 and TYMS - potential toxicity risk factors in fluoropyrimidine-based chemotherapy

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Background: A variant in enolase superfamily member 1 (*ENOSF1* c.742-227G>A) has recently been suggested to account for the previously reported association between genetic variation in thymidylate synthase (*TYMS* 5'VNTR 2R/3R and 3'UTR 6 bp ins-del), a gene adjacent to *ENOSF1*, and toxicity in cancer patients treated with capecitabine (Cp), an oral prodrug of 5-fluorouracil (5-FU). This association, however, has so far only been investigated in Cp-treated patients, whereas the impact of this variant on 5-FU toxicity risk is unknown. The aim of this study was thus to assess the association of *ENOSF1* and *TYMS* variation with severe early-onset toxicity in patients receiving Cp-based and 5-FU-based chemotherapies. **Methods:** The common *TYMS* polymorphisms 5'VNTR 2R/3R (rs34743033), 3'UTR 6 bp ins-del (rs34489327), the *ENOSF1* variant c.742-227G>A (rs2612091), and a rare dihydropyrimidine dehydrogenase mutation (*DPYD* rs12132152) were genotyped in 144 Cp-treated patients and in 403 patients receiving 5-FU-based chemotherapy. The association of genotyped variants with overall toxicity, hand-foot syndrome (HFS) and diarrhea was assessed. **Results:** In Cp-treated patients, the associations of c.742-227G>A in *ENOSF1* and rs12132152 in *DPYD* with toxicity were replicated ($P_{adjusted} = 0.027$; OR = 1.61; 95% CI 0.99-2.63, OR for G allele; and $P_{adjusted} = 0.025$; OR = 4.63; 95% CI 1.01-21.4). Moreover, carriers of the *TYMS* 3'UTR 6 bp insertion experienced more Cp-induced HFS ($P_{adjusted} = 0.017$; OR = 2.63; 95% CI 1.08-6.67) with a stronger association compared to the *ENOSF1* variant. Conversely, no associations of the same candidate variants with toxicity were found in 5-FU-treated patients. Finally, a common haplotype, including the strongly linked protective alleles, c.742-227A and the 3'UTR 6 bp deletion, was associated with decreased risk of developing HFS ($P_{adjusted} = 0.019$; OR = 0.30; 95% CI 0.11-0.82). **Conclusions:** A haplotype encompassing the neighboring genes *TYMS* and *ENOSF1*, and *DPYD* rs12132152 may be risk factors for toxicity in Cp-based, but not 5-FU-based chemotherapy. Further investigation of the strongly linked polymorphisms in *ENOSF1* (c.742-227G>A) and *TYMS* (3'UTR 6 bp ins-del) is needed to determine the causal variant(s) underlying the observed effect on the individual susceptibility to Cp-related toxicity.

DGKL-P076

Use of liquid profiling/liquid biopsy to detect RAS mutations in cfDNA of patients with metastatic colorectal cancer (mCRC)

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Background:
Colorectal cancer is the second most common cancer and cause of cancer related death in many countries, including Germany. Advances in chemotherapy and the recent development and implementation of monoclonal antibodies improved the survival rate and increased the progression-free time of stage-four patients that until then only used to be treated palliatively.
Aims & Objectives:
One possible target for antibodies is the epidermal growth factor receptor (EGFR). By binding to the EGFR and competing with EGF the antibodies, cetuximab or panitumumab, lead to a stop of cell proliferation and to apoptosis. Chemotherapy in combination with an antibody is more effective than chemotherapy alone.

In about 50% of all colorectal cancers there are mutations in either *KRAS* or *NRAS*. Yet, only patients with wild type *RAS* benefit from administration of the antibodies. Since there may be an emergence of new mutations in response to antibody treatment, rendering therapy inadequate, it is crucial to know the *RAS* status of the patient before and during antibody therapy in order to provide optimal medication.
Material & Methods:
Usually, a biopsy of tumor tissue is used to determine *RAS* status. Alternatively it is now possible to detect *RAS* mutations in the cfDNA of a blood sample. The so called OncoBEAM® RAS CRC assay (by Sysmex Inostics) was developed to detect 34 mutations (16 in *KRAS*, 18 in *NRAS*) in plasma samples. In short, cfDNA is isolated from plasma samples of patients and then analyzed via digital droplet PCR and subsequent flow cytometry. By use of sequence specific hybridization probes it is possible to distinguish between wild type and mutant DNA and thus to determine the mutant fraction in the cfDNA.
Conclusion:
This method is highly sensitive, minimally invasive and allows monitoring of the patient. Even patients with tumors that are not accessible for biopsy can be examined for *RAS* mutations. Another very important aspect is the possibility of detecting new mutations many months before progression of the disease is evident with optical methods like CT and measurement of tumor DNA (ctDNA) is more reliable concerning tumor burden than the standard biomarker CEA. Thus, resistance or therapy failure can be discovered and adjustment of medication can occur much earlier.

DGKL - New Methods and Parameters

DGKL-P077

UPLC-MS/MS method for simultaneous measurement of apixaban, dabigatran, edoxaban and rivaroxaban in human plasma

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Background: Although coagulation assays are usually used for determination of direct oral anticoagulants (DOACs), there are special circumstances in which direct determination of drug concentration in patient blood is essential.
Issues: The fast, precise, and direct measurement of DOACs such as apixaban, dabigatran, edoxaban, and rivaroxaban in patients' plasma gives information about the drug level in patients' blood and is therefore helpful in situations when results of coagulation assays are not valid.
Methods: The method combined straightforward sample preparation, consisting of protein precipitation with methanol containing internal standards (IS), and a purification step of the analytes by a flush/back-flush cycle before the analytical chromatography. After ionization of the analytes by electrospray ionization (ESI) as positively charged ions; molecules were detected using tandem mass spectrometry. 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 enhancement 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 ^{13}C-labeled IS were used to compensate these matrix effects. All DOACs were stable in citrate plasma at -20°C, 4°C, and even at RT for at least 1 month.
Conclusion: We successfully developed and validated an UPLC-MS/MS method for fast, sensitive, and specific measurement of the new generation of oral anticoagulants such as apixaban, dabigatran, edoxaban, and rivaroxaban.

DGKL-P078

Nanodiscs: a novel technology for functional reconstitution and characterization of membrane proteins

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Introduction
Nanodiscs are self-assembled bilayers of phospholipids, stabilized by synthetic, amphipathic helical membrane scaffold protein (MSP). Due to the native-like lipid environment, reconstitution of isolated membrane proteins into nanodiscs enables the stability and functionality of membrane proteins in solution. These reconstituted membrane proteins can be characterized by standard detection methods and are accessible from both the intracellular and extracellular sides.
Motivation
Membrane proteins are involved in many essential cellular processes, however, structural and functional studies of membrane proteins are very challenging due to difficulties generating full functional and native membrane proteins in solution. The aim of our investigation is to isolate membrane proteins directly from human cell lines or other model systems and reconstitute the protein of interest into nanodiscs for diagnostic applications.
Methods
The required cell population or overexpressing cell line was isolated and harvested. Membrane isolation was processed with Dounce homogenization and centrifugation and ultracentrifugation steps. The isolated membrane pellet was solubilized with compatible detergent. MSP, synthetic lipids and stabilizing polymers were added to the pre-solubilized protein target of interest. Removal of detergent was performed with hydrophobic bio-beads and the self-assembled nanodiscs were analyzed and purified via size-exclusion chromatography (SEC) and electron microscopy. Detection of inserted membrane protein was performed with western blot and optical methods.
Results
We established this novel concept with erythrocyte membranes and could successfully insert different integral and peripheral erythrocyte membrane proteins into nanodiscs. The nanodisc fraction was clearly detected and purified via SEC. The insertion of membrane protein of

interest was confirmed by immunological detection methods. Further functional assays as well as interaction assays showed encouraging results.

In addition, initial experiments with human cell culture overexpressing membrane protein target were carried out successfully. Generated nanodiscs with inserted membrane proteins of interest constitute a novel stable format to conserve membrane proteins in their native state, which could be applied for clinical diagnostics.

Conclusion and Outlook

Based on the first results, we show that the nanodisc technology is a promising tool for functional reconstitution of membrane proteins with a native-like lipid environment. We successfully inserted different membrane proteins into nanodiscs. The functionality of inserted proteins could be proved with different interaction biosensor techniques.

This novel technology is a valuable tool for diagnostic applications such as for autoimmune diseases and immune oncology. The transfer of the protein of interest from the cell membrane into nanodiscs with correct folding and full functionality enables innovative possibilities of cell-free assay formats in the future.

DGKL-P079

Non-invasive detection of erythrocyte zinc protoporphyrin by tissue fluorescence spectroscopy on the lip

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Introduction

Worldwide, more individuals have iron deficiency than any other health problem. Most of those affected are unaware of their lack of iron, in part because detection of iron deficiency has required a blood sample. Here we report a non-invasive method to optically measure an established indicator of iron status, erythrocyte zinc protoporphyrin (ZnPP), in the microcirculation of the lower lip.

Methods

For non-invasive measurements, an optical fiber probe is placed in gentle contact with the red vermillion of the lower lip and slowly moved over the surface. Spectral fitting of the blood absorption characteristics to the acquired tissue fluorescence spectra yields the "blood absorption index". The blood absorption index identifies sites on the lower lip where mucosal tissue properties (including hemoglobin concentration, light scattering coefficient, blood vessel size and epithelial thickness) permit quantitative measurement of erythrocyte ZnPP. When such a site is found, it is indicated to the examiner by illumination of a light-emitting diode. The examiner can then initiate the ZnPP measurement, where dual-wavelength excitation (425 nm and 407 nm) is used to reduce the fluorescence background in the difference spectrum by more than 90%. Spectral fitting is then used to quantitatively determine the ZnPP fluorescence, providing immediate results.

The non-invasive method was evaluated in a clinical study on 56 women after childbirth and compared to a reference determination of ZnPP in blood samples by HPLC.

Results

In 56 women, 35 of whom were iron-deficient, the sensitivity and specificity of optical non-invasive detection of iron deficiency were 97% and 90%, respectively. The robust limits of agreement (1.96 x robust standard deviation) were 19 µmol ZnPP/mol heme (95% confidence interval: 14-24, bias: -1). For comparison, the robust limits of agreement between separate determinations by the HPLC method were 16 µmol ZnPP/mol heme (95% CI: 13-19, bias: 4).

Discussion

These results provide proof-of-concept validation for non-invasive detection of erythrocyte ZnPP by optical fiber probe fluorescence spectroscopy of the lower lip. Close quantitative agreement was found between the non-invasive measurements in vivo and a standard HPLC reference assay in blood samples in vitro. Three technical innovations were integrated in the device. First, spectral analysis of the effect of heme absorption on the tissue background fluorescence spectrum identifies suitable tissue sites. Second, the dual-wavelength excitation method reduces the contribution of the background fluorescence by more than 90%. Third, spectral fitting extracts the ZnPP component from the resulting spectrum. The combination of these elements makes possible quantitation of erythrocyte ZnPP in the lower lip in vivo with a precision approaching that of the HPLC method in a blood sample in vitro.

Conclusion
This fluorescence method potentially provides a rapid, easy to use means for point-of-care screening for iron deficiency in resource-limited settings lacking laboratory infrastructure.
Reference
Hennig, G. et al. Non-invasive detection of iron deficiency by fluorescence measurement of erythrocyte zinc protoporphyrin in the lip. Nat. Commun. 7:10776 doi: 10.1038/ncomms10776 (2016).

DGKL-P080

Differential Eicosanoid Response on Gene Expression and Mediator Level as Marker of Coronary Artery Disease

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Background: Eicosanoids are important lipid mediators generated from arachidonic acid (AA) out of membrane phospholipids. Enzymatic metabolization by cyclooxygenases and lipoxygenases mediates the generation of a broad spectrum of eicosanoids. As proinflammatory molecules, platelet aggregation factors and contractors of smooth muscle cells (SMC), these cell derived mediators have been implicated in atherogenesis.
Objectives: Based on the hypothesis that differential regulation of the eicosanoid response on gene expression and mediator level may affect coronary artery disease (CAD), we aimed to investigate the individual eicosanoid response in patients with or without catheter diagnosed CAD as potential marker of minimal and invasive atherosclerosis.
Material and Methods: Heparinized whole blood from patients with (n = 20 (<50 % stenosis)/ 32 (≥50 % stenosis)) or without (n = 40) angiographically confirmed CAD was incubated with or without LPS (100 ng/mL) for 4 h and 24 h. RNA was isolated and target genes of the arachidonic acid (AA) pathway (cyclooxygenase (COX) 1 and 2, prostaglandin E synthase (PGES), PGFS, thromboxane synthase (TXS), 5-lipoxygenase (LOX), 5-LOX activating protein (FLAP) and 12-LOX) were determined by quantitative fluorogenic RT-PCRs. Corresponding metabolites (AA, PGs and hydroxyeicosatetraenoic acids (HETEs)) were analyzed in supernatants using LC-MS/MS.
Results: Patients with CAD ≥50 % showed an increased COX-1 and FLAP mRNA expression after 24 h ($P<0.01$) and a reduced COX-2 mRNA expression after 4 h LPS activation ($P<0.05$) compared to patients without CAD. Patients with CAD revealed a reduced release of AA, 12-HETE and 5-HETE after 4 and 24 h LPS activation ($P<0.05$). Receiver operating characteristic (ROC) curve analysis combining differentially regulated target genes (COX-1, COX-2), mediators (AA), age and gender revealed an area under the curve (AUC) of 83.6 when comparing patient with and without CAD. On the contrary, ROC curve analysis of hsTroponin T revealed an AUC of 61.0, indicating that eicosanoid response in the whole blood activation model might be a better marker for CAD.
Conclusion: Differentially regulated eicosanoid response on gene expression and mediator level in CAD patients suggest that analysis of AA metabolism in an in vitro whole blood activation model may be a promising approach for estimating cardiovascular risk. We are currently standardizing the whole blood activation model for application and validation in large-scale clinical studies.

DGKL-P081

Early serological diagnosis of acute pancreatitis by analysis of serum glycoprotein 2 – lost and found

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Background: Pancreatic glycoprotein 2 (GP2), the major zymogen granule membrane glycoprotein, was reported to be elevated in acute pancreatitis (AP) animal models. Enzyme-linked immunosorbent assays (ELISAs) were developed to evaluate human GP2 isoform alpha (GP2a) and total GP2 (GP2t) as specific markers for AP.
Methods: Monoclonal antibodies to GP2a and polyclonals to GP2t were generated for the development of ELISAs for the detection of GP2a and GP2t in sera of 153 patients with AP of differing disease duration and staging, 24 with chronic pancreatitis, 125 with pancreatic cancer, 118 with

liver cancer, 126 with gastrointestinal cancer, 40 with neuroendocrine tumors, 40 with sarcoma, 109 patients with benign liver/biliary disease, 27 with peptic ulcer, 40 with peritonitis, and 101 blood donors. GP2a and GP2t levels were correlated with procalcitonin (PCT) and C-reactive protein (CRP) in 152 and 146 follow-up samples of AP patients, respectively.
Results: In contrast to GP2t, the GP2a ELISA possesses a significantly better assay accuracy with a sensitivity of 91.7% within the first 3 disease duration days and a specificity of 96.7% (positive likelihood ratio (LR): 24.6, -LR: 0.09). Concentration and prevalence of GP2a were significantly elevated in early AP in contrast to all control cohorts including patients with chronic pancreatitis ($p<0.05$, respectively).
GP2a and GP2t levels were significantly correlated with PCT (rho=0.21, 0.26; p=0.0110, 0.0012; respectively) and CRP (rho=0.37, 0.40; $p<0.0001$; respectively)
Conclusions: Serum GP2a is a specific early marker of AP and analysis of GP2a can aid in the differential diagnosis of acute abdominal pain.

DGKL-P082

Total Hemoglobin – implementation of a reference measurement procedure

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Currently, international standardization for the determination of total Hb (t-Hb) in blood does not exist. Only one entry is found in the JCTLM-data bank of internationally accepted reference measurement procedures (RMP). In Germany the standard DIN 58931 describes two RMPs for t-Hb: the cyanmethemoglobin method and the alkaline haematine detergent (AHD) method. Up to now, the target values for t-Hb in EQA-schemes of RfB-DGKL and INSTAND are reference measurement values (RMVs) certified by the German metrology institute (PTB). Recently it was agreed to transfer the AHD-RMP from PTB to the calibration laboratories of RfB-DGKL and INSTAND. This will enable both EQA organizers to certify t-Hb in EQA samples. Accreditation of the RMP and listing at JCTLM are further objectives. In addition to running comparisons between PTB and a network of three laboratories, the Hannover Calibration Laboratory (RfB) had performed further investigations to verify its RMP.

The photometric AHD-method is based on hemolysis and subsequent complex building of all Hb variants using Triton X-100 (10 g/L) in aqueous NaOH (0.2 M) as detergent. The haematin complex at 574 nm after a delay of ≥ 5 min (endpoint method) was monitored with a UV/VIS-Spectrophotometer Cary 300 (Agilent). The t-Hb concentration was calculated using the published molar absorption coefficient $\varepsilon_{574} = (694.5 \pm 1.9)\ m^2/mol$. The procedure does not need a calibrator.

Intermediate measurement imprecision was found to be 0.4 % and 0.5 % (CV of mean values, measurement on 4 days, 10 single values per day) for aqueous chlorhaemine solution and for blood as samples, respectively. Solutions of Triton X-100 spiked with chlorhaemin are stable and might be used as control materials. A method comparison (n = 40) to a clinical routine measurement system revealed a Pearson's correlation coefficient of r = 0.989. Slope and intercept were 1.03 (SD = 0.02) and -0.1 (SD = 0.3), respectively based on linear regression. Pooled human blood was stored at -20 °C testing the stability as a long term control material. No trend was found during the observation period of 169 days (n = 13 mean values, 10.676 g/dL to 10.459 g/dL). The mean values of measurements of five days of two EQA samples (RfB-DGKL HA 2/16) were 13.88 g/dL and 13.29 g/dL. CVs of respective daily means were 0.8 % and 0.6 %. The recoveries of the RMVs determined by PTB (13.8 g/dL ± 0.02 g/dL and 13.1 g/dL ± 0.02 g/dL) for these materials were 100.6 % and 101.5 %. Standard reference material (JCCRM 912-2, ReCCS, Japan) of 3 certified values (7.80 g/dL, 13.72 g/dL, 17.95 g/dL) was analysed on 4 days (12 single values per material). The recoveries were 99.7 %, 99.1 % and 99.0 %.

The presented results of the Hannover calibration laboratory will strongly support the joint activity for the implementation of an internationally accepted and applied reference system for t-Hb.

DGKL-P083

Nationwide CF implementation in Germany- chances and potential pitfalls

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Objectives
In August 2015, the G-BA voted for the nationwide implementation of the Cystic fibrosis newborn screening (CF-NBS) in Germany. Until it's final realization in 2016, Mecklenburg-Vorpommern (MV) is the only German state, with a CF-NBS for all its newborn in due course of an EU founded Interreg IVa project.

The experience, gained since 2012 is an additional valuable pool of information concerning the optimization of processes in accordance to the proposed CF-NBS screening protocol and for analytical drawbacks.

Methods
The same filter paper as for the regular newborn screening was used for the CF-NGS. A two-step strategy is used since 2012 in MV: first the analysis of the immunoreactive trypsinogen (IRT) and second of the pancreatitis associated protein (PAP). If both analysis are elevated or if IRT is higher than the > 99.9th percentile, the CF-NBS is considered as positive and a sweat test is recommended. The positive CF-NBS reports were communicated latest three weeks after sample receipt and primarily on Mondays in order to ensure a short time span between the report and the actual sweat test. The positive CF-NBS report includes detailed information about the four, in MV existing CF-ambulances. Here, the sweat tests are performed according to the CF guidelines, applying the quantitative pilocarpine iontophoresis (Gibson-Cooke).
Results and Discussion
A profound data base with more than 55.000 screened newborns was established. The acceptance of the CF-NBS reached soon after its introduction more the 99 %. The mean time between birth, positive CF-NBS reports and sweat tests range within the European cystic fibrosis society standards of care recommendations. Furthermore, the applied cut- offs for IRT and PAP detected all 7 positive CF-NBS newborns.

In contrast to our applied two-step strategy, the G-BA added a third diagnostic step- a molecular genetic analysis. In the future, CF-NBS will be positive in case of an elevated IRT and PAP and with at least one mutated CFTR-gene or if IRT is higher than the > 99.9th percentile.

The challenges for the nationwide implementation are divers. Challenging will be the inclusion of the third analytical step, the resulting tight time schedule until the sweat test and the safe use of the altered IRT cut-off to reduce the false positives. Furthermore, reimbursement concerning the sweat tests, the additional laboratory analytic and the tracking are not solved yet.

DGKL-P084

Vitamin D levels in patients with end-stage liver disease are associated with mortality

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Background: The liver plays the central role in many metabolic pathways like protein synthesis and detoxification. Patients with advanced liver cirrhosis and associated complications have a dramatically increased mortality. Aim of our study was to investigate the association between vitamin D levels and parathyroid hormone (PTH) in serum and mortality in patients with liver cirrhosis. Methods: Vitamin D, PTH and calcium as well as albumin, INR (international normalized ratio), bilirubin and creatinine levels were measured (Roche, Germany) in serum and plasma samples of 660 patients on the waiting list or in evaluation for liver transplantation with liver cirrhosis caused by different aetiologies. Results for MELD-score (model of end-stage liver disease) were calculated. Follow up time was 365 days. Results: Vitamin D levels were significantly correlated with albumin (r=0.270; p<0.001), INR (r=-0.249; p<0.001) and bilirubin (r=-0.165, p<0.001) but not with creatinine (r=-0.07; p=0.06). PTH was correlated with vitamin D levels (-0.276; p<0.001) and creatinine (r=0.333; p<0.001) but not with the other parameters. Vitamin D and the parameters of the MELD-Score: bilirubin, INR and creatinine were analyzed in a logistic regression for 365 day mortality. Vitamin D serum concentrations were significantly associated with mortality independently from MELD parameters in uni- and multivariate logistic regression analyses (p=0.006). Conclusion: Lower vitamin D levels were independently associated with mortality. A substitution of vitamin D could be considered in patients with liver cirrhosis and may improve clinical outcome. However further intervention studies are needed for proof of principle.

DGKL-P085

Standardization of Functional Assays for Peripheral Blood Mononuclear Cells for the Use in Clinical Studies

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Background: Peripheral blood mononuclear cells (PBMCs) are composed of lymphocytes and monocytes, which play an important role in the pathogenesis of atherosclerosis. Since differential PBMC functions, such as apoptosis, proliferation, and adhesion, might predispose to atherosclerosis susceptibility, we aim to investigate these cell functions in a standardized manner in different clinical studies.
Objectives: It was the aim of the present study to establish functional cell culture assays for PBMCs in high through-put formats, which are easy-to-use and reproducible in routine laboratory settings. Here, freshly isolated PBMCs instead of cryopreserved cells were used in proliferation, apoptosis and adhesion assays.
Material and Methods: PBMCs were collected in mononuclear cell preparation tubes (BD Vacutainer CPT; BD Biosciences) and isolated according to the manufacturers' instructions. Before functional experiments, the cells were either kept on ice or in an incubator at 37°C (5% CO_2) for 1-6 hours. Cell proliferation was measured by CellTiter-Glo Luminescent Cell Viability Assay (Promega) on day 1 to 6 after isolation of the cells. Apoptosis was induced by staurosporine and quantified using the Caspase-Glo 3/7 Assay (Promega) after 24 hours. Adhesion was quantified by CellTiter-Glo Luminescent Cell Viability Assay (Promega) in relation to standard curves.

Results: Proliferation and apoptosis assays were established in 384-well plates. Adhesion assays were performed in 96-well plates. An immediate processing of the cells was necessary to avoid cell death. Cells, which were stored on ice, revealed a shortened survival compared to cells that were kept at 37°C before processing. Quantification of apoptosis was robust and reproducible also after different lengths of incubation (24 h + 0, 2, 4 or 6 h). The same held true for proliferation experiments (24-120 h + 0, 2, 4 or 6 h) whereas for adhesion experiments, exact timing was essential.
Conclusion: Preliminary results of the current study suggest that high through-put functional cell culture assays for PBMCs are feasible and that special attention should be paid to the immediate processing of the cells. In ongoing work, the assays are further standardized to allow application in clinical routine settings.

DGKL-P086

Immunoturbidimetric Procalcitonin Assay on Abbott Architect c8000

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Background
Procalcitonin (PCT) is a relatively new marker used in diagnosis of bacterial infections; especially for monitoring patients with severe systemic infections. The availability of a latex-enhanced immunotubidimetric assay offers new perspectives with regard to fast accessibility and a high degree of automation. In our study, a modified method on Abbott Architect c8000 was evaluated in order to prepare the CE marking.
Materials and Methods
Performance characteristics of the latex-enhanced immunoturbidimetric PCT- assay (6-point calibration) were evaluated on Abbott Architect c8000. Linearity, analytical sensitivity, precision, calibration stability and on-board stability of the reagent were tested with four different serum sample pools and 2 controls (high and low) provided by the manufacturer. New immunoturbidimetric-assay was compared to immunological method on miniVidas (Biomérieux) using routine sera. Samples were divided into 3 groups depending on the PCT concentration: < 0.5 ng/mL, 0.5-2.0 ng/mL and >2.0 ng/mL.
Results
Calibration stability was given for 14 days, without any deviation. Inter-assay precision show CVs of 4.5% (PCT 4.7 ng/mL), 5.0% (PCT 2.9 ng/mL), and,12.7% (PCT 0.5 ng/mL) respectively.

Using the zero-calibrator, 'limit of blank' (LoB) was 0.0 ng/mL and 'limit of detection' (LoD) was 0.12 ng/mL. Linearity, given up to 35 ng/mL, was tested by diluting a high PCT patient serum sample (37.4 ng/mL) with a patient serum sample with a low PCT content (0.53 ng/mL). Recovery was 67% (5:95 dilution) and 98% (95:5 dilution).

The comparison of the new immunoturbidimetric assay to the miniVidas using 134 patient sera samples showed an acceptable correlation. Equivalent interpretation was found for 73.9% of all samples. 16.4% were assigned to a higher group of the miniVidas whereas 9.7% of the samples were classified higher using the new immunoturbidimetric assay.
Conclusion
The new latex-enhanced immunoturbidimetric PCT test provided by DiaSys Deutschland Vertriebs-GmbH offers a fully automated assay on Abbott Architect c8000 for the first time. Test shows a convincing performance for use in the hospital setting. Data evaluated in this study allow a CE marking of the instrument application. For better comparability, the standardization of the PCT- assays needs to be improved, especially in the decision range of 0.5 ng/mL. Unfortunately, there is no international standard preparation available at the moment.

DGKL-P087

Is there a different systemic response for stress serum biomarkers in patients undergoing minimal-invasive and conventional colorectal surgery?

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Background
Minimal colorectal surgery has become a routine treatment option for colorectal cancer patients. Compared with open surgery minimal invasive approaches has advantages in reduced blood loss, shorter stay in hospital and reduced overall mortality. However, there is only limited knowledge about the stress burden and consequences in both surgical treatments.
Objective
We studied for the first time the dynamic course of copeptin, a preprohormone of antidiuretic hormone (ADH) during and after minimal-invasive and open surgery.
Material and Methods
22 patients (15 m, 7 w) with indication for colorectal surgery were consecutively included (minimal-invasive n=12, open n= 10), median age 64,5 ys (26-88 ys). Blood was taken before, intraoperatively and postoperatively. Copeptin (pmol/L) was measured in serum using trace technology (Brahms, Hennigsdorf). Clinical chemistry was analyzed with standard methods (Roche-Diagnostics, Mannheim; Sysmex, Norderstedt).
Results
Copeptin levels showed a significant and parallel rise (x 10) in both surgical approaches without relevant differences between the treatments. 24 hours after surgery serum level were still x2-2,5 compared to baseline. 4 patients showed no increase in copeptin levels.
Conclusion
In both surgical treatments copeptin was released to a comparable extent indicating a systemic stress reaction of the patients. However, some patients appear to be resistant to stress by surgery, despite open or minimal-invasive treatments. The non-responder did not differ in blood pressure or other analyzed biomarkers.

Presently we are analyzing steroid stress hormones and inflammatory biomarkers to elucidate the different stress response followed by surgical treatment. Long-term studies with more patients will be necessary to evaluate the role of stress and inflammatory biomarkers on the clinical outcome.

DGKL-P088

Enhanced analytical performance of two assays for the determination of 1,25 (OH)2 Vitamin D in serum that feature automated extraction, benchmarked by LC-MS as a reference

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Background Determination of 1,25 (OH)2 Vitamin D (1,25(OH)2VitD) serum levels is analytically challenging. In the past, only radio-immuno assays were available for the determination of 1,25(OH)2VitD with sufficient sensitivity. Our current assay requires a manual purification step prior to measurement of this parameter, making the workflow less robust and more labor-intensive. Now two tests that include an automated extraction step - one assay from DiaSorin (on the immunoassay analyzer LIAISON) and another assay from IDS (on the IDS iSYS immunoanalyzer) - are commercially available.
Method We compared both tests for the quantitative determination of 1,25(OH)2VitD serum levels with a mass spectrometry-based test as a reference method. 1,25(OH)2VitD has been measured in remnant patient samples sent to our institute for determination of this parameter.
Results 1,25(OH)2VitD serum concentrations ranged from 5.0 - 184 pg/mL for the Diasorin and from 7.5 to 202.5 pg/mL for the IDS iSYS test, respectively. Particularly in the higher concentration range, measurement with the IDS assay revealed higher 1,25(OH)2VitD concentrations compared to the Diasorin test. To evaluate the accuracy of both tests, we compared the results with those obtained via LC-MS (liquid chromatography – mass spectrometry) as a reference method. The concentrations measured with the Diasorin test correlated better with the mass spectrometry results.
Conclusion In comparison to the assays available so far, the DiaSorin and the IDS iSYS test include an automated extraction step that allows for a faster determination of 1,25(OH)2VitD serum levels with less hands-on time. 1,25(OH)2VitD serum concentrations obtained by the Diasorin test were in better agreement with the LC-MS reference method.

DGKL-P090

A novel Interpretation of S100B in Plasma Samples as a Quick and Sensitive Marker for Minimal Brain Injury

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S100-beta (S100B) is a member of the S100 family proteins, which are known as calcium binding proteins with various regulatory functions. It has been shown that the glial cell and especially astrocytes in the central nervous system are the source of S100B. A release of this factor after a trauma leads to an elevated S100B level in the blood stream. Various studies have characterized the role of S100B in the diagnostics and monitoring of brain trauma.

A well-established application of S100B is as a tumor marker in patients with malignant melanoma. The various reagents available on market to test S100B concentrations are based on ELISA assay and thus are not suitable for time critical diagnostics. Unfortunately the offered automated system is only validated for diagnostics in serum samples.

In order to optimize the work-flow in cases of suspected brain damage an optimal laboratory approach should be establish, considering the timing of the ready result. Because the serum samples require a certain intermission for the complete blood coagulation these samples are not suitable for emergency diagnostics. Avoidance of technical complications such as micro thrombus influence does not allow shortening of the pre-analytical coagulation period. In our study we aim to assess the plasma concentration of S100B in patients with brain injury. The reduced turn-around-time of plasma samples compared to serum are of a great value during the assessment of patients with head trauma. Thus the result of the laboratory test can be available together with or even earlier than CT. An elevated S100B might prompt the radiologist and the physician to reevaluate the findings of a patient with a mild trauma and normal GCS-score and to search more carefully for minimal injuries which might cause a secondary intracranial bleeding.

Additionally, we present here a case report of one of our patients where the elevation of S100B appeared to be a more sensitive marker for the risk of secondary bleeding compared to CT-imaging.

Furthermore, we would like to propose a different interpretation of S100B plasma level in the algorithms for management of patients with suspicion of minimal brain injury.

DGKL-P091

Conventional constituents in cerebrospinal fluid (CSF) are new parameters of CSF analysis, revealed with Marburg CSF model

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Background: Leukocytes, lymphocytes, total protein are conventional CSF constituents to diagnose inflammations / barrier disturbances in human central nervous system (CNS). In CSF of healthy humans, these constituents vary: leukocytes (mainly lymphocytes): 0-1/µl in ventricle V-CSF, up to 2/µl in cisternal C-CSF, up to 5/µl in lumbar L-CSF; total protein (medians): 110 mg/l in V-CSF, 200 mg/l in C-CSF, 325 mg/l in lumbar L-CSF. – Questions: What is the cause for diversity in leukocytes / protein in CSF? From where do come leukocytes / proteins in human CSF? – Material: V-CSF is collected by drainage of lateral ventricles (Vs); C-CSF / L-CSF by suboccipital / lumbar punctures, peripheral venous blood by cubital vein puncture; thoracic lymph by thoracic duct puncture. – Methods: In CSF leukocytes / erythrocytes are counted unstained in calibrated Fuchs-Rosenthal chamber under microscope using 400-fold magnification: coefficient of variation (CV) <40%. Total leukocytes in blood and lymph are analyzed with fluorescence-activated cell sorter: CVs <9%. Total protein is detected with the Biuret method in 0.5 ml CSF, precipitated with 30 g/l trichloroacetic acid: CV <8%; detection limit: 40 mg/l. – Results: Protein in V-CSF is secreted by 4 choroid plexus: Special tight junctions in apical plexus epithelium and a basement membrane screen blood plasma according to molecule size of proteins: albumin > IgG > IgA > IgM. Total protein increases from 200 mg/l in C-CSF to 421 mg/l in lumbar CSF by reflux of thoracic lymph which contains ˜40 g/l lymph proteins. Leukocytes (lymphocytes) are pressed from blood through 7 barrier-free circumventricular organs (CVOs) into V-CSF; since blood-brain barrier, localized in CNS capillaries, is firmly locked and blood-CSF barrier (bCSFb) in choroid plexus is impermeable to blood leukocytes. Lumbar leukocytes are the sum of CSF blood lymphocytes plus lymph cells refluxed from thoracic duct into lumbar CSF. – Discussion: V-CSF proteins are diluted with intramural CSF (protein-free filtrate of CNS capillaries and oxidation-water of CNS cells) in relationship >80% blood plasma proteins (filtered through bCSFb in choroid plexus) and <20% intramural CSF. L-CSF is the sum of V-CSF plus refluxed thoracic lymph. Oscillating CSF drains along cranial and spinal nerves: small proteins (albumin) drain out easier than large ones (IgM), producing the CSF sink-effect. – Summary: The conventional CSF components leukocytes / lymphocytes and total protein are new

parameters for analysis of CSF normality revealed with the Marburg CSF model: hyper-CSF-, hypo-CSF production syndromes (brain water syndromes); CVO permeability syndrome, tight junction syndrome; in lumbar CSF: CSF flow syndromes: SOP CSF/ lumbar CSF-, CSF outflow-, lymph back flow- (thoracic duct)-syndromes; CSF loss syndrome: CSF outflow along spinal nerves.

DGKL - POCT

DGKL-P092

Point-of-care convenient testing of activated partial thromboplastin time (aPTT) in whole blood and in plasma: A comparison with standard laboratory plasma coagulation method

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Background. Coagulation measurement of activated partial thromboplastin time (aPTT) is generally performed in the central laboratory and can take more than 1h. The delay in obtaining the test results limit their use in monitoring of coagulation in coagulation disorders after blood loss, in perioperative settings or in the intensive care unit. To identify risk of bleeding or thrombotic complications in these patients in time it needs more rapid coagulation testing. Looking for a reliable POCT analyzer of activated partial thromboplastin time (aPTT) we compared the coagulation measurements of two point-of-care convenient devices with those of the standard laboratory plasma coagulation method.
Method. Comparison was performed by paralled measurement of aPTT in 100 venous Citrate anticoagulated whole blood samples from hospital patients left after routine clinical laboratory investigations. aPTT was measured in patient near devices both in whole blood by Hemochrom jr. signature (Keller Medical GmbH, Bad Soden, Germany) and in plasma by one-channel-ball coagulometer Merlin Medical MC1 (ABV Medizin und Technik, Lemgo, Germany) paralled by the determination of aPTT in plasma by the usual laboratory testing (ACL Top, Fa. Werfen, Kirchheim, Germany). T-test was used to determine differences in aPTT values for the 3 methods. The Bland-Altman difference analysis and the Passing-Bablok agreement test were performed to compare the results data.
Results. In the range of 17 sec to 70 sec laboratory aPTT results in plasma differed significantly from point-of-care values in whole blood ($t(\text{Hemochrom jr. signature};99) = 9{,}2$; $P < 0.001$) and in plasma ($t(\text{Merlin Medical MC1};99) = 12.3$; $P < 0.001$). When compared with laboratory by Bland-Altman method, Hemochrom jr. signature was an average of +3.9 sec higher, but the 95% limit of agreement was very broad at 51 sec approximately (95% CI -21.4 to 29.1). Merlin Medical MC1 values fared better, being on average +7.3 sec higher, with the 95% limit of agreement being approximately 31 sec (95% CI -8.3 to 22.9). Passing-Bablok analysis of data determined a low agreement of measurement with a poor Pearson's correlation coefficients of 0.66 for the correlation between Hemochrom jr. signature /laboratory and 0.85 for the correlation of Merlin Medical MC1/laboratory.
Conclusion. In the present study, whilst aPTT point-of-care measurement in whole blood correlated poorly with laboratory standard, aPTT in plasma with POC convenient device revealed even better agreement. However, the bias of both POCT devices could lead to clinically relevant differences in aPTT values between laboratory –based and POCT-based measurement. With these limitations in mind, aPTT values obtained by POCT devices should be interpreted with caution and should be verified with a laboratory analyser especially when using in diagnosis and treatment.

DGKL-P093

Evaluation of the performance of two point-of-care glucose meters in a paediatric hospital

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Background: POCT glucose measurements are often performed in clinical routine in patients, both ambulatory and on ward. Reliable results are essential not only in the normoglycemic range. Physiologically low values in preterm babies and newborns are a challenge to the accuracy of measurements. The results of the primary control must also be present immediately.
Aims: Evaluation of clinical accuracy and performance in glucose meters in routine use in our hospital, in supplementation to our previous study published in 2013.
Methods: The Accu-Chek inform II (Roche) and the StatStrip Glucose Hospital Connectivity Meter (Nova Biomedical) were tested on 126 (Roche) and 157 (Nova Biomedical) whole blood samples from patients with a simultaneous blood gas analysis (heparinized capillary whole blood) in 2015. Accuracy (bias) of the meters were examined by comparing the results of the meters with the results of blood gas analyzers (Radiometer ABL90) routinely used for glucose measurements in our hospital.

Results: The results of the Accu-Chek and StatStrip glucose meter correlated very well with the reference routine method across a wide glucose concentration range (33-363 mg/dL). The 95% confidence intervals for slope
(Nova: 0.9825, CL 0.9542 – 1.0073;
Roche: 0.9452, CL 0.9207 – 0.9740) are similar, the intercepts are slightly different (Nova -3.2982, 95%-CL -5.6204 – -1.2137;
Roche +5,7123, CL +3.3182 – +7.8567 mg/dL).
Mean Nova glucose 123.92 versus ABL90 130.75 mg/dL,
Mean Roche glucose 106.23 versus ABL90 105.90 mg/dL),
Median Nova 101 versus ABL90 108 mg/dL,
Median Roche 82 versus ABL90 80 mg/dL).
Conclusions: Both glucose meters showed good clinical accuracy and performance for measuring and monitoring glucose levels in our patients, with special respect to preterm infants and diabetic patients, and therefore can act as a perfect alternative to a blood gas analyzer for measuring blood glucose in these patients.

DGKL-P094

Comparison of point-of-care troponin assays with fully automated laboratory high sensitivity troponin assays in the diagnose of myocardial infarction

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Measurement of cardiomyocyte injury biomarker, preferably high sensitive cardiac troponin (hs-cTn), complements clinical assessment and resting 12-lead ECG in the diagnosis, risk stratification and therapy of patients with acute coronary syndrome (ACS). The European Society of Cardiology (ESC) recommends 0 h /3 h NSTEMI rule-out algorithm using hs-cTn assays in the assessment of patients with acute chest pain, where absolute change in hs-cTn level over three hours is evaluated in conjunction with chest pain and clinical presentation [1]. Due to the short turnaround time, point-of-care (POC) tests may facilitate faster managing and triaging patients out of the emergency department. However, POC tests for cTn are not yet as thoroughly evaluated as fully automated cTn assays which form the basis of current ESC guidelines. Therefore, this study aims at:

1) assessment of the diagnostic performance of POC tests for cTn according to the 0 h/3 h NSTEMI rule-out algorithm [1] and in comparison with two fully automated laboratory tests (hs-cTnT Elecsys and hs-cTnI Architect)
2) assessment of the analytical quality of POC tests to classify their clinical utility according to the scorecard designations proposed by F.S. Apple [2]
3) evaluation of user-friendliness

Following POC tests for cTn are included in this evaluation: Triage Cardio with Next Generation TnI (Alere), AQT90 FLEX TnI and TnT (Radiometer), Pathfast TnI (Mitsubishi), and Stratus TnI (Siemens).

DGKL-P095

Implementation of a Point-of-Care concept at the University Hospital Bonn

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Background: Point-of-care testing (POCT) has steadily increased in recent decades. POCT allows rapid measurements right next to the patients and is usually performed by non-laboratory staff. Correct and legally binding execution of measurements is of great importance.
Aim: Implementation of a legally binding and effective POCT concept at the University Hospital Bonn.
Materials and Methods: The University Hospital Bonn is a maximal care hospital with 1250 beds. Currently, 550 000 POCT measurements are carried out annually by approximately 2600 POCT users, while 400 000 laboratory measurements are carried out in the central laboratory. In line with the guidelines of the German Medical Association (RiliBÄK), we developed and implemented a POCT concept regarding blood gas analysis, blood glucose and coagulation tests.
Results: Responsibility levels were assigned as follows: The medical director of the central laboratory is responsible for the internal and external quality controls. A POCT coordination team was established for user administration and initial and follow-up training of POCT users.

Training sessions are carried out weekly and upon individual appointment. POCT users are activated for measurements only after successful completion of the training. POCT users are responsible for the patient and control sample measurements and for refilling, ordering and discharging of consumables. POCT device manufacturers are responsible for maintenance and repairs. To appoint a capable committee for the constructive and trustful supervision of the entire POCT diagnosis, a POCT commission was established by the clinical director of the University Hospital Bonn. Its members are the medical director of the central laboratory, the physician and medical technical assistants of the central laboratory as part of the POCT coordination team and members of the purchasing department, POCT users (physicians and nurses), the clinic pharmacy, the IT department for medical engineering, the Nursing Director and the department for quality and risk management. The POCT commission reports directly to the executive board.

Conclusion: Against the background of the legally binding RiliBÄK we have not only clearly defined the necessary areas of responsibility for the required continuous internal and external quality controls, but also implemented a working user training concept with high administrative requirements.

DGKL - Therapeutic Drug Monitoring - Toxicology

DGKL-P096

An enzyme-linked immunosorbent assay for therapeutic drug monitoring of vedolizumab

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BACKGROUND:
Vedolizumab (VLZ), an a4ß7 integrin antagonist, is a therapeutic monoclonal antibody recently approved for use in moderate to severe ulcerative colitis (UC) and Crohns disease (CD). Part of the interindividual differences in response to VLZ treatment may be explained by interindividual variability in pharmacokinetics.
METHODS:
Microtiter plates were coated with anti-VLZ specific monoclonal antibody. Samples diluted 1:200 were added on a microtiter plate for specific binding, and bound VLZ was detected using peroxidase-conjugated goat anti-human immunoglobulin G Fc fragment (HRP-anti hIgG). Trough serum concentrations of VLZ were analyzed in 86 samples of 21 adult UC patients and compared to concentrations measured by in-house developed LC-MS/MS assay (Christ et al., J Crohn's Colitis 2016, S1).
RESULTS:
Linearity testing of the ELISA was performed by analysis of two serially diluted patient samples; the coefficients of variation (CV%) were below 8%. The limit of quantification (LoQ) for VLZ determination in human serum samples was 0.0071µg/mL. The intra-assay variation (n=20) was 8.57% for 9.55 microg/mL and 6.54% for 18.9µg/mL. The inter-assay variation (n=40) was 7.10% for 28.5µg/mL and 8.33% for 35.7µg/mL. No false positive signals were detected in samples spiked with TNFa blockers (infliximab, adalimumab, golimumab). In the samples of patients treated with VLZ the trough level ranged from 0.02 to 71.01µg/mL. VLZ results of ELISA and an in-house developed LC-MS/MS assay showed a correlation coefficient (R^2) of 0.96 (Fig.1).
CONCLUSION:
This developed ELISA method is rapid, accurate and reproducible, and may be useful for pharmacokinetic-pharmacodynamic studies, as well as in therapeutic drug monitoring of vedolizumab.

DGKL-P097

Simultaneous measurement of Piperacillin, Levoflaxacin and Meropenem serum levels on amaZon speed (Bruker Daltronik)

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Background
Since microbial resistances are a major problem especially in intensive care units, LC/MS therapeutic drug monitoring is increasingly used for antibiotics to provide an individual therapy and prohibit new resistances.
Objective
The aim of the study was to establish a LC/MS based in-house method for antibiotic quantification, which is easy to integrate into clinical workflow the simultaneous measurement of Piperacillin, Levoflaxacin and Meropenem.
Material and Methods
ICU patient serum samples were analyzed with the amaZon speed (Bruker Daltronik).

Calibrators and Samples were separated on a Hypersil Gold C8 Column (Thermo Scientific) using a 5 min isocratic HPLC method on a Dionex Ultimate 3000.

The amaZon speed (Bruker Daltronik) was equipped with the standard electrospray source (nebulizer pressure 30 psi; drying gas 300 °C at a flow of 9 L/min) and operated in positive ion mode. Full Scan MS/MS spectra were acquired from *m/z* 120 to 550, in UltraScan (32,500 m/z s-1) mode.
Results
This method provides a simple extraction procedure, as well as a valid, sensitive, and specific liquid chromatography-tandem mass spectrometry assay for the simultaneous quantification of Piperacillin *m/z* 518 ® 359, levofloxacin *m/z* 362 ® 318 and meropenem *m/z* 384 ® 340 in human plasma.

The method is accurate and precise, the intra-assay and inter-assay precision for quality control samples ranged within 2.8 and 5.8 % respectively.
Conclusion & Discussion
Over 800 patient samples were analyzed till June 2016.

The described method is a fast, cost efficient and highly reliable assay for the quantification of antibiotic serum levels. The assay requires adequate hardware and skilled staff. The implementation of the assay into the clinical routine provides an individual therapeutic drug monitoring.

DGKL-P098

Integration of Time in Therapeutic Range (TTR) into Lab Reports to improve care of patients with oral anticoagulant therapy. A first experience report

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Introduction
Oral anticoagulation with vitamin K antagonists (VKA) e.g. with warfarin is the standard therapy. Therapeutic monitoring is performed by measuring the prothrombin time (PT), results are reported as the International Normalized Ratio (INR). However, during therapy many patients are above the therapeutic range (increases the risk of bleeding) or below the therapeutic range (increases the risk of thromboembolic complications).
Method
To support physicians in management of oral anticoagulant therapy and to improve coagulation lab reports we integrated the time in therapeutic range (TTR) according to Rosendaal, a method which INR-specific person-time is calculated by incorporating the frequency of INR measurements and their actual values, assuming that changes between consecutive INR measurements are linear over time. We investigated the added value of cumulative tabular TTR values with a questionnaire sent to physicians in Hamburg.
Result
420 physicians in Hamburg were contacted to complete a questionnaire on cumulative tabular TTR reporting. 119 (28.3 %) physicians responded.

79 % of physicians knew about TTR values
90 % of physicians were able to interpret TTR values
46 % of physicians stated that cumulative tabular TTR values enabled a more rapid therapy adjustment
99 % of physicians stated a cumulative tabular TTR report is self-explaining
87 % of physicians a cumulative tabular TTR report provides a better monitoring of therapy
76 % of physicians recommend to include TTR in lab reports
69 % of physicians will change to direct oral anticoagulant therapy (non-vitamin K antagonists) if the cumulative tabular TTR report shows unstable VKA therapy
44 % of physicians use cumulative tabular TTR values routinely.

Conclusion
Our survey showed that a graphical TTR report is providing additional information and easier monitoring of oral anticoagulation with vitamin K antagonists. However, only 44 % of physicians adopted the use of TTR values in patient care. Therefore, further training of physicians is required.

DGKL - Diagnostics of non-blood based Specimens (Urine, CSF, others)

DGKL-P099

Implementation of a novel urinary biomarker TIMP-2 x IGFBP7 for early detection of acute kidney injury risk in surgical ICU patients

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Background: The prognosis of patients with acute kidney injury (AKI) is still poor. Intervention for prevention and therapy of AKI are currently only initiated in the late phase of already established injury; therefore, benefits remain limited. Since serum creatinine and cystatin C, standard laboratory parameters of kidney function, are too insensitive for early recognition of AKI, biomarkers of early cellular injury were prioritized as a research target in the last decade. Recently, combination of two novel renal cell cycle arrest proteins, i.e. urinary tissue inhibitor of metalloproteinases-2 and insulin-like growth factor-binding protein 7 (TIMP-2×IGFBP7), was described and validated for prediction of moderate and severe AKI risk in critically ill patients.
Objectives: Implementation and validation of TIMP-2×IGFBP7 in daily laboratory routine as part of the "Biomarker guided intervention for prevention of acute kidney injury (BigpAK) Study" for early detection of AKI risk in high risk surgical ICU patients.
Methods: The BigpAK Study (NCT02500394) is a single-center prospective study evaluating the impact of early intervention (correction of hypovolemia and avoidance of nephrotoxins) on the development of AKI in patients after major surgery. Eligible patients are screened for increased level of urinary TIMP-2×IGFBP7 and randomised into a standard care group or an interventional group. The biomarker measurement occurs after admission to ICU and after 12 hours. Using Astute Medical NephroCheck® Test, a unit-use immunofluorescence assay on the ASTUTE140® Meter with a 20-minute reaction time, AKI risk (TIMP-2×IGFBP7) is derived from $(c_{TIMP\text{-}2} \times c_{IGFBP7})/1000$ with a 0,3 $(ng/ml)^2/1000$ cutoff. In a pre-analytical phase, precision and accuracy of the test were checked, SOPs prepared and laboratory staff trained for 24-hour test availability.
Results: Successful integration of the test into the established laboratory QM system was achieved by one-month training of the 26 MTAs comprising shift duty staff with intelligible SOPs, as well as, by sufficient analytical precision and accuracy. In total, 169 patients were screened between May 2015 and May 2016. 79 patients had pathological values of TIMP-2×IGFBP7 and were randomized into standard or interventional group. Analysis of the LIMS dataset revealed only one erroneous result out of 269 total measurements (0,4 %) and a median turnaround time of 70 min.
Conclusions: Implementation of novel biomarkers, such as TIMP-2×IGFBP7, in the daily laboratory routine requires standardization of care and coordinated interdisciplinary approach from both, clinical and laboratory medicine. Improved logistics of the analytical process from blood draw to automatic result transmission and integration of the test into large clinical chemistry platforms in the future may decrease the turnaround time and enable very fast therapeutic intervention in AKI risk patients leading to improved clinical outcome.

DGKL-P100

A novel automated turbidimetric platform for cerebrospinal fluid diagnostics

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BACKGROUND: Nephelometric methods for diagnostics of barrier dysfunction and inflammatory diseases of the CNS are well established. However, there is limited experience about automated turbidimetric analysis of cerebrospinal fluid (CSF) and serum proteins for CSF diagnostics.
OBJECTIVE: We evaluated the recently introduced turbidimetric Optilite® analyser (The Binding Site) in comparison to the established BN ProSpec® (Siemens) nephelometer in a clinical routine setting.

MATERIALS AND METHODS: CSF and serum samples of 66 patients with medical indication for lumbar puncture were consecutively analyzed using the BN ProSpec and Optilite. Results for barrier dysfunction and inflammation (Reiber scheme) were compared and evaluated in the clinical context. Measurements for accuracy and precision were performed using the European Reference Material ERM-DA470k/IFCC.
RESULTS: Albumin, IgG, IgA and IgM in serum and CSF showed a good accordance between the Optilite and BN ProSpec results. Bland-Altman analysis of the quotients QAlb, QIgG, QIgA and QIgM revealed a mean difference (±1.96 SD) of 4.3% (±16), 1.6% (±23.3), 5.7% (±20.8) and 7.3% (±45.8), respectively (Optilite-BN ProSpec). Reiber schemes showed concordant results in the evaluation of the barrier function in 61 out of 66 patients (92.4%), 3 cases revealed a barrier dysfunction only on the Optilite, 2 cases only on the BN ProSpec. Intrathecal synthesis (IF >10%) of IgG/IgA/IgM was determined in 6/0/3 cases by the Optilite compared to 5/1/4 on the BN ProSpec, resulting in 1/1/3 divergent cases, respectively. Results for CSF were below detection limit in 6 vs. 2 for IgA and in 10 vs. 24 cases for IgM on the Optilite vs. BN ProSpec, respectively. Measurements with the European Reference Material ERM-DA470k/IFCC showed high accuracy and precision of the Optilite.
DISCUSSION: The divergent results between the two methods concerning barrier dysfunction in 5 patients can be explained by imprecision variabilities near the cutoff. One case with intrathecal IgG synthesis was detected by the Optilite only. This patient suffered from multiple sclerosis and oligoclonal bands were positive. Another patient with intrathecal IgA synthesis was detected by the BN ProSpec only, IgA in CSF was below the Optilite detection limit in this case. Concerning intrathecal IgM synthesis, 2 patients were detected by the BN ProSpec only, another case by the Optilite only. In the latter patient IgM in CSF was below the BN ProSpec detection limit. The other two patients suffered from acute disseminated encephalomyelitis and a suspected vasculitis of the CNS, which might cause an intrathecal IgM synthesis. The divergent results are most likely due to known limitations of precision in terms of IgM detection in CSF.
CONCLUSION: The novel turbidimetric Optilite analyser performed well in comparison to the established BN ProSpec nephelometer in terms of diagnostic performance and seems to be a valid platform for routine CSF diagnostics.

DGKL-P101

Automated immunostaining of bone marrow specimens

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Background: Evaluation of bone marrow morphology is crucial for the diagnosis of a variety of hematologic diseases. Additional immunostaining is important for several aspects of alterations in bone marrow, e.g. to evaluate tumor cell infiltration of bone marrow and to assess subsets of lymphocytes or immature cells.

To facilitate cytology processing (staining of peripheral blood smears, cytospin specimens and others) an automated or semi-automated solution has been developed. Smears of peripheral blood are processed in a way that reduces hands-on time and costs but still provides a satisfactory quality. Easy to achieve for standard staining of peripheral blood samples (e.g. pappenheim staining) technological advances now focus on automation of immunological staining on a broad variety of sample types.

Bone marrow specimens proved to be very challenging if the automated procedure has to meet both requirements preserving the morphology and being compatible with automated sample processing.

We identified factors causing deterioration of the morphological quality of the specimens during the staining protocol and provide a strategy how to circumvent these problems.
Material and Methods: Bone marrow specimens were subjected in parallel to both manual processing and automated staining and quality of morphology and immunostaining was assed afterwards. Variation of individual protocol steps (e.g. fixation protocol, staining time und buffers) have led to identification of one major deterioration cause in automated bone marrow staining.
Results: Many fixation protocols for immunostaining of bone marrow specimens strive to preserve immunoreactivity completely to make the most of this technique. These protocols usually use methanol or acetone based fixatives and tend to avoid formalin and other protein crosslinking agents. What works well in manual processing gives unsatisfactory results in automated processing. We identified unipolar carbohydrate substances used to act as liquid cover slip as cause for the majority of deteriorating effects specifically in bone marrow specimens. To avoid this and similar effects, introduction of formaldehyde is usually recommended at a concentration of 4-10% (w/v). Unfortunately, this fixation although preserving morphology does interfere with immunoreactivity of bone marrow cells which renders this technique quite useless for hemato-oncologists. We evaluated the factors affecting protein cross-linking during cell fixation in bone marrow specimens, using immunostaining for CD34 and CD138 as model case.
Discussion: We found that adjustment of the formaldehyde concentration and reduced temperature prevents some of the cross-linking effects with the formaldehyde still helping to maintain the morphology. Furthermore, the efficiency of this protocol adaption relies on bone marrow cellularity and specimen age.

DGKL - Laboratory Management and Quality Assurance

DGKL-P102

Chancen und Risiken von e-Health in der Labormedizin: Vorschläge für Laborbefunde in der persönlichen Krankenakte durch die AG Labormanagement der DGKL

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Aus dem Lifestyle- und Wellnessbereich werden den Kunden vielfältige Apps angeboten, die Kundendaten ansprechend präsentieren. Auch der politische Wille fordert die Digitalisierung in der Medizin mit dem sog. „E-Health-Gesetz". In der nationalen elektronischen Patientenakte sollen dazu auch Laborbefunde gespeichert werden. Dafür notwendig ist allerdings eine ausreichende Harmonisierung von Prä-Präanalytik (Terminologie, Testprofile, Testungsintervalle), Präanalytik (Abnahmezeiten, Patientenvorbereitung, Probentransport und Probenlagerung), Analytik (Probenqualität, Methode, Kalibrator, Qualitätssicherung) bis hin zur Postanalytik (Einheiten, Datenformate, Referenzintervalle, Entscheidungswerte). Diese Harmonisierung kann aufgrund der vielen verschiedenen Laboruntersuchungen und testspezifischen Besonderheiten trotz sehr vieler nationaler und internationaler Aktivitäten noch nicht als abgeschlossen gelten. Andere Herausforderungen elektronischer Patientenakten liegen bei der Datensicherheit (d.h. der Integrität der Laborbefunde) und dem Datenschutz unter Berücksichtigung der informationellen Selbstbestimmung der Patienten und weiterer Gesetze wie dem Gendiagnostikgesetz (GenDG).
Schlussfolgerung: Wir empfehlen aus Gründen der Patientensicherheit, sich bei der nationalen elektronischen Patientenakte auf wenige ausgewählte Laborbefunde zu beschränken, die unmittelbar zur Dosisanpassung von Medikamenten notwendig sind und die so den elektronischen Medikationsplan unterstützen. Die AG Labormanagement der DGKL hat eine konkrete Liste entwickelt, die den einheitlichen patientenbezogenen Medikationsplan der Arzneimittelkomission der Deutschen Ärzteschaft ergänzen könnte.

DGKL-P103

Retrospectively estimated reference limits for total protein

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Background
Manufacturers of total protein in blood often report reference limits from the literature. In this study, we evaluated the applicability of the Excel-Tool "Reference Limit Estimator" to the Biuret-method for measuring total protein in serum.
Materials and Methods
We used a retrospective analysis of patient data from two hospital-laboratories (Bremen/Bremerhaven) in comparison with a laboratory predominantly for outpatients (Villach). In Villach and Bremen the total protein was measured by cobas c501 (Roche) and in Bremerhaven by the Architect c8000 (Abbott). Both manufacturers operate with "Biuret-method". The "Reference Limit Estimator" (version 20151017) was used for calculation of reference intervals. If possible patients from intensive care units and pregnant women were excluded from the analysis and only the first value was used for the estimation.
Results
We included more than 107.219 patients from Villach (28.690 patients, 2012 - 2015), Bremen (51.901 patients 2010-2014) and Bremerhaven (26.628 patients, 2008 – 2012) aged 21 to 80 years.
The estimated reference intervals were (all in g/dl):
Age gender Villach Bremen Bremerhaven
21-40 all 6,2-7,8 6,1-8,1 6,1-8,1
21-40 male 6,4-8,0 6,3-8,2 6,1-8,2

21-40 female 6,2-7,7 5,6-8,1 5,7-8,1
41-80 all 6,1-7,7 5,9-8,0 5,9-7,9
41-80 male 6,1-7,7 5,9-8,0 5,9-7,9
41-80 female 6,1-7,8 5,9-7,9 5,9-7,9
The differences between the laboratories were within the acceptable range regarding the imprecision of the method.
Discussion
The Reference Limit Estimator can be applied to total protein for the estimation of reference intervals. Although the laboratories used different manufactures and focus on different patient cohorts the estimated reference limits were very similar. The limits utilized by the manufacturers are from Josephson et al. (1957) (adults 6,6-8,7 g/dl, cited by Roche) or from the Tietz Textbook (adult ambulatory 6,4-8,3 g/dl, adults recumbent 6,0-7,8 g/dl, cited by Abbott and Roche) are mostly higher than our estimations. However the new estimations fit better with the recent published consensus results from the Australian Association of Clinical Biochemist using 6,0-8,0 g/dl.

DGKL-P104

Risikobewertung: die Sprache der Mitarbeiter nutzen

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The evaluation of risks takes on an important role in daily business: in many normative regulations the installation and progression of an risk management is requested. In many circumstances, using a risk based approach, only a uniform and understandable risk evaluation system will assure that it will be used by the employees in daily work.

One of the oldest risk evaluation systems is the FMEA (Failure Mode and Effects Analysis).

The FMEA based on the concept of evaluation of a fact in view of three main aspects: probability (of the event occurring), severity (of the event) and detection (probability that the event would not be detected before the user was aware of it). For each of these aspects a 10-point scale is defined, mainly in form of a potency system and the user has to assign a rating using the three scales. He will get three key-notes and the risk level is provided by multiplication of the three key-notes. This will result in the Risk Priority Number (RPN). On the basis of predefined limit-values one can decide whether a fact is high or low-risk and how to proceed.

The FMEA is furthermore useable in prospective considerations of facts, e.g. in forejudging whether a planned action will be an improvement compared to actual approach.

While implementing a classical FMEA into the field of a transfusion center we ascertained, that the potency-based system with its 10-point-classification (e.g. describing the detection in the way of 1:10, 1:100 etc) will be met with refusal by the employees. In the process of evaluation of alternatives our staff noticed, that the classification tables are not useful referring to their daily work. A better way would be transferring the classification in form of words. While developing such a word-based classification we mentioned, that a 10-point graduation has a grade of detail that cannot be reflected by words. We chosen a 5-point graduation. In addition it was important for us to define each step of the classification with words.

Our own classification of risks: (Classification.jpg)

We defined lines of action:

Risk Priority Number (RPN):
High: 51-125 actions have to take place absolutely and they have to implemented
Middle: 26-50 possibly actions have to take place
Low: 01-25 living with the remaining risk

This system of risk evaluation is in function since 2011. With this scheme, 246 events of different topics (deviations on processes, external complaints, events with financial backgrounds etc.) were analysed. For each event the risk analysis contains the rating in the three main aspects on the base of our classification and the construction the RPN. This analysis will happen twice: once in the acute situation by the staff and twice later by the QM- team, as a form of outstanding evaluation.

Each planned action was analyzed by the same strategie assuming that an implementation only makes sense if the RPN will be lower than the RPN of the origin case.

Following all the years we found, that the used formulations will be sufficient for the whole staff: in the phase of acute assessment, while secondary reflection by the QM-team or in evaluate the planned measures in point of success.

With this system we found an objectifiable form of risk description which allows us, e.g. in discussion with external boards like audits, to illustrate our procedure in a transparent and comprehensible way.

This way of traceability became more important for us especially in events with a low-risk-classification where we had to explain, why we did not initiate an action.

DGKL - Laboratory Management and Quality Assurance

DGKL-P105

Method comparison between LOCI™-based tumor marker assays for Dimension™ VISTA 1500 and immunoassays for Cobas™ e411

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Background: Although LOCI™-based tumor marker assays have been established in daily routine, evidence is sparse regarding method comparison with established immunoassays.
Aim: We performed a method comparison between LOCI™-based tumor marker assays for Dimension™ VISTA and tumor marker immunoassays for Cobas™ e411.
Materials and Methods: AFP, CEA, CA 125, CA 15-3, CA 19-9, PSA and free PSA (fPSA) were analyzed in 1088 sera on the Dimension™ Vista 1500 and Cobas™ e411 analyzer. Spearman's correlation coefficients were calculated. Bland-Altman plots were generated and Passing-Bablok regressions analyses were performed.
Results: Strong correlations were found for PSA (r=0.999), AFP (r=0.994) and CEA (r=0.993). Both methods achieved similar results with slopes of 1.05 (PSA), 1.02 (AFP) and 0.94 (CEA), respectively. However, differences in correlations were found for CA 125 (r=0.976), CA 19-9 (r=0.960), fPSA (r=0.950) and CA 15-3 (r=0.940). In addition, considerably different slopes were observed for these markers with 1.50 (CA 19-9), 0.76 (CA 15-3), 0.75 (fPSA) and 0.64 (CA 125).
Conclusion: We found excellent correlations and similar values for AFP, CEA, PSA, while correlations were weaker for fPSA, CA125, CA 15-3 and CA 19-9. The slope for the latter markers has to be considered when changing methods in a laboratory.

DGKL - Nachwuchsarbeit, Lehrkonzepte

DGKL-P107

Dynamic Visualization of Clinical Chemistry Data to improve Education

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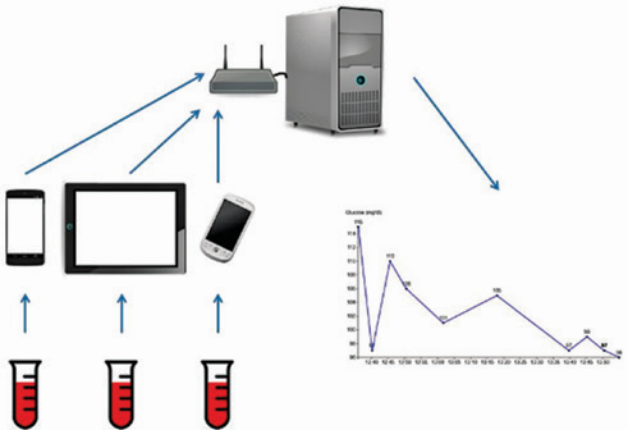
Background:
Research in medical education has conclusively shown that the dual presentation of verbal and visual learning material improves effectiveness of education [1]. In practical courses for laboratory medicine, students can conduct their own measurements and experience typical problems hands-on. However, many important concepts in laboratory medicine like quality control or statistics require a joint view of many measurements. This work describes and evaluates a robust technical framework for visualizing measurements in practical courses for laboratory medicine.
Material and Methods:
Technical framework: Software for operating a generic server was installed on a dedicated PC. A web application was written with two main functions. The data entry form allowed the insertion of measured values. The data display page visualized all inserted data in a graph. The D3.js framework [2] and AJAX technology were used to update the graph after new data entry in real-time. The web application was installed on the server and made accessible via a wireless router.



Application in practical course: Students were instructed to measure glucose concentration in an EDTA blood sample with a point-of-care glucometer over a time period of two hours. To insert the result into the server database students could use their own smartphone or tablet. To do so, they had to connect to the wireless network and access the webpage with the data entry form. Clear hygiene instructions were provided. Instructors could also insert data for their students. The data display page provided a graph of joined glucose measurements over time. It was shown via projector to visualize glycolysis in the blood sample and reliability of point-of-care glucose measurements.

Students were asked to evaluate the described course with an anonymous questionnaire. Answers options ranged from 1 to 5 (fully agree to fully disagree).

Results:

A total of 168 students participated in the practical course on three days. 89 students returned their questionnaire.

Most students fully agreed that data entry was sufficiently simple (mean of answers = 1.49) and disagreed that it interfered with measurements (mean = 4.18). Instructors had to enter data for the students less than 5 times. Answers were more heterogeneous regarding the use of an own device. The majority of students preferred to use their own device (mean= 2.45). However, answers to this question had the largest spread of all answers (sd= 1.38). In addition many free-form comments dealt with the own-device issue approvingly as well as disapprovingly ("cool new idea", "hygiene problem").

Many students (~15%) failed to correctly use the glucometer. Incorrect sample application was a common problem. As a result values varied by more than 200%. The effect of glycolysis in the sample was hardly visible because of these errors. Most students agreed that the dynamic display of measurements improved their understandings (mean = 2.29).

Discussion:

The described technical framework proved to be suitable to visualize measurements in practical courses of laboratory medicine. Data entry using an own device remains controversial. Therefore good hygiene education and alternative ways for data entry are required. To meaningfully visualize measurements a high frequency of measurement errors by the students has to be taken into account.

References:

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DGKL-P108

Team based learning in laboratory medicine - a modern teaching concept at the Karl Landsteiner University

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The established Austrian syllabus of medical studies contains little information about laboratory medicine so far.

The 1st academic year of the Human Medicine Master programme (following the Health Sciences Bachelor programme) at the Karl Landsteiner University in Krems will offer students much more opportunities for exploring the theoretical and practical laboratory aspects in endocrinology, rheumatology and nephrology in the future.

The concept of team-based-learning provides a good possibility to realise this aim.

Each group consists of only three students to grant a high level of individual development.

Clinical routine often makes it impossible that only one person is responsible for teaching activity. Therefore teaching scripts were designed to establish a guideline for standardized teaching.

The teaching script and additional teaching material such as paper cases, study abstracts and role play scripts are stored in an accessable sharepoint file.

13 standardized skill sectors that mainly refer to practical skills like anamnesis were outlined to classify the didactic value of the team-based-learning concept.

One unit of team based learning lasts three hours and contains three subunits: theoretical part, practical part and assisted self studies.

„Theoretical part“ should be used for a short interactive assessment to check the students‘ theoretical knowledge, but also for brainstorming.

„Practical part“ is the main subunit and enables the students to get in contact with clinical laboratory routine. They can train technical skills as well as diagnostical skills.

„Assisted self studies“ is determined for establishment of illness scripts, which should be depicted in a mind map.

The team-based-learning concept in laboratory medicine will be launched in march 2017 at the Karl Landsteiner University.

DGKL - Foundation for Pathobiochemistry and Molecular Diagnostics

DGKL-P109

Cellular and Molecular Characterization of the Effects of Different Anticoagulants on the Regulation of Matrix metalloproteinase-9

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Background
Matrix metalloproteinase 9 (MMP-9) is a subject of growing interest in human pathology, e.g. stroke, cancer, and neurological diseases. It has been reported that blood sampling with different anticoagulants (e.g. high-/low- molecular-weight heparin (H-/L-MWH), EDTA, or citrate) alters the expression of MMPs differentially.
Objective
The aim of the study was to evaluate the direct and indirect effects of different anticoagulants on the regulation of MMP-9. Furthermore, to identify those involved molecular and cellular mechanisms (i.e., involved cell types, soluble mediators), so to assess the impact of the respective substances on the suitability of MMP-9 as a biomarker.
Design and Methods
MMP-9 mRNA and protein expression was assessed in response to different anticoagulants in co-culture experiments using THP-1, Jurkat, and HT cells (representing monocytes, T-, and B-cells). The cytokine profile secreted in response HMWH was assessed in the supernatant of the co-culture experiments using the Proteome Profiler Human XL Cytokine Array. The influence of cytokines on MMP-9 production was analyzed using individual-/-combinations of cytokines.
Results and Discussion
Direct anticoagulant stimulation has no influence on MMP-9 expression of monocytes, T-, and B-cells. Co-culture experiments of monocytes/T-cells or monocytes/T-/B-cells revealed that stimulation with HMWH increased MMP-9. Supernatant of HMWH-stimulated T-cells significantly induces MMP-9 expression of monocytes and activates different monocytic cell functions such as proliferation, phagocytosis, and apoptosis. Cytokine profiling of T-cell supernatant revealed that IL-8 (i.e. monocyte- derived), IL-16 and sICAM-1 (T-cells-derived) are specifically increased in response to HMWH.
Conclusion
Monocytes show increased MMP-9 production following stimulation with T-cell-derived mediators secreted in response to HMWH. In consequence, the use of HMWH as an anticoagulant may affect the MMP-9 expression in blood samples and its suitability as a biomarker in certain diseases.

DGKL-P110

Comprehensive molecular profiling in acute aortic dissection: seeking diagnostic biomarkers

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Acute aortic dissection (AAD), the most common form of acute aortic syndromes, is associated with extremely high morbidity and mortality. Despite recent advances in the diagnostic procedures and better understanding of the pathophysiology of the acute aortic syndromes, misdiagnosis rate in AAD is still high. However, prompt and proper diagnosis is vital to increase a patient's chance to survive and to prevent life-threatening complications. Modern imaging technologies provide a robust foundation for the diagnosing and treating aortic diseases, but easily accessible and cost-effective blood tests only play a minor role. Therefore, we primarily aim for the discovery and initial clinical validation of blood biomarkers to improve diagnosis and clinical management of AAD. Such biomarkers may also be of clinical interest in the prognostic surveillance of patients at increased risk for the AAD, for example patients with Marfan syndrome or with thoracic aortic aneurysm (TAA).

To reach these goals, we compared protein abundances in the secretomes which are produced by tissue culture of aortic samples from patients with AAD type A and from patients who underwent elective surgery due to the aortic valve replacement (AVR) or thoracic aortic aneurysm (TAA). In total almost 1500 secretome proteins were identified. First standard classification of proteins with fold change greater than two revealed 163 differentially regulated proteins between AAD and AVR, 96 between AAD and TAA as well as 25 between AVR and TAA. Further computational analysis via regularized classification based on fold change identified platelet factor 4, intelectin-1 and platelet basic protein as the best discriminating between AAD and both control groups. In the following steps, initial clinical validation of these selected biomarker candidates will performed in blood samples.

In the second part of the project, we compared the transcriptome profiles (mRNA and long noncoding RNAs) isolated directly from the aortic tissues. More hundreds transcripts were significantly differentially expressed in aortas of patients with AAD when compared to the both control groups. Moreover, sixteen differentially expressed transcripts with fold change greater than eight and false discovery rate (FDR) lower than 0.6% differed between dissected and preserved parts of aortas of AAD patients and three transcripts with fold change greater than eight and FDR lower than 1% differed between TAA and AVR. In the succeeding steps, computational analysis via regularized classification based on fold change will be applied to identify biomarker candidates for the initial clinical validation in the blood samples.

Postersitzung DGKL - Other Topics

DGKL-P111

Novel genetic variants in carboxylesterase 1 predict early-onset capecitabine-related toxicity

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Background: Capecitabine (Cp), the oral prodrug of 5-fluorouracil (5-FU), is a commonly prescribed anticancer drug. Its clinical use, however, is complicated by severe adverse effects occurring in 20-35% of patients at standard doses, in particular the hand-foot syndrome (HFS) and diarrhea. The aim of this study was to perform a comprehensive assessment of Cp drug pathway-associated genetic variability as a predictor of Cp-related early-onset toxicity. **Patients & Methods:** The coding and exon-flanking regions of the cytidine deaminase gene (CDA) were sequenced in 144 Cp-treated patients, in whom Cp-related toxicities in the first two chemotherapy cycles were recorded. For all other investigated candidate genes (CES1, CES2, TYMP, UPP1, and UPP2), genotyping was performed in a discovery subset of 48 patients, and associated candidate variants were subsequently genotyped in the full cohort of 144 patients. **Results:** We identified a haplotype in the carboxylesterase 1 gene (CES1) associated with Cp-related toxicity ($OR_{additive} = 2.3$, 95% CI: 1.25-4.32, $P_{adjusted} = 0.008$; $OR_{recessive} = 16.6$, 95% CI: 2.78-98.7, $P_{adjusted} = 0.002$). This common haplotype (frequency = 14%) encompassed five noncoding variants, including an expression quantitative trait locus (rs7187684) for CES1. In addition, the association of two common linked CDA promoter variants (c.1-451C>T: $OR_{dominant} = 4.29$, 95% CI: 1.30-14.2, $P_{adjusted} = 0.017$; and c.1-92A>G: $P_{adjusted} = 0.015$; $OR_{dominant} = 4.40$; 95% CI 1.34-14.5) with increased risk of Cp-induced diarrhea was replicated. For all other investigated genes, no significant association of genetic variants with Cp-related toxicities was detected. **Conclusions:** This is the first study to identify an association of genetic variation in CES1 with Cp-related toxicity. Given that a variant (rs2244613) of the same CES1 haplotype was previously associated with trough concentrations and bleeding from the CES1-metabolized anticoagulant dabigatran, this

finding provides important evidence for the existence of a common regulatory CES1 variant with possible clinical relevance for carboxylesterase-metabolized drugs. Combined with genetic variation in CDA, this novel risk variant may contribute to an improved prediction of adverse effects from Cp-based chemotherapy.

DGKL-P112

ABCC6 deficiency in liver induces cholesterol biosynthesis in peripheral tissue

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Objectives: Pseudoxanthoma Elasticum (PXE) is a heritable rare disorder and is caused by mutations in the ATP-binding cassette sub-family c member 6 (ABCC6) gene. PXE is primarily associated with progressive calcification of elastic fibers in soft connective tissue of the eye, skin and cardiovascular system and has been postulated to be a metabolic disease. Furthermore, there are several hints, for example increased 3-hydroxy-3-methylglutaryl-CoA reductase activity in PXE patient cells as well as alterations in lipoprotein concentration of blood in PXE patients and Abcc6 knockout mice, which reveal that lipid metabolism might play a pivotal role in development of PXE. So the aim of this study was to deepen our insights into PXE pathomechanisms concerning the involvement of lipid metabolism.
Methods: By means of relative quantitative real time PCR gene expression of key enzymes of cholesterol biosynthesis and lipoprotein metabolism such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), farnesyl diphosphate synthase (FDPS), farnesyl-diphosphate farnesyltransferase 1 (FDFT1), geranylgeranyl diphosphate synthase 1 (GGPS1), lanosterol synthase (LSS), low density lipoprotein receptor (LDLR), proprotein convertase subtilisin/kexin type 9 (PCSK9) and apolipoprotein E (Apo E) was investigated in healthy and PXE human dermal fibroblasts as well as in healthy and PXE murine liver tissue. The accumulation of neutral lipids was analyzed by oil red o staining.
Results: Our data show that expression levels of genes involved in cholesterol biosynthesis were increased in human fibroblasts from peripheral skin, but decreased in the murine liver tissue. As a result of induced cholesterol biosynthesis PXE fibroblasts expressed significantly more PCSK9, but the gene expression of LDLR was not affected. As opposed to that the expression level of LDLR was significantly enhanced in PXE mouse liver whereas gene expression of PCSK9 was not regulated. ApoE was downregulated in ABCC6 deficient fibroblasts as well as in Abcc6 knockout liver tissue. Murine liver tissue accumulated more lipids than healthy controls.
Conclusion: In summary, our results support the metabolic hypothesis and further characterize the link between PXE and lipid metabolism. The absence of ABCC6 transporters in the liver might lead to an accumulation of the ABCC6 substrate in the liver, leading to a downregulation of cholesterol biosynthesis. In contrast, the ABCC6 substrate is missing in the periphery resulting in an upregulation of cholesterol biosynthesis in peripheral tissue, e.g. skin. In consequence of a disturbed cholesterol biosynthesis also lipoprotein metabolism seems to be affected.

DGKL-P113

The vitamin D status of 5-15 age children in Zonguldak, Turkey Der Vitamin-D-Status von Kindern im Alter von 5-15 in Zonguldak, Türkei

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Introduction: Vitamin D deficiency is an important health problem in children. The study aimed to determine vitamin D status of 5–15 age children living in Zonguldak, Turkey.
Methods: A total of 153 (81 girl, 72 boy) children aged between 5 to 15 years, who had presented to the outpatient clinic at the end of the winter and the summer, were included in the study. The subjects were divided into two groups according to age: 5-10 years and 11–15 years. Plasma 25-hydroxyvitamin D (25[OH]D) levels were determined by HPLC method and categorized as < 15 (deficiency), 15-29 (insufficiency), and ³30 ng/ml (sufficient).
Results: Our data showed that the plasma 25[OH]D levels decreased when the children got older (p=0.016). Mean plasma 25[OH]D levels were significantly higher in boys than in girls in each age group (5-10 age group, p=0.004; 11-15 age group, p=0.04). There was a marked seasonal effect on 25[OH]D levels (p=0.03) (Figure 1). The prevalence of vitamin D deficiency and vitamin D insufficiency were 19% and 42%, respectively. The prevalence of vitamin D deficiency and insufficiency showed significant difference in relation to age and season. The prevalence of vitamin D deficiency were higher in girls than boys, when was analyzed in the 11-15 age group (p=0.006)
Conclusions: Vitamin D supplementation should be recommended to Turkish adolescence girls in the winter. However, the main problem is the increasing risk of vitamin D insufficiency in all children. This is an important health problem, which requires focus and preventive strategies should be developed.

DGKL-P114

Occupational field analysis in biomedical analytics

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Background
The rising demands towards professionals, the increasing complexity of activities and the necessity for biomedical scientists (BMS) to qualify for assuming higher management functions as well as the growing difficulties in recruiting qualified personnel have prompted the professional association, labmed switzerland, to initiate in 2014 a reassessment of the whole occupational field of biomedical analytics by means of an occupational field analysis. The project was significantly supported with contributions from the State Secretariat for Education, Research and Innovation.
Research Question
The research aims to analyse, in qualitative and quantitative terms, the carrying out the profession in the present and in the future, the required qualification profile(s) and the classification within the vocational training system.
Research Methods
The research encompasses 77 problem-focused expert interviews. The interviews were transcribed and authorised by the interviewees. Subsequently, an activity analysis was carried out by means of a qualitative content analysis. In this process, the main and secondary functions in the six professional areas were listed and described. Moreover, the practical professional skills relevant for the labour market were derived.

In order to obtain a comprehensive overview of the occupational field, various qualitative and quantitative methods from empirical social research were also applied.
Results
Biomedical analytics in Switzerland show a heterogeneous occupational field with six partly different professional areas and a low vertical and horizontal mobility.

The main and secondary functions were divided up according to the taxonomy of cognitive, affective and psychomotor levels. The activity analysis shows the high share of challenging cognitive and affective levels in the profession of the BMS. Psychomotor function's share shifts in favour of the cognitive levels without sparing the motor function. Large parts of the profession are application-related, practical activities based on a challenging theoretical background. The challenging intellectual features are identified in self-assessment and external assessment as the most important characteristics of a BMS.

In general, there are few clearly defined and established hierarchical levels or role and function designations with a clearly recognisable permeability.
Conclusion regarding future vocational training system
The upper-secondary specialised school is recommended as the provider of qualifications in biomedical analytics. However, other options for qualifying also remain possible.

The training at PET colleges has in greater part proven to be successful. However, a qualified track at university level in management functions and/or in-depth professional occupations with correspondingly higher requirements as well as a course of studies in the field of applied research is lacking. As a result, a university-track is recommended as a second education path.

Thereby the principle of permeability shall be respected. The connection to the international development and comparable professions shall be achieved. The access to international master programmes shall be ensured. The differentiation of the vocational training system allows to structure management functions and to create development possibilities within the occupational field.

DGKL-P115

Determination of the Validity to the Assessment of Oligoclonal Bound (OB) Patterns in Liquor-Serum-Pairs by means of the Capillary Electrophoresis in Comparison to the Isoelectric Focusing

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Background: Neurologic disorders often appearing with unspecific clinical symptoms what complicates the differential diagnostics of illnesses of the central nervous system. To the support to make a diagnosis laboratory investigations of the cerebrospinal fluid (briefly Liquor)

deliver important diagnostic information as for example the qualitative analysis of oligoclonal bounds. Presently for the proof of oligoclonal bound counts the time consuming isoelectric focusing as a golden standard. An alternative to the quicker diagnostics could show the capillary electrophoresis.
Issue/Aim: At first in the emphasis is the inquiry of the ability for assessment of oligoclonal bound patterns (OB-type 1 to type 5) in liquor-serum-pairs by means of the capillary electrophoresis in comparison to the isoelectric focusing, on basis of the availability / not-availability of oligoclonal bounds in the liquor.
Material and Methods: For this purpose a total of 432 liquor-serum-pairs of different oligoclonal bound patterns (OB-type 1 to type 5) were analysed by means of the analysis device MINICAP of the company sebia capillary electrophoretic and were evaluated statistically. To allow a comparison, the raw data of the capillary electrophoresis were moved in an evaluation script written for it graphically and were compared to the results of the isoelectric focusing. With the help of this comparison the diagnostic sensitivity and specificity, the Likelihood ratio which predictive value and the diagnostic efficiency or was determined was calculated.
Results: Thus the first statements could be make to the validity of the capillary electrophoresis on basis of the medical statistics. Besides, the probability lies for right-positive statements with about 63% and for wrong-negative with about 61%. Indeed, the determination of right OB-type explains currently with the help of the capillary electrophoresis still as problematic.
Conclusion: Even if the negative likelihood ratio from about 0.61 confirm the excellency of the test, the applied methodology shows currently still no alternative to the isoelectric focusing. In general the capillary electrophoresis absolutely owns the potential, for the identification of oligoclonal bound including classification to become a quick and reliable alternative to the isoelectric focusing. Indeed, these even other laboratory investigations require.
Keywords: oligoclonal bounds, capillary electrophoresis, isoelectric focusing, medical statistics, validity

DGKL-P116

Detection of Total Protein and IgG from Serum and Citrated Plasma

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Aim: In blood services, where whole blood and plasma donations are taken, the detection of several parameters from serum and plasma helps to safe RBCs. To ensure that samples with TP or IgG values below the allowed limit, the conversion of plasma levels into serum levels has to be validated according to current GMP and ICH guidelines.
Methods: Validation plan: From a donor population (n=49) a serum and a citrated whole blood sample should be drawn. After Separation of serum or plasma both parameters, IgG and TP (ThermoFischer, Protein Total 981826 / 981827 and IgG 981937) should be detected using the methods as described by the manufacturer. By using the Passing and Bablok Regression as well as the method by Bland-Altman, the methods should be compared.
Results: Validation Report: Both parameters IgG and TP were found normally distributed in the evaluated donor population. For IgG a sample dilution by factor 1.33 was found (y = -16.978286 + 1.331245 x). The intercept did not differ significantly from "0". Also the requirements for TP measurements were fulfilled (y = -0.450806 + 1.312122 x). Also the results from the evaluation according to Bland-Altman did not showed any systematic deviations.
Conclusions: The validation of the conversion formula from citrated plasma into serum valued showed the expected results. Both methods, IgG and TP showed a good comparability so that this methods can be used in routine for serum and citrated plasma.

DGKL-P117

Heavy/Light Chain Immunoassays for Monitoring of a Multiple Myeloma Patient

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Background: Common assays for identification and monitoring of monoclonal gammopathy are the combination of serum protein electrophoresis (SPEP), serum and urine immunofixation electrophoresis (sIFE/uIFE) as well as the quantification of the total immunoglobulins and the serum free light chains (FLC). The development of the heavy/light chain (HLC) immunoassay (Hevylite®, The Binding Site, UK) allows the identification and quantification of the different light chain types of each immunoglobulin (Ig) class (for example IgAκ and IgAλ). Clonality

can be assessed by calculation of the ratio (rHLC). The HLC assays are used for diagnosis, response assessment, monitoring and prognosis of monoclonal gammopathies.
Objectives: Comparison of the performance of the HLC assays with SPEP and total Ig assays in monitoring of a multiple myeloma patient with a biclonal gammopathy.
Methods: The patient in this case study had multiple myeloma with a biclonal gammopathy IgAκ and IgGκ. He received maintenance therapy with Lenalidomid until progression. HLC were quantified using polyclonal antisera assays on the BN™II nephelometer (Siemens Healthcare Diagnostics Inc.). Absolute values of IgG and IgA were also analyzed on the BN™II nephelometer.
Results: During monitoring, the patient showed total IgA and IgAκ levels above normal range whereas IgAλ, total IgG, IgGκ and IgGλ levels remained below normal range. Progression of the disease was detected with IgG rHLC earlier than with SPEP and total immunoglobulin. This additional sensitivity had been gained through the suppression of IgGλ (pair suppression). Since the IgAλ values of this patient were below the detection limit, analysis of the IgA rHLC was not possible. IgG monoclonality could not be detected by total immunoglobulin measurements, but by an abnormal IgG rHLC (confirmed by IFE). In this case of biclonal gammopathy the different clones were not able to be monitored by SPEP but by HLC measuring.
Conclusion: HLC measurement was a helpful and sensitive method to evaluate progression of multiple myeloma after therapy. It was more sensitive than SPEP and total immunglobulin analysis. In this case study, the increase of the rHLC showed a good correlation with the patient's outcome.

DGKL-P118

Effects of preanalytics on the quantification of lymphocyte subsets by flow cytometry

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Background: Infrequent quantification of different lymphocyte subsets as regulatory and cytokine producing T lymphocytes by flow cytometry is time-consuming. Therefore, the integration of such analyses in daily laboratory measurements requires freezing of samples and measurement after thawing or sample collection. The most important questions that have to be answered in this context are: Is it possible to freeze samples and quantify lymphocyte subsets after thawing? And if not, what effect do duration and temperature of sample storage have on sample material and measurement results using flow cytometry?
Methods/Results: Heparinized whole blood of healthy blood donors was analyzed for regulatory T lymphocytes (CD4+CD25+CD127lo/-FOXP3+), stimulated interleukin-2 (CD3+CD4+/CD8+CD69+IL-2+) and interferon-γ (CD3+CD4+/CD8+CD69+IFN-γ+) producing T lymphocytes by flow cytometry (BD FACS Canto II). For comparison of fresh and frozen/thawed samples, blood of 15 donors was prepared and measured. Stabilization of white blood cells was for interleukin-2 and interferon-γ assay performed after stimulation and for regulatory T lymphocytes during permeabilisation step. The percentage variance between fresh and frozen/thawed samples range from 0 % to 59.3 % for cytokine producing T lymphocytes and from 42.1 % up to 500 % for regulatory T lymphocytes.
To show the impact of sample storage, material from six blood donors was analyzed. Sample processing was implemented at 0, 3, 24, 48 and 72 hours after blood collection and storage at 4 °C or room temperature. Deviations from baseline values (measurement directly after blood collection) were shown after 3 hours of storage at both temperatures. No correlation with baseline values could be demonstrated 24 hours after sample collection. Imprecision studies were carried out showing excellent correlation for all analyses investigated.
Conclusion: Here we show for the first time, the lack of correlation for the quantification of different lymphocyte subsets using flow cytometry between fresh and frozen/thawed samples and, furthermore, between fresh and stored samples. Sample processing must be guaranteed within 3 hours after blood collection to avoid profound changes of sample material. In consequence the drawback of preservation of fresh samples is challenging and data based on the upper described protocols should be critically scrutinized. It is necessary to develop less time-consuming and more standardized protocols for sample processing in order to integrate such quantification methods into daily laboratory measurements.

DGKL-P119

Alcohol consumption and cholesteryl ester transfer protein (CETP) activity – a possible link for increased HDL-cholesterol levels

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Background: Chronic alcohol consumption reduces the carbohydrate content of several N-linked glycoproteins. In clinical care the carbohydrate-deficient transferrin (CDT) is the most specific (above 90%) with good sensitivity (50-90%) and is frequently used as a biomarker for

average alcohol consumption. The cholesteryl ester transfer protein (CETP) is a plasma glycoprotein which plays an important role in reverse cholesterol transport. A reduced glycosylation could possibly influence the CETP activity, with the consequence of increased HDL-cholesterol levels.
Materials and Methods: In the period from 01.01.2013 to 31.01.2015 the database of the laboratory was searched for patients who simultaneously had results for HDL cholesterol and CDT. The collective were grouped into quintiles of CDT. Based on the quintiles serum samples were collected to measure CETP activity (CETP Activity Fluorometric Assay Kit II, BioVision, USA) and the CETP protein concentration (CETP ELISA Kit, antibodies, USA).
Results: Overall, in the 25 month studied 637 patients were found with results for CDT and HDL cholesterol in the database. The HDL cholesterol correlated positively with the CDT. Because the HDL-cholesterol levels were not significantly different in the 1st and 2nd quintiles of CDT concentration, these two quintiles were combined for further collection. The CETP activity and concentration were measured in a total of 37 serum samples. CETP activity showed a statistically significant negative correlation with HDL. CETP activity was also negatively correlated with CDT. CETP activity and CETP concentration showed a positive correlation, but this correlation was statistically not significant
Conclusion: We conclude that alcohol consumption affects the CETP activity. Thus, increased HDL-cholesterol levels under alcohol consumption may be due to a reduced CETP activity. The reduced CETP activity may be explained by changes in the glycosylation pattern

DGKL-P120

Comparison of PST tubes(Greiner PST, Improve PST) with SST tube(Greiner SST) for clinical chemistry assays in emergency laboratory

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Background
It is very important to supervise Turn Around Time(TAT) of the specimens for clinical chemistry assays in emergency laboratory, which enables to make exact results as soon as possible. To improve TAT we considered Plasma Separation Tube(PST) with lithium heparin and Gel as the replacement of the existing Serum Separation Tube(SST).
Method
Total 20 subjects who had volunteered for the clinical trial were conducted between July and August 2015 in National cancer center. We compared Greiner PST tube(Geiner Bio-one, Germany) and Improve PST tube(Guanzhou Improve Medical Instruments Co., Ltd, China), with the existing Greiner SST tube for 18 clinical chemistry assays and 1 cardiac marker. The results were statistically analyzed using the paired t-test and Bland-Altman plot.
Result
Paired t-test analysis revealed that statistically significant differences were not showed to all three comparison tubes in 17 assays (calcium, aspartate aminotransferase, alanine aminotransferase, gl-ucose, total bilirubin, lactate dehydrogenase, lipase, amlyase, C-reactive protein, Albumin, blood ureanitrogen, creatinine, sodium, chloride, phosphate, CK-MB, creatine kinase: $p>0.05$) except for 2 assays (total protein, potassium: $p<0.05$).Bias(%) of total protein was 4.2%(Greiner PST tube and Greiner SST tube) and 4.63%(Improve PST tube and Greiner SST tube).Bias(%) of K was -8.99%(Greiner PST tube and Greiner SST tube) and -9.44%(Improve PST tube and Greiner SST tube). In addition, 2 brands of PST tubes were statistically no significant differences in all assays.
Conclusion
PST tube provided acceptable results in comparison to the existing SST tube except Total Protein and K in emergency laboratory. In case of using PST tube, Total Protein and K should be recommended the correlation analysis with clinical chemistry assays in routine laboratory. As a result, PST tube was considered to be available to supervise TAT due to reducing the clotting time and the error which was caused by fibrin.

DGKL-P121

Dried blood spot contamination- an underestimate risk in Newborn screening

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Objectives:
Filter paper with dried blood is the standard specimen used for newborn screening all around the world. The convenient transportation by regular mail and the reasonably stability of metabolites of interest support its use. The heel pick procedure as the method of sample

acquisition is well standardized. The use of native blood without anticoagulants (e.g EDTA) is required since they are known to interfere with screening laboratory methods.

However, other invisible contaminations of the filter paper prior, during or after the sample collection are often not visually detectable and do have, nevertheless a significant influence on the screening result. In order to emphasize the correct pre-analytical phase within the Newborn screening, nine different contamination sources, which are present in our neonatal ward, were applied on filter papers. The lack of reports and further information about the impact of such contaminants lead to realization of this study, aiming on the understanding and proper risk evaluation of so far not well investigated contaminants and their impact on the NBS result.

Methods:

Capillary blood was obtained from 10 volunteers and applied on screening filter papers. Right after the blood collection, the still moist spots were intentionally contaminated with the nine different substances: (1) disinfectant, (2) feces, (3) urine, (4) baby cream, (5/6) two different types of baby food, (7) ultrasonic gel, (8) breast milk and (9) baby wipes. One spot was left out of any contamination for control purposes.

TSH, 17-OHP, GALT, Biotinidase, IRT, amino acids and acylcarnitines were analyzed.

Results:

Eight of nine applied contaminants affected the performed NBS tests. Altogether 81 analyts were significantly altered, resulting in false positive results. In practice, this would lead to an unneeded second blood collection procedure for the newborns and consequently to increased costs. Furthermore, the parents undergo an unnecessary elevated psychological stress, due to the false positive screening report.

Conclusion:

Mishandling of filter papers in the pre-analytical phase is an underestimated source of false positive screening results. Our study underlines the importance of regular training of the medical staff involved in the process in order to obtain reliably newborn screening results.

Postersession DVTA

DVTA-P02

Saccharopine and Pipecolic Acid in Laboratory Diagnostics

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Both saccharopine and pipecolic acid occur as intermediates in the pathways of lysine biosynthesis in nature.

Certain metabolic diseases as lack of saccharopine dehydrogenase can cause increased appearance of saccharopine. Metabolic disorders of pipecolic acid may lead to epileptic seizures. Pipecolic acid attestation in urine and blood offers the possibility of controlling the clinical course of therapeutic B6-vitamin- administration.

The complexity of saccharopine evidence is actually manifested in the wordwide clinical method comparison of ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) determined by the minor participation (33%) contrary to the participations in other evidences like valine and tyrosine. At these more common amino acids 261 laboratories participated even with further methods as tandem MS, reverse phase HPLC and GC/MS apart from the predominantly represented cation exchange chromatography. The complexity of pipecolic acid evidence is actually exceled in a more less participation (20% in 2013).

The analysis of saccharopine and pipecolic acid by cation exchange chromatography with an extended 210 mm separation column in a lithium-gradientsystem and added ninhydrin- or OPA-after-column-derivatization is an exact possibility to verify these amino acids. Whereas there is an overlapping with cystine on shorter separation columns, an exact separation of pipecolic acid, cystine, saccharopine, homocitrulline, cystathionine, methionine and allo-isoleucine in a row takes place on the 210 mm-separation column. For preanalytics solely deproteinization with sulfosalicylic acid and following ultracentrifugation is necessary before diluting the specimen with lithium-citratebuffer (pH 2,2).

The participation of our laboratory on the quantitative ERNDIM schemes resulted in an 0 to 0,5fold standard deviation on saccharopine in the first circuit. Over 2016 there will be 8 serum specimens to be analysed with each time 28 different compounds of amino acids. Only 85 of total 261 of the worldwide participating laboratories in the amino acid schemes analysed saccharopine. 75 of these laboratories determined saccharopine by cation exchange chromatography; but 12 of them were out of 2fold standard deviation. Verification of pipecolic acid was within the same scope of standard deviation in the last scheme.

Concerning clinical evidence of metabolic anomalies by detecting saccharopine and pipecolic acid cation exchange chromatography is an advantageous alternative for practice studies to MS-analysis. It offers high precision after low preparation time.

It is hardly possible to detect these both components on common separation columns in classical amino acid analysis up to a length of 150 mm in an overall-time from 100 – 120 min because of overlapping with given above components. Thus application of an extended separation column results in more unfailingly results.

DVTA-P05

Comparison of two bimodal, retrospective methods (Reference Limit Estimator, quantile-quantile-plot) for the determination of intra-laboratory reference intervals using the example of ferritin and lipase

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Background: Accurate results of quantitative measurements are presented as useless or even dangerous, if the biological variability and the factors are not taken into account, when establishing reference limits. The current gold standard to determine reference intervals according to CLSI is based on a prospective, unimodal method. Normal ranges, which distinguish "not sick" from "sick", are provided by various internet sites or in specialized literature of diagnostic manufacturers. Many laboratories take on this without further testing. In this empirical work two bimodal, retrospective, statistical methods for determining reference intervals easily are evaluated and compared. On the one hand the quantile-quantile-plot by Hoffmann and on the other hand the Reference Limit Estimator from the DGKL. Furthermore, the factors of age- and sexual-function are correlated to the reference areas to check their impact on the reference limits.
Methods: Based on an empirical work, a comparison of the two statistical methods quantile-quantile-plot and Reference Limit Estimator was created and analyzed following the impact of age- and gender-dependent ability factors to reference intervals.
Results: The calculated reference intervals are comparable with the CLSI targets. However, it turned out that not all measured values can be calculated with statistical process. In the calculation of reference intervals there is a age and gender dependency.
Conclusion: The bimodal, retrospective concept is partly suitable to determine intra-laboratory reference intervals. Some parameters cannot be calculated accurately with this method. There is a clear advantage over the prospective, unimodal method. The guiding principle would be to combine the retrospective with the prospective approach.

DVTA-P06

Methodenvergleich zweier Möglichkeiten der Resistenztestung für Staphylococcus aureus

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Secondary resistance to antibiotics is given increasing importance in recent decades. One of the most feared multi-resistance relates to the gram-positive bacterium *Staphylococcus aureus*, which is known as methicillin-resistant *Staphylococcus aureus* (MRSA). To ensure effective therapy, it is mandatory to perform solid susceptibility tests.

This bachelor thesis was focused on testing the susceptibility of 502 *Staphylococcus aureus* isolates with the VITEK® 2 Compact (BioMérieux) and the disk diffusion method. The aim of the empirical study was to compare the two different methods of susceptibility testing and to identify possible resistance patterns.

The results of the analysis showed a conformity of 98.18%, which speaks for an equal equivalent of both possibilities of susceptibility testing. It turned out that guidelines and standardized work are obligatory for the reproducibility of the results. Regarding the resistance patterns, no significant results were achieved, because only 8 of the tested isolates turned out to be methicillin-resistant.

DVTA-P07

Molecular-based screening of sexually transmitted diseases (STD screening) in urogenital specimens

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Objective
The goal of this study was to evaluate the performance of the fluorescence-based DNA microarray STI complete (Euroimmun, Germany) and the flow-through hybridization assay GenoFlow STD array (DiagCor, China) for the simultaneous detection of several sexually transmitted pathogens.

Methods
82 urogenital samples were analyzed in parallel with both hybridization assays after automated nucleic acid extraction using the QIAsymphony SP (Qiagen, Germany). The results were directly compared with the routinely used real-time PCR-based detection methods.
Results
32.9% (27/82) of the samples were tested positive with the Euroimmun microarray and 18.3% (15/82) with the DiagCor assay. Compared to routine diagnostics 4.8% (4/82) of the Euroimmun test results were false negative with a sensitivity - depending on pathogen - of 70-100% and a specificity of 100%. With the DiagCor test 15.9% (13/82) of the samples were false negative and 1.2% (1/82) false positive. The test showed a sensitivity of 55-100% and a specificity of 77-98%.
Conclusion
Both detection methods were easy to implement into daily routine procedures and provided an efficient workflow. The performance of the Euroimmun microarray was similar to the routinely used molecular detection methods, while the DiagCor system showed a weaker correlation due to a lower sensitivity concerning various pathogens. Thus, optimization of the detection limit and the software-based evaluation are required.
Keywords:
STD screening, sexually transmitted diseases, real-time PCR, microarray, fluorescence, flow-through hybridization

DVTA-P09

Establishing of the Multilex-PCR for the Detection of MRSA by the genes *mecA, mecC, nuc* und *lukS/F*

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MRSA has a resistance against methicillin acquired by the genes *mecA* and *mecC*. In addition some MRSA have the virulence factor Panton-Valentine leukocidin. This is encoded by gene *lukS/F* and induces to serious skin and soft tissue infections. Genes *nuc* and *spa* serve the species proof of Staphylococcus aureus. Aim of this study is the establishment of a Multiplex-PCR for genes *nuc, mecA, mecC* and *lukS/F* to proof of MRSA. Tests to the establishment of the PCR were made available by the Helmholtz-Zentrum in Braunschweig. At the beginning genes *nuc, mecA, mecC* and *lukS/F* were established as single locus PCR. Afterwards the establishment of the Multiplex-PCR was executed to the proof of these genes. Among the 504 study samples from Braunschweig, 483 were yielded positive nuc. Among the 21 nuc negative, 16 yielded *spa* positive amplification. Among 499 Staphylococcus aureus isolates, nine yielded *mecA* positiv amplification. Under the samples no one yielded *mecC* and *lukS/F* amplifications. MRSA's prevalence is 1,8% in our study. The occurrence of mecC is described to positive strains in the literature with less than 1%. We couldn't prove any *mecC* due to a too small sample. Persons who showed infections within the last six months were excluded from the study. Therefore the chance to detect any *lukS/F* positive strains was minimal. In one setting up it is possible to detect the species, MRSA evidence and appearance of Panton-Valentine leukocidin. The valuable method could be collected epidemiological data. By a temporal optimization of this Multiplex-PCR it would be possible to detect MRSA in screening settings.

Autorenverzeichnis