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P-01-01

Serum ghrelin is positively associated with physiological anxiety but negatively associated with pathological anxiety in humans: Data from a large community-based study

Dirk Wittekind

Universitätsklinikum Leipzig, Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Leipzig, Germany

The orexigenic hormone ghrelin is being increasingly recognized as a stress hormone being involved in anxiety regulation. In animals, ghrelin effects on, and responses to acute stress differed from those in chronic stress, an animal model for anxiety and depression. In humans, elevated ghrelin levels were reported in pathological anxiety (e.g. panic disorder). However, no reports exist on physiological anxiety in mentally healthy subjects. In addition, reports on generalized anxiety symptoms, both in mentally healthy subjects (e.g. worrying) or in adult patients, are lacking. Total serum ghrelin was determined in 1666 subjects of a population-based cross-sectional study ('LIFE'). The 7-item Generalized Anxiety Disorder Scale (GAD-7), detecting also other anxiety disorders, was administered. For multiple linear regression analyses, 1091 subjects were finally included. Serum ghrelin and GAD-7 scores were positively but not significantly associated in the total group (ß=0.00025, standardized β = 0.039, 95% CI: −0.00006; 0.0006; p = 0.144), in subjects with no more than mild anxiety, there was a sig-nificant positive association (GAD-7 ≤9: n = 1061, 97.25%, β = 0.00032; standardized β = 0.060; 95% CI: 0.000023; 0.00062; p = 0.036). In contrast, there was a negative association in subjects with anxiety symptoms above the GAD-7 cut-off (GAD-7 ≥10: n = 30, 2.75%, ß=−0.003, standardized β = −0.462; 95% CI: −0.006; 0.0001; p = 0.045). Ghrelin levels were only numerically (p = 0.23) higher in subjects with clinically relevant anxiety symptoms (963.5 ± 399.6 pg/ml; mean±SD) than in those without (901.0 ± 416.4 pg/ml). In conclusion, the positive association between ghrelin and no more than mild anxiety is an initial indication for a role for ghrelin in the regulation of physiological anxiety in humans. This association and the opposed associ- ation in pathological anxiety resemble findings in animals showing diverging ghrelin effects in acute and chronic stress.

P-01-02

Association between self-rating depression scores and total ghrelin and adipokine serum levels in a large population-based sample

Dirk Wittekind

Universitätsklinikum Leipzig, Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Leipzig, Germany

Background: Ghrelin and the adipokines leptin and adiponectin have been suggested to be involved in mood and anxiety regulation and to be altered in affective disorders. However, studies investigating the association between ghrelin, leptin and adiponectin and depressive symptomatology are scarce but might contribute to a better under-standing of their involvement in mood regulation. We thus aimed investigating the association between depressive symptomatology and total ghrelin as well as leptin and adiponectin serum levels in a large population-based sample.

Methods: Total serum ghrelin, adiponectin and leptin levels were determined in 1666 subjects of a population-based cross-sectional study ('LIFE'). The Center for Epidemiological Studies Depression Scale (CES-D) and the Inventory of Depressive Symptoms – Self Rating (IDS-SR) were administered. Multiple linear regression analyses were conducted to examine the association between total serum ghrelin, leptin and adiponectin and the intensity of depressive symptoms.

Results: In the total sample (n=1092), neither ghrelin nor leptin or adiponectin serum levels showed a significant association with CES-D or IDS-SR sum scores in the total sample (N=1092) or in depressed/non-depressed subjects. Leptin serum levels showed a significantly positive association with IDS-SR sum scores in elderly men (≥60 years; β = 0.122, 95% CI: 0.009; 0.236; p = 0.035).

Conclusion: Our study suggests that peripheral levels of ghrelin and adipokines in a cross-sectional study design might not be sufficient to measure their involvement in depression, suggesting that associations are more complex and multi-layered.

P-01-03

Differences in biomarker profiles between heart failure patients with preserved versus reduced ejection fraction from the DIAST-CHF study

Abass Eidizadeh[1]; Moritz Schnelle[1]; Andreas Leha[2]; Frank Edelmann[3]; Andreas Fischer[1]; Lutz Binder[1]; Rolf Wachter[4]

[1]Universitätsmedizin Göttingen, Institut für Klinische Chemie, Göttingen, Germany; [2]Universitätsmedizin Göttingen, Institut für Medizinische Statistik , Göttingen, Germany; [3]Charité-Universitätsmedizin Berlin, Institut für Innere Medizin und Kardiologie, Berlin, Germany; [4]Universitätsmedizin Göttingen, Klinik für Kardiologie und Pneumologie, Göttingen, Germany

Introduction: Chronic heart failure (HF) is a common disease and one of the leading causes of death worldwide. Heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) are different diseases with distinct as well as comparable pathophysiologies and diverse responses to therapeutic agents. We aimed to identify possible pathobiochemical signaling pathways and biomarkers in HFpEF and HFrEF by using a broad proteomic approach.

Methods: 180 biomarkers in the plasma of a representative subgroup of HFpEF and HFrEF patients (n = 127) from the DIAST-CHF trial were examined with a proximity extension assay and compared with a healthy control group (n = 40). Biostatic analyses were performed to identify possible differences in biomarker profiles and signalpathways between HFpEF and HFrEF, always compared to the control group.

Results: Thus, we were able to identify 35 proteins that were expressed significantly different in both HF groups compared to the control group. It was also able to determine 29 unique proteins expressed in HFpEF and 33 unique proteins in HFrEF. Additionally network analyzes showed a special role of platelet-derived growth factor subunit A (PDGF-A), Dickkopf-related protein 1 (Dkk-1) and tumor necrosis factor receptor superfamily member 6 (FAS) in HFpEF patients, while perlecan (PLC) and junctional adhesion molecule A (JAM-A) stood out in the HFrEF group. Overall, signaling pathways of metabolic processes, cellular stress and iron metabolism seemed to be important for HFrEF, while for HFpEF oxygen stress, hemostasis, cell renewal, cell migration and cell proliferation are in the foreground.

Conclusion: The identified proteins and signaling pathways offer new therapeutic and diagnostic approaches for patients with chronic heart failure.

P-01-04

Mechanistic insights into the progression of systolic heart failure in aortic stenosis patients

Moritz Schnelle[1]; Dawid Lbik[2]; Miriam Puls[2]; Elisabeth Zeisberg[2]; Andre Fischer[3]; Andreas Fischer[1]; Gerd Hasenfuß[2]; Karl Toischer[2]

[1]Universitätsmedizin Göttingen, Institut für Klinische Chemie, Göttingen, Germany; [2]Universitätsmedizin Göttingen, Abteilung für Kardiologie und Pneumologie, Göttingen, Germany; [3]Deutsches Zentrum für Neurodegenerative Erkrankungen, Abteilung für Epigenetik und Systemmedizin, Göttingen, Germany

The process of transition to heart failure (HF) in humans is poorly understood. This study was designed to identify key mechanistic features at different HF stages in severe human aortic stenosis (AS) in order to better understand the pathophysiological process of HF progression. The study included 57 AS patients presenting with different degrees of systolic dysfunction at the time of aortic valve implantation. Echocardiographic phenotyping, Next Generation Sequencing (NGS) as well as histological analyses were performed in left ventricular (LV) myocardial biopsies of respective patient subsets. Principal Component Analysis (PCA) of gene expression data combined with cardiac remodeling assessment identified three major stages of HF progression: i) AS with normal ejection fraction (ASnEF, EF ≥ 55%) and concentric LV hypertrophy due to increased myocyte size, ii) AS with mildly reduced EF (ASmrEF, EF 45-54%) and LV dilatation, and iii) AS with reduced EF (ASrEF, EF < 45%). NGS was used to characterize the three groups. On a cellular level, inflammatory mast cells dominated in ASmrEF, whereas monocytes and gene expression related to T-lymphocyte activity were found in ASrEF. At the level of epigenetic regulation, microRNAs and DNA methylation/hydroxymethylation were dominant in ASrEF. Myocardial fibrosis was significantly increased only in ASrEF. This study identified LV dilatation as an early feature of HF in human AS that is followed by development of cardiac fibrosis and further contractile impairment. Changes in the inflammatory response, epigenetic modifications and extracellular matrix remodeling appear to be critically involved in a stage-specific manner. Our results highlight the need for a more personalized concept of HF therapy that takes into account individual disease stages and distinct, underlying mechanisms.

P-01-05

Hypercoagulability impairs plaque stability in diabetes-induced atherosclerosis

Saira Ambreen[1]; Sameen Fatima[1]; Ahmed Elwakiel[1]; Rajiv Rana[1]; Kunal Singh[1]; Anubhuti Gupta[1]; Dheerendra Gupta[1]; Hamzah Khawaja[1]; Jayakumar Manoharan[1]; Christian Besler[2]; Ulrich Laufs[3]; Shrey Kohli[1]; Berend Isermann[1]; Khurrum Shahzad[1]

[1]University Hospital Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostic, leipzig, Germany; [2]University Hospital Leipzig, Klinik und Poliklinik für Kardiologie, leipzig, Germany; [3]Universität Leipzig, Cardiology, Leipzig Heart Center, leipzig, Germany

Introduction: Diabetes mellitus, which is largely driven by nutritional and behavioral factors, is characterized by accelerated atherosclerosis with impaired plaque stability. Atherosclerosis and associated complications is the major cause of mortality in diabetic patients. Efficient therapeutic concepts for diabetes-associated atherosclerosis are lacking. Atherosclerosis among diabetic patients is associated with reduced endothelial thrombomodulin (TM) expression and impaired activated protein C (aPC) generation. Here, we demonstrate that atherosclerotic plaque stability is reduced in hyperglycemic mice expressing dysfunctional TM (TMPro/Pro mice) which have a pro-coagulant phenotype due to impaired thrombin inhibition and markedly reduced aPC generation.

Methods: Female ApoE-/- or TMPro/Pro ApoE-/- mice (age 6 to 8 weeks) were fed a normal chow diet and were made diabetic (DM) by injecting streptozotocin. After 22 weeks of age, the mice were sacrificed and analyzed for different blood parameters. Oil Red O, MOVAT, MOMA-2 or α-SMC actin stainings were conducted on thoracic aortae (opened longitudinally) or frozen sections of the brachiocephalic arteries.

Results: The vessel lumen and plaque size of atherosclerotic lesions in the truncus brachiocephalic were decreased in diabetic TMPro/Pro ApoE-/- mice compared to diabetic ApoE-/- mice. While lipid accumulation in lesions of diabetic TMPro/Pro ApoE-/- mice was lower than that in diabetic ApoE-/- mice, morphometric analyses revealed more prominent signs of instable plaques, such as a larger necrotic core area and decreased fibrous cap thickness in diabetic TMPro/Pro ApoE-/- mice. Congruently more macrophages and fewer smooth muscle cells were observed within lesions of diabetic TMPro/Pro ApoE-/- mice.

Conclusion: Thus, impaired TM function reduces plaque stability, a characteristic of hyperglycemia-associated plaques, thus suggesting the crucial role of impaired TM function in mediating diabetes-associated atherosclerosis.

P-01-06

Association of Plasma Chemerin with All-Cause and Disease-Specific Mortality – Results from a Population-Based Study

Stephanie Zylla[1]; Katharina Noppes[1]; Stefan Groß[2]; Marcello R.P. Markus[2]; Martin Bahls[2]; Henry Völzke[3]; Marcus Dörr[2]; Matthias Nauck[1]; Nele Friedrich[1]

[1]Universitätsmedizin Greifswald, Institute of Clinical Chemistry and Laboratory Medicine, Greifswald, Germany; [2]Universitätsmedizin Greifswald, Department of Internal Medicine B, Greifswald, Germany; [3]Universitätsmedizin Greifswald, Institute for Community Medicine, Greifswald, Germany

Background and objectives: Various cross-sectional studies have observed an association between high circulating chemerin concentrations and an unfavorable metabolic profile. However, the actual prognostic value of chemerin for the risk of associated diseases and mortality was examined only in few studies mostly using small and specific patient populations. Therefore, this study aimed to analyze the association between plasma chemerin concentrations and all-cause as well as cause-specific mortality in a general population.

Study design and methods: From the Study of Health in Pomerania (SHIP) 2903 SHIP-START-1 and 4111 SHIP-TREND-0 participants were followed up for 15 and 9 years (median), respectively. The association between plasma chemerin and all-cause mortality was analyzed using Kaplan-Meier survival curves and multivariable Cox proportional hazard regression models. Additionally, cause-specific hazards for cardiovascular diseases (CVD) and cancer were modelled considering competing events.

Results: A total number of 372 and 126 deaths occurred during follow-up in SHIP-START-1 and SHIP-TREND-0, respectively. Unadjusted Kaplan-Meier survival curves illustrated in both study cohorts that subjects with chemerin ≥ 109 ng/mL (66.67%-percentile) had a lower survival function than subjects with lower chemerin. Multivariable regression analyses revealed that this association was independent of major confounders. Each increase of chemerin per 30 ng/mL was associated with a 23% higher risk of all-cause mortality (95%-confidence interval: 1.13 – 1.35). Cause-specific analyses have further shown that the effect estimates for cancer were greater than those for CVD.

Conclusion: The present study detected a positive association between plasma chemerin concentrations and mortality from all-causes, cancer, and CVD in a large population-based study sample. In comparison, the highest effect estimates were found for cancer as cause of death suggesting that the association between chemerin and mortality is mainly attributed to cancer related deaths.

P-01-07

Comparison of multi-steroid LC-MS/MS assays used for routine operation in five laboratories in Switzerland and Germany for the simultaneous analysis of 9 steroids

Valentin Braun[1]; Christoph Seger[2]; Manfred Rauh[3]; Matthias Weber[4]; Alexander Gaudl[5]; Uta Ceglarek[5]; Joana Gawinecka[6]; Daniel Müller[6]

[1]Dr Risch Ostschweiz AG, —, Buchs, Switzerland; [2]Universität Innsbruck, Department of Pharmacy, Innsbruck, Austria; [3]University Hospital Erlangen, Department of Pediatrics and Adolescent Medicine, Erlangen, Germany; [4]Bioscientia MVZ Labor Karlsruhe GmbH, —, Karlsruhe, Germany; [5]University Hospital Leipzig, Institute of Laboratory Medicine, Clinical

Chemistry and Molecular Diagnostics, Leipzig, Germany; [6]University Hospital Zurich, Institute of Clinical Chemistry, Zürich, Switzerland

Introduction: Regarding selectivity and specificity, LC-MS/MS is the best technology available for the quantification of steroids in human serum in a clinical setting. By simultaneous analysis of multiple steroids, it can provide the clinicians with more information for better diagnosis. For quality assurance, accuracy estimation and between-method standardization laboratory (lab) comparisons are a valuable tool. We present results from an inter-lab comparison study for 9 steroid analytes with 5 participating labs.

Methods: The sample set consisted of 40 pooled human serum samples generated by targeted mixing of pre-analyzed leftovers to get a well-balanced distribution across reference ranges of each steroid. All 5 labs measured a sample set once using own protocols and calibrators for their multi-steroid LC-MS/MS assay. 4 labs used in-house developed tests, from which 3 were using IVD-CE certified calibrators (Chromsystems, Munich, Germany), while the 5th lab was using the whole LC-MS kit of that manufacturer. All labs reported results for 17OH-progesterone (17P), androstenedione (A), cortisol (F) and testosterone (T) and 4 labs for 11-deoxycortisol (S), corticosterone (B), cortisone (E), DHEAS (DS) and progesterone (P). Results of each lab were compared against the mean of all labs using Bland-Altmann (BA) and Passing-Bablok (PB) statistics.

Results: Mean differences in BA-analysis were within ±10% for 17P, A, DS, E, P, S and T and within ±15% for B and F. Slopes of individual labs in PB analysis were inside the 95% CI of the slope calculated from all values. Significant deviations from the all-lab mean were found only in one site for 17P and A (mean differences 33.1% and -18.9%). BA 2 SD intervals, indicative of the variability of result differences, were < ±15 % for all analytes and labs except for one site (±17 to 30 %) and analytes P and T at all sites due to matrix effects from gel-barrier tubes and sensitivity and linearity issues at concentrations < 1 nM, respectively. Mean inter-lab CV was < 10% for E, F and S; < 15% for A, B, DS and T; 20.0% for 17P and 21.5% for P.

Conclusions: Comparable results (concordance of PB slopes) were found for B, E, F, P, S, and T but also for 17P and A, if one lab was excluded. Reference method assigned target values are missing hence the study delivers no evidence on the absolute trueness of the assays. The number of samples, measurements and participating labs was limited, but study design would allow for upscaling and repetition, due to the use of pooled instead of single donor samples. Nevertheless, results of this study indicate good overall standardization for the LC-MS/MS measurement of 9 steroids between 5 labs and some sources of higher variance and bias like operator errors, non-supported matrix types and higher imprecision at lower ends of measuring intervals could be identified, thereby helping the participating laboratories enhancing their assays and delivering better results to clinicians.

P-01-08

Analytical interference of different hemoglobin variants on HbA1c measurements comparing high-performance liquid chromatography with whole blood enzymatic assay.

Bozena Zechmeister[1]; Tanja Erden[2]; Berit Kreutzig[3]; Matthias Weber[4]; Philippe Joly[5]; Jürgen Erdmann[6]; Christine Brockmann-Honig[7]; Andreas Fischer[8]; Abass Eidizadeh[8]

[1]Universitätsmedizin Göttingen, Interdisziplinäres UMG-Labor, Göttingen, Germany; [2]Tosoh Bioscience, Diagnostic Business Unit-Europe, Göttingen, Germany; [3]Amedes MVZ wagnerstibbe , Laboratoriumsmed. med. Mikrobiologie und Immunologie, Göttingen, Germany; [4]4Bioscientia MVZ Labor Karlsruhe GmbH, Bioscientia MVZ Labor Karlsruhe, Karslruhe, Germany; [5]Laboratoire Interuniversitaire de Biologie de la Motricit´e (LIBM) EA7424, Team « Vascular Biology and Red Blood Cell », Lyon, France; [6]Laborgemeinschaft Rottweil G.b.R., Laborgemeinschaft Rottweil G.b.R., Villingen-Schwenningen, Germany; [7]Aesculabor Hamburg GmbH, Aesculabor Hamburg GmbH, Hamburg, Germany; [8]University Medical Center Göttingen, Institute for Clinical Chemistry/Interdisciplinary UMG Laboratory, Göttingen, Germany

Introduction: The concentration of glycated hemoglobin (HbA1c) is an essential diagnostic and therapeutic biomarker in diabetes mellitus. However, it is known that Hb structural variants and synthesis disorders, can affect the HbA1c measurement in different assays. Although the analytical interference of various hemoglobinopathies on the chromatographic measurement of HbA1c using HPLC has been well studied, data on the interference on the enzymatic assay are few.

Methods: In this multi-center study, a large number (n = 104) of 33 different hemoglobin variants were collected over a period of one year and compared between an HPLC (Tosoh G8 and G11) and an enzymatic assay (Abbott Alinity c).

Results: A good comparability between ion-exchange HPLC and the Alinity assay for most Hb variants was found. However, we were able to determine for the first time that certain Hb variants (Hb Okayama, HbAE, Hb Lepore) can lead to clinically relevant discordant results. HbF (>5%) can already cause a relevant aberration.

Conclusion: Overall, using the Abbott HbA1c assay in the presence of certain hemoglobin variants can induce clinically relevant interference that can affect diagnosis and therapy monitoring decisions, mainly because the enzymatic assay cannot provide any information about Hb variants.

P-01-09

Sex-specific Comparison of Reference Intervals for 13 Serum Steroids Determined by Direct or Indirect Methods

Sophie Anker[1]; Jakob Morgenstern[1]; Jakob Adler[2]; Maik Brune[1]; Sebastian Brings[1]; Thomas Fleming[1]; Elisabeth Kliemank[1]; Markus Zorn[1]; Andreas Fischer[3]; Julia Szendrödi[1]; Lars Kihm[1]; Johanna Zemva[1]

[1]University Hospital Heidelberg, Department of Internal Medicine I and Clinical Chemistry, Heidelberg, Germany; [2]Medical Laboratory for Clinical Chemistry, Microbiology, Infectious Diseases and Genetics Prof. Schenk/Dr. Ansorge & Colleagues, Clinical Chemistry, Magdeburg, Germany; [3]University Hospital Göttingen, Department of Clinical Chemistry, Göttingen, Germany

Aims: For the quantification of steroids, mass spectrometry offers a higher degree of specificity and sensitivity than conventional immunoassays. However, as reference intervals have not been adjusted accordingly [1], laboratories are required to determine their own values which is time- and cost-consuming due to the recruitment of a sufficient number of healthy reference subjects [2]. In this study, we addressed the question whether reference intervals for serum steroids can be established by an indirect approach. Furthermore, we wanted to contribute to the methodological harmonization of reference intervals.

Materials and Methods: We used data of 9801 individuals, of whom only age and gender were recorded, to establish post-hoc reference intervals for androstenedione, dehydroepiandrosterone, testosterone, progesterone, dihydrotestosterone, 17α-hydroxyprogesterone/-pregnenolone, corticosterone, 11-deoxycorticosterone, 11- and 21-deoxycortisol, aldosterone, cortisol, and cortisone. Analyses were performed on a Waters® Acquity UPLC class I system coupled to a Waters® XEVO TQ-S LC-MS using a MassChrom® Steroids kit (Chromsystems). The indirect reference interval algorithm [3,4], includes three robust quantile-based steps which were executed in R (Version 4.1.1): 1. Bowley's quartile skewness predicts whether a normal or a lognormal distribution should be assumed. 2. An iterative boxplot method is applied to remove obvious outliers. 3. A normal quantile-quantile plot provides the 2.5th and 97.5th percentiles, calculated from the intercept and slope of the linear regression line according to the formula RI = intercept±1.96•slope [4]. Post-hoc reference intervals were compared to reference intervals derived from studies using a direct approach.

Results: The majority of reference intervals generated by the indirect method showed a very good overlap with those derived from a direct approach. Significant deviations were seen for sex hormones such as progesterone, 17α-hydroxypregnenolone/-progesterone, and testosterone. This was reflected by skewed quantile-quantile-plots, which indicated an inhomogeneous distribution of the underlying data. The indirect approach yielded complete reference intervals also in cases, in which the lower reference limit was not provided by the direct method, e. g. in the case of aldosterone, 17-OH-pregnenolon and 11-Deoxycorticosterone.

Discussion/Conclusion: Our results suggest that it is a valid approach to verify and establish reference intervals by an indirect method. It should, however, be noted that reference intervals for widely varying sex hormones may differ significantly between direct and indirect methods due to missing background information on female cycle, menopause and stages of puberty when the indirect approach is applied to routine laboratory results. Further, this study contributes a substantial set of data to the methodological harmonization of steroid reference ranges using mass-spectrometry.

P-01-10 / FV-01

Critical appraisal of the influence of anti-Thyroglobulin on the measurement of Thyroglobulin and Thyroglobulin recovery

Robert Markewitz; Klaus-Peter Wandinger; Ralf Junker

Universitätsklinikum Schleswig-Holstein, Institut für Klinische Chemie, Kiel, Germany

Background: Antibodies against Thyroglobulin (Anti-Tg) are known to be able to cause interferences in the measurement of Thyroglobulin (Tg). Measuring Tg recovery after adding a known amount of Tg to the sample is a popular method to identify samples that are affected by this interference.

Methods: Results of clinical samples in which Tg, anti-Tg and Tg recovery were measured in a high-throughput clinical laboratory via immunometric assays (by Roche, Switzerland) were retroactively anonymized and analyzed. Specifically, the associations between the analytes Tg, anti-Tg and Tg recovery with one another and with the variables age and sex were statistically evaluated.

Results: 8871 samples were collected, of which only 60 (0.0068%) exhibited pathologically decreased Tg recovery, of which 47 contained quantifiable and 24 pathological levels of anti-Tg. Quantifiable levels of anti-Tg were associated with significantly decreased overall levels of Tg recovery and Tg itself (both: $p < 0.0001$), with the strongest decreases being associated with pathological levels of anti-Tg. But even for pathological anti-Tg, median Tg-recovery is still well within the reference range at 94±10.4 %. Effect sizes of all detected differences or associations were small to very small.

Conclusion: Tg recovery appears neither sensitive nor specific enough to detect interference of anti-Tg in the measurement of Tg on a single-specimen basis. This interference can nevertheless be detected with statistical methods both for Tg recovery and for Tg itself, albeit with small effect sizes. Methods other than Tg recovery are needed to reliably detect samples with impaired Tg measurement.

P-01-11 / PV-02

Activated protein C reduces maladaptive unfolded protein response (UPR) to ameliorate diabetes-accelerated atherosclerosis

Sameen Fatima; Saira Ambreen; Ihsan Gadi; Kunal Singh; Anubhuti Gupta; Ahmed Elwakiel; Shruthi Krishnan; Hamzah Khawaja; Akash Methew; Shrey Kohli; Berend Isermann; Khurrum Shahzad

Institut für Laboratoriumsmedizin, Klinische Chemi, Department of molecular diagnostics, Leipzig, Germany

Background: The mechanisms underlying the different atherosclerosis course in diabetic and non-diabetic patients remain unknown. Hyperglycemia causes endothelial cells dysfunction, a key disease driver. Atherosclerotic plaques display markers of senescence and unfolded protein response (UPR). UPR has been linked with the atherosclerosis. One of the outcome of UPR is to control the cell senescence hallmarks including cell cycle arrest, DNA repair capacity, morphological changes, metabolic changes, the secretory pathway, and changes in membrane lipid composition. Plasma levels of coagulation protease, activated protein C decline in diabetes and atherosclerosis.

Aim of the Study: In this project we hypothesized that high glucose induces UPR causes cell senescence, results in endothelial dysfunction which leads to vulnerable plaques. Activated protein C or IRE1 alpha inhibitor prevent diabetes induced accelerated atherosclerosis by reducing maladaptive UPR induced endothelial cell senescence.

Methods: To gain insights into pathomechanisms of diabetes induced atherosclerotic plaque development we cultured human coronary artery endothelial cells (HCAECs) under hyperglycemic (HG) or hyperlipidaemic (HL) conditions. ApoE-/- mice (age 8 weeks) was made either diabetic by streptozotocin injections (A mouse model type 1 diabetes) or fed them HFD to induce hyperlipidemia. Mice were analyzed after 20 weeks of treatments.

Results: HG conditions induced strong barrier disruption as compared to HL (TEER, FITC dextran leakage) and protein expression of the senescence markers (p21, p16, p53) and UPR markers (XBP1, IRE1α and ATF6). Activated protein C restored barrier integrity, reduced glucose induced expression of senescence and UPR markers in vitro. Targeting IRE1α RNAase activity prevented HG induced cellular senescence. Ex vivo, diabetic ApoE-/- mice revealed increased expression of senescence and UPR markers within atherosclerotic lesion as compared with non-diabetic ApoE-/- mice. Activated proetin C significantly reduced expression of senescence and UPR markers within atherosclerotic lesions of diabetic ApoE-/- mice. Thus, senescence associated inflammation and UPR are associated with glucose-dependent endothelial cells dysfunction and loss of endothelial barrier integrity.

Conclusion: These results demonstrate that diabetes-induced atherosclerosis is associated with cellular senescence and UPR. Targeting cellular senescence and UPR (with aPC or IRE1α inhibitor) may be a useful therapy of atherosclerosis in diabetic patients.

P-02-01

Fasting plasma glucose concentrations in different sampling tubes measured on different glucose analyzers

Stefan Pleus; Alexandra Beil; Annette Baumstark; Cornelia Haug; Guido Freckmann

Institut für Diabetes-Technologie, Forschungs- und Entwicklungsgesellschaft mbH an der Universität Ulm, n.a., Ulm, Germany

Introduction: The German Diabetes Association recommends use of sampling tubes with citrate and fluoride additives for the diagnosis of diabetes by oral glucose tolerance test. In this study, venous blood was sampled in different tubes with and without citrate/fluoride additives. Glucose concentrations were subsequently determined on different laboratory analyzers in order to estimate the potential effect of these additives.

Materials and Methods: Venous samples were obtained from 42 persons without anamnestically known diabetes mellitus in a fasting state. Samples were collected in lithium-heparinized tubes (LH) and in tubes with citrate/fluoride additives: GlucoEXACT 66x11mm (GE1), GlucoEXACT 75x13mm (GE2), and Vacuette FC Mix (VA). For each participant, venous blood was sampled 3 times per tube type for centrifugation after different periods (immediately (≤5 min), 20 min, and 240 min). All measurements were performed in plasma. Measurements for LH and GE tubes were performed on 3 hexokinase-based laboratory analyzers: Cobas Integra 400 plus with GLUC2 Glucose HK application (LAB1; Roche) and two Cobas pro c503 with GLUC3 Glucose HK Gen.3 application (LAB2a, LAB2b; Roche). VA tubes were measured only on LAB1.
Relative differences between glucose concentrations in immediately centrifuged LH tubes (LAB1) and glucose concentrations in all other samples were calculated. Since glucose concentrations in GE1, GE2 and VA tubes were similar for the three incubation times when measured on the same analyzer, results were pooled.

Results: Fasting glucose concentrations in immediately centrifuged LH samples were 94.0±5.9 mg/dl (mean±standard deviation), ranging from 80.0 to 105.2 mg/dl (n=42). For LAB1, relative differences in tubes with citrate/fluoride additives were -5.9±1.2% (GE1, n=118), -6.1±1.3% (GE2, n=119), and -1.0±1.4% (VA, n=104). For LAB2a, differences of -1.5±0.5% (LH, n=41), -0.7±1.3% (GE1, n=123), and -1.8±1.4% (GE2, n=118) were found; differences for LAB2b were -0.1±0.5% (LH, n=41), +0.6±1.3% (GE1, n=123), and -0.4±1.4% (GE2, n=118). LH samples centrifuged after 20 min exhibited some glycolysis (-2.4±1.1% (LAB1, n=41), -3.1±1.1% (LAB2a, n=40), -1.8±1.2% (LAB2b, n=40)), although the effect was much more pronounced after a delay of 240 min (-17.8±3.6% (LAB1, n=41), -18.9±3.5% (LAB2a, n=41), -17.7±3.6% (LAB2b, n=41)).

Conclusion: Systematic differences of about -6% were found for GE tubes compared to LH tubes on analyzer LAB1, whereas no relevant differences between GH and LH tubes were found on analyzers LAB2a and LAB2b. These results suggest that additives in GE tubes might affect glucose measurement with the hexokinase application used in analyzer LAB1. VA samples did not show relevant differences on analyzer LAB1. In LH samples, delayed centrifugation of 20 and 240 min led to systematic differences of up to -3% and -19%, respectively.

P-02-02

Differences between venous, capillary and interstitial glucose concentrations during an oral glucose tolerance test

Stefan Pleus; Alexandra Beil; Annette Baumstark; Sebastian Schauer; Manuela Link; Delia Waldenmaier; Cornelia Haug; Guido Freckmann

Institut für Diabetes-Technologie, Forschungs- und Entwicklungsgesellschaft mbH an der Universität Ulm, n.a., Ulm, Germany

Introduction: In this study, glucose concentrations in venous blood, capillary blood, and interstitial fluid (ISF) obtained during an oral glucose tolerance test (oGTT) were compared. Capillary and venous blood glucose (BG) concentrations are known to be not necessarily comparable. This study aimed at quantifying potential differences between venous BG, capillary BG, and ISF glucose concentrations before and after glycemic load.

Materials and Methods: An oGTT was performed on 41 persons without anamnestically known diabetes mellitus. Venous and capillary blood sampling was performed in a fasting state before consumption of a standardized 75-g glucose solution as well as 60 and 120 minutes afterwards. In parallel, ISF glucose was recorded with a FreeStyle Libre system for continuous glucose monitoring (CGM). Venous and capillary blood samples were collected in lithium-heparinized tubes for immediate centrifugation (median: 3 min) and subsequent plasma glucose (PG) measurement on a laboratory analyzer (LAB; Cobas Integra 400 plus). Capillary BG measurements were also performed with a BG monitoring system (BGMS; Contour Next One). Relative differences from venous PG (LAB) were calculated for capillary PG (LAB), plasma-equivalent capillary BG (BGMS) and ISF glucose (CGM).

Results: Fasting glucose concentrations at the start of the oGTT showed only minimal differences from venous PG (LAB). Results are provided as mean ± standard deviation. Capillary PG (LAB) was +3.9% ± 3.6% higher than venous PG, capillary BG (BGMS) was +9.1% ± 5.2% higher, and ISF glucose (CGM) was +1.4% ± 12.3%. After 60 minutes, marked differences with increased variability were found: +34.9% ± 23.8% for capillary PG (LAB), +42.8% ± 25.5% for capillary BG (BGMS), and +30.8% ± 25.7% for ISF glucose (CGM). After 120 minutes, slightly smaller, but still marked differences with increased variability were found: +27.0% ± 15.6% for capillary PG (LAB), +33.2% ± 17.7% for cap BG (BGMS), and +17.6% ± 19.5% for ISF glucose (CGM).

Conclusion: After consumption of the oGTT glucose solution, capillary BG/PG and ISF glucose concentrations were substantially higher than venous PG concentrations. Fasting glucose concentrations showed minimal differences. The post-prandial differences exhibited marked inter-individual variability.

P-02-03

HbA1c-usage under intensified preanalytical scrutiny

Norbert Ostendorf

St. Franziskus-Hospital Münster, Institut für Laboratoriums- und Transfusionsmedizin, Münster, Germany

Introduction: We established an algorithm to question the reliability of HbA1c-measurements in a tertiary care hospital by looking at concomitant data in the laboratory information system, especially full blood count and transfusion record. After one year, data obtained by this strategy were evaluated.

Methods: Results of HbA1c testing were validated together with haemoglobin (Hb) concentration and reticulocyte counts using the following algorithm: Hb concentrations of 10 g/dl or more were considered unsuspicious. When Hb concentrations were below 10 g/dl but not lower than 8 g/dl, a comment was added to the HbA1c-result, stating that the value was questionable. When Hb concentrations were below 8 g/dl, the HbA1c result was deleted and replaced by a commentary stating that the result was questionable and reporting the HbA1c value within the comment only. Moreover, in these cases the patient record was checked for erythrocyte transfusions within the current hospital stay. Finally, in cases where the HbA1c value was implausibly low in the first place, a reticulocyte count was performed to look for hyperregenerative states. HbA1c was considered implausibly low in patients above 80 years of age and a HbA1c result below 5%.

Results: From 01.05.2021 to 30.04.2022, 2718 measurements were evaluated. Of these, 87 or 3.2% were replaced by a comment because a preanalytical impairment was very likely. In 29 cases, transfusions of erythrocytes were recorded prior to the requested HbA1c-measurement. From the remaining 2631 values, 165 or 6.3% were commented to highlight the possibility of preanalytical impairment.

Conclusion: HbA1c-measurement has a special preanalytical issue: a normal erythrocyte turnover in the weeks before the investigation and avoidance of recent erythrocyte transfusions. Using a haemoglobin concentration of 10 g/dl as an indicator for bleeding or impaired erythropoesis, 9.5% of the investigations were suspicious for preanalytical errors. Erythrocyte transfusions interfered with 1% of HbA1c-measurement.

P-02-04

Plasma-glycerol determinations: comparison of an enzymatic assay versus NMR spectroscopy

Mohammed Abdullah[1]; Kathrin Budde[1]; Ann-Kristin Henning[1]; Astrid Petersmann[2]; Matthias Nauck[1]

[1]Uni.Med.Greifswald, Institut für Klinische Chemie und Laboratoriumsmedizin, Greifswald, Germany; [2]Universitätsmedizin Oldenburg, Institut für Klinische Chemie und Laboratoriumsmedizin, Oldenburg, Germany

Background: Glycerol is a trivalent alcohol (1,2,3-propanetriol) composing the backbone of the triglyceride molecule. The determination of plasma glycerol is not applied in general patient care, but it is the main reaction of the enzymatic determination of triglyceride concentrations after the hydrolysis is performed. In vivo, glycerol is metabolized quickly, so that the plasma concentrations are below 20 mg/dl in general. But the concentrations of glycerol represent the products of in vitro lipolysis. The hydrolysis of triglycerides can be reduced by storing samples refrigerated or even frozen in the biobanking context. The amount of glycerol is a marker for hydrolysis and therefore a biomarker for sample quality.

Methods: In the presented study, we evaluated the performance of two different methods for glycerol concentration measurements: Classical enzymatic method and NMR spectroscopy. Fifty plasma samples from patients with normal and abnormal triglyceride concentrations were collected randomly. Glycerol concentration has been directly estimated (T0) and after two weeks of storage at room temperature (T1). The correlation and agreement between these methods have been analyzed according to laboratory standards.

Results: The glycerol concentration increased from 0.22 ± 0.14 mmol/l (T0) to 1.3 ± 0.45 mmol/l (T1), demonstrating the hydrolyses of triglycerides during this time period. Both glycerol measurements showed a strong correlation and agreement according to Passing-Bablok. Bland-Altman analysis confirmed the good agreement between both methods, which was more pronounced at the higher concentration range. Conclusions: Due to its high reproducibility, robustness, sensitivity and good agreement with glycerol concentrations measured by enzymatic method, the NMR spectroscopy is a valuable analytical tool for glycerol determination in plasma samples. Keywords: Glycerol, Nuclear Magnetic Resonance (NMR), Triglyceride.

P-02-05

Association of suPAR and cardiovascular risk factors in a young and healthy population

Niklas Wohlwend[1]; Kirsten Grossmann[1]; Martin Risch[2]; Ornella C. Weideli[1]; Stefanie Aeschbacher[3]; David Conen[4]; Lorenz Risch[2]

[1]Labormedizinisches Zentrum Dr. Risch, Forschung, Vaduz, Liechtenstein; [2]Labormedizinisches Zentrum Dr. Risch, Geschäftsleitung, Vaduz, Liechtenstein; [3]University Hospital Basel, Cardiovascular Research Institute Basel and Division of Cardiology , Basel, Switzerland; [4]McMaster University, Population Health Research Institute, Hamilton, Canada

Aims: The increasing global burden of cardiovascular diseases led to further intensive research in its cause, its development, its therapy and more and more in possible preventive measures including individual risk stratification strategies. The biomarker soluble urokinase plasminogen activator receptor (suPAR) is one candidate of potential biomarkers which could be used for the evaluation of the individual cardiovascular risk.

Materials and Methods: The current analysis is based on data from the GAPP (Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors) study, a population-based cohort-study investigating young and healthy adults (aged 25-41 at baseline) living in the Principality of Liechtenstein. In this analysis we included the following cardiovascular risk factors and laboratory parameters: BMI, physical activity, alcohol consumption, smoking status, blood pressure parameters, glucose status, lipid levels, liver enzymes, and kidney function. Additionally, the Framingham Score and the Healthy Lifestyle Score as indicators of the overall cardiovascular risk were included. We compared these factors using sex-specific quartiles and multivariate regression analysis in relation to suPAR serum levels.

Results: Comparing the baseline characteristics, it was shown that female participants have higher levels of serum suPAR levels than the male participants (1.73 vs 1.50, p-value < 0.001). An inverse correlation between serum suPAR levels and HDL-cholesterol in men (p-value < 0.001) and women (p-value < 0.001) was found. Furthermore, smoking participants showed higher levels of serum suPAR levels (p-value < 0.001). For male participants, a higher lifestyle score resulted in lower serum suPAR levels (p-value < 0.001). The HbA1c (p-value 0.008) and the Framingham Score (p-value < 0.001) correlated with serum suPAR levels for female participants.

Discussion/Conclusion: The current analysis shows statistically significant differences in average serum suPAR levels for male and female participants. The correlation of suPAR and cardiovascular risk factors differs among male and female participants. suPAR as a biomarker for future cardiovascular risk in the general population can support clinical pre-diagnostics.

P-02-06

Standardizing laboratory parameters in different models and stages of experimental cirrhosis and in healthy control male Sprague-Dawley rats

Ramona Christina Dolscheid-Pommerich[1]; Franziska Schneider[2]; Marc Hebest[2]; Lino Teichmann[3]; Ruth-Miriam Koerber[4]; Michael Praktiknjo[2]; Birgit Stoffel-Wagner[1]; Johannes Chang[2]

[1]University Hospital Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, Bonn, Germany; [2]University Hospital Bonn, Department of Internal Medicine I, Center for Cirrhosis and Portal Hypertension Bonn (CCB), Bonn, Germany; [3]University Hospital Bonn, Department of Medicine III, Bonn, Germany; [4]University Hospital Bonn, Department of Medicine III, Mildred Scheel School of Oncology, Bonn, Germany

Introduction: Cirrhosis is the common end-stage of liver disease. Established animal models of experimental cirrhosis have been widely used to study human pathogenesis of chronic liver disease and portal hypertension. Two of the most successful and reproducible models are a cholestatic model of bile-duct ligation (BDL) and a toxic model induced by exposure to carbon tetrachloride (CCL4). In the context of these models, some of the liver-related laboratory parameters have been investigated in the past. However, there are no standard laboratory parameter ranges for these models.

Material and Methods: To induce cholestatic cirrhosis (BDL), in male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) the bile duct was exposed after median laparotomy, ligated twice and dissected between the two ligatures. For toxic cirrhosis, the animals were exposed to CCL4 inhalation twice weekly in increasing intervals. Blood samples from these models and from healthy control animals were taken at defined timepoints. Analyses were carried out at the central laboratory. Sodium and potassium were determined potentiometrically (cobas 8000 ISE, Roche Diagnostics). Total protein, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase, triglycerides, cholesterol, high and low density-lipoprotein cholesterol, direct and total bilirubin, creatinine, and urea nitrogen were determined with cobas c702 or c502 (Roche Diagnostics). Mean, median, standard deviation, minimum and maximum, 2.5-97.5, 5-95 or 10-90 percentiles (depending on group size) were calculated.

Results: AST, ALT were slightly elevated in healthy controls compared to human reference ranges. Different stages of cirrhosis in BDL and CCL4 models showed distinct differences in liver-related parameters. In BDL rats, the development of clinical and laboratory features, such as weight changes, hypalbuminemia and hyperbilirubinemia, was similar to that of human cirrhosis. Kidney function worsened with progression of cirrhosis. In CCL4 rats, bilirubin was only slightly elevated, even in more advanced stages, compared to healthy controls. Over time, liver-related parameters worsened. In general, changes in laboratory parameters were more pronounced in the BDL model than in the CCL4 model. Interestingly, sodium levels did not differ between control animals and models of cirrhosis nor between compensated and more decompensated stages of cirrhosis.

Conclusion: Standardizing laboratory values in experimental cirrhosis in rodents is of utmost importance. However, existing data mostly focus on few parameters in treated groups. With our data, we provide a much needed overview of standard laboratory values in experimental cirrhosis models and healthy controls in male Sprague Dawley rats. Our data show that some laboratory features behave in a similar manner to human cirrhosis (albumin, liver parameters), while others, such as sodium, do not.

P-02-07

The extent of life-style induced weight loss determines the risk of prediabetes and metabolic syndrome recurrence during a 5-year follow-up

Silke Zimmermann; Akash Mathew; Berend Isermann; Ronald Biemann

Universitätsklinikum Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany

Aims: Lifestyle-induced weight loss (LIWL) is regarded an efficient therapy to reverse or ameliorate metabolic syndrome (MetS). However, weight loss is difficult to maintain. Studies have shown, that 4.5 years after a structured weight loss program the average sustained weight loss was only a -3.2 % of the initial weight. The aim of our study was to determine (i) whether the initial LIWL-induced weight loss predicts the sustained weight loss, (ii) the relation of weight initial loss changes to parameters characteristic of the MetS, and (iii) whether the extent of initial weight loss in a controlled LIWL predicts the risk of prediabetes and recurrence of the MetS at 5 years of follow-up.

Methods: The study is embedded in a prospective, controlled, monocentric, 6-month LIWL intervention trial in individuals with MetS (ICTRP Trial Number: U1111-1158-3672). Following LIWL, 61 participants were split based on the initial weight loss (WL) into four quartiles: Q1 (WL 2.0-8.1 %, n= 15), Q2 (WL 8.1- 13.0 %, n= 15), Q3 (WL 13.0- 16.6 %, n= 15), Q4 (WL 16.6- 27.5 %, n= 16) and followed up for 5 years. Changes of body weight and parameters reflecting MetS i.e. fasting plasma glucose (FPG), triglycerides (TG), HDL-cholesterol and blood pressure were analyzed before and after LIWL and at annual follow-up visits over 5 years.

Results: The mean weight loss was 12% after completing the LIWL and 3.6% at 5 years follow-up. Weight gain after LIWL was comparable between the quartiles. A differentiation of the quartiles shows that participants with a higher initial weight loss (> Q1) were able to maintain their body weight below the initial weight after 5 years. In addition to weight gain, the magnitude of the FPG increase was more pronounced in Q1 compared to Q4 (54% versus 21% at 5-year follow-up). Accordingly, recurrence of prediabetes frequency (glycated hemoglobin 5.7 to 6.4 %) was increased during follow-up in Q1 as compared to quartiles with higher weight loss. The frequency of MetS was reduced after LIWL to 73 % (Q1), 43 % (Q2), 27 % (Q3) and 31 % (Q4). Whereas a significant reduction of MetS frequency was maintained in Q3 and Q4 during the 5-year follow-up, the number of participants with MetS increased in Q1 and Q2 already after 1 (Q1) and 2 years (Q2) post LIWL.

Discussion: Our current results indicate that LIWL of more than 8% reduces the risk of prediabetes and recurrence of MetS up to 5 years.

P-02-08

Effects of PCSK9 inhibitors on apolipoproteins

Anne Mieritz[1]; Julia Dittrich[1]; Ilijana Begcevic Brkovic[1]; Markus Scholz[2]; Ulrich Laufs[3]; Uta Ceglarek[1]

[1]University Hospital Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany; [2]Leipzig University, Institute for Medical Informatics, Statistics and Epidemiology (IMISE), Leipzig, Germany; [3]Universitätsklinikum Leipzig, Klinik und Poliklinik für Kardiologie, Leipzig, Germany

Introduction: Apolipoproteins are promising biomarkers for atherosclerotic cardiovascular disease (ASCVD), which represents one of the main causes of death in western society. Hyperlipidemia, especially of LDL, plays a major role in the pathogenesis of ASCVD. Therefore, reduction of atherosclerotic lipoproteins in blood is an important option in the prevention of cardiovascular events.
In addition to conventional lipid-lowering drugs, PCSK9 inhibitors represent a new therapeutic approach. These monoclonal antibodies decrease the degradation of the LDL-receptor resulting in an increased LDL intake and, consequently, in a reduction of LDL cholesterol levels by 50-60%. However, information on effects on other lipoprotein constituents like apolipoproteins are missing, so far. Therefore, we studied apo profiles before and after administration of PCSK9 inhibitors.

Methods: In this observational study, 92 patients with diagnosed hyperlipidemia were included. Blood samples were drawn in fasted state prior and 4-8 weeks after administration of the PCSK9 inhibitors Evolocumab and Alirocumab. Two different dosages (75 mg vs. 140/150 mg) were applied. Simultaneous quantification of the apos A-I, A-II, A-IV, B-100, C-I, C-II, C III, D, E, H, J, and M was performed from 3 µl serum by LC-MS/MS. Group comparisons were performed using paired sample t-test, Kruskal-Wallis H test, Mann-Whitney-U-test and multivariate regression.

Results: PCSK9 administration reduced serum levels of the LDL- and triglyceride-rich lipoproteins-associated apos B-100, C-I, C-II, C-III, and E by 12% to 46%. Furthermore, concentrations of apos D, and M were also decreased by up to 15%. These effects were independent from the subjects' potential lipid-lowering premedication, naming statins, ezetimibe or a combined administration, as well as from PCSK9 inhibitor-induced changes in LDL cholesterol. Interestingly, the effects of PCKS9 inhibitor administration on LDL cholesterol and apos were not dose-dependent.

Conclusion: Apolipoprotein profiles are altered by PCSK9 inhibitors independently from total and LDL-cholesterol levels and provide additional information of PCSK9 effects on lipoprotein metabolism.

P-02-09

Adjusted Calcium – It is the cutoff, not the equation!

Felix Eckelt[1]; Maria Schmidt[1]; Anke Tönjes[2]; Berend Isermann[1]; Thorsten Kaiser[1]

[1]Universitätsklinikum Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany; [2]Universitätsklinikum Leipzig, Clinic and Polyclinic for Endocrinology, Nephrology, Rheumatology, Leipzig, Germany

Introduction: Calcium (Ca) disorders are common and adverse events such as coma, cardiac arrest or tetany occur if left untreated. Severe calcium disorders can be treated, but are easy to miss due to multifaceted symptomatics. Diagnostic options available are: total calcium (tCa), free calcium (fCa) and adjusted calcium (aCa). fCa is the analytical gold standard, but blood sampling is laborious, prone to preanalytic error and expensive. tCa is the routine parameter at hospital admission for screening of calcium disorders, yet interpretability is hampered by its dependence on primarily albumin and secondarily anion concentrations. The relationship between tCa and albumin is known to physicians and incorporated into interpretations. aCa calculation is based on published equations (e.g. Payne, Smith), yet, it is consensus not to use published equations at other hospitals without validation. It is clinical practice nonetheless. Our objective at the University Medicine Leipzig (UML) was to develop our own equation for aCa, reevaluate its decision cutoffs based on the gold standard fCa and compare them to established tCa cutoffs.

Methods: The first German adjusted Ca (aCa) equation was developed using UML measurements of Ca in whole blood (fCa) and serum (tCa), and albumin (2014-2019). Set fCa cutoffs for severe hypocalcemia (< 0.9mmol/L) and severe hypercalcemia (> 1.6mmol/L) were translated to concordant aCa and tCa cutoffs. In a retrospective analysis of inpatient laboratory results and clinical data at UML (2018-19) the equation and cutoffs were applied and alerts for hypo- and hypercalcemia detected accordingly.

Results: The presented optimized aCa cutoffs show best concordance with fCa and were similar for our equation and established ones by Payne et al. and Smith et al. Cutoffs differed considerably between tCa and aCa. Also, our optimized cutoffs do not match the recommendations of the European Society of Endocrinology for hypo- (1.9mmol/L) and hypercalcemia (3.5mmol/L). Retrospective application to UML inpatients (598,592 Ca measurements in 59,209 cases with ≥5y of age) revealed severe hypocalcemia (tCa < 1.6 mmol/L, aCa < 1.9mmol/L) in 2,154 cases (61% female) and severe hypercalcemia (tCa > 2.8 mmol/L, aCa > 3.1mmol/L) in 353 cases (47% female). Hospital length of stay was increased for severe hypocalcemia and hypercalcemia (8.3d and 17.2d vs. 4.7d) as well as admission to ICU treatment (52% and 40% vs. 15%).

Conclusions: The largest cohort is presented to evaluate aCa. Translating fCa cutoffs to aCa and tCa equivalents reveals the first evidence-based decision thresholds for severe Ca disorders. Optimized cutoffs have greater influence potential on interpretation quality than optimized equations. We suggest to calculate an aCa and, moreover, to critically assess it with cutoffs tailored to the used equation for best results. Results have been incorporated into the clinical decision support system AMPEL and our laboratory information system.

P-02-10 / FV-02

The reliability of rapid immunoassay for the detection of anti-PF-4 antibodies in patients suspected of vaccine-induced immune thrombotic thrombocytopenia after COVID-19 vaccination

Günalp Uzun; Karina Althaus; Stefanie Hammer; Yvonne Wanner; Stefanie Nowak-Harnau; Sigrid Enkel; Tamam Bakchoul

Zentrum für klinische Transfusionsmedizin, Transfusionsmedizin, Tübingen, Germany

Aims: Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare but serious complication of vector based COVID-19 vaccines. Similar due heparin-induced thrombocytopenia (HIT), antibodies reacting to platelet factor 4 (PF4) are responsible from platelet activation in VITT. The diagnosis of VITT includes the detection of anti-PF4 antibodies. Particle gel immunoassay is one of the rapid tests, that is commonly used in the diagnosis of HIT to detect anti-PF4 antibodies. The aim of the current study was to investigate the diagnostic performance of rapid immunoassay in patients suspected of VITT.

Materials and Methods: In this retrospective, single-center study, the correlation between rapid immunoassay, enzyme-linked immunosorbent assay (ELISA) and modified heparin induced platelet aggregation assay (HIPA) in patients with findings suggestive of VITT was investigated. A commercially available PF4 rapid immunoassay (ID PaGIA H/PF4, Diamed) and an anti PF4/Heparin ELISA (Zymutest HIA IgG, Hyphen) were used according to manufacturer´s instructions. A sample was considered reactive in ELISA if the optical density (OD) was ≥0.500. Modified HIPA was accepted as the gold standard test.

Results: Between March 8th and May 20th, 21 samples from clinically well-characterized patients were analyzed with rapid immunoassay, ELISA and HIPA. Of these sera 8 revealed positive results and 13 tested negative in rapid immunoassay. Sensitivity and specificity of rapid immunoassay were 50% and 69%, respectively. On the other hand, sensitivity and specificity of ELISA were 88% and 92%, respectively.

Discussion: Particle gel immunoassay is not reliable in the detection of anti-PF4 antibodies in patients suspected of VITT. Commercially available PF4 rapid immunoassays are validated for the diagnosis of HIT. They should not be used to rule out VITT.

P-02-11 / PV-03

Elevated cholinesterase activity and the metabolic syndrome - dissecting fatty liver, insulin resistance and dysglycemia

Martin Heni[1]; Julia Hummel[2]; Louise Fritsche[2]; Robert Wagner[3]; Lasse Relker[1]; Jürgen Machann[4]; Fritz Schick[4]; Andreas Birkenfeld[3]; Hans-Ulrich Häring[3]; Norbert Stefan[3]; Andreas Fritsche[3]; Andreas Peter[1]

[1]Universitätsklinikum Tübingen, Institut für Klinische Chemie und Pathobiochemie, Tübingen, Germany; [2]Helmholtz Zentrum München, IDM, Tübingen, Germany; [3]Universitätsklinikum Tübingen, Medizinische Klinik IV, Tübingen, Germany; [4]Universitätsklinikum Tübingen, Diagnostische und Interventionelle Radiologie, Tübingen, Germany

Aims: While low plasma cholinesterase activity (CHE) is a well-established marker of reduced hepatic synthesis capacity, the clinical significance of elevated activity is not clear. High CHE was long suspected to be present in hepatic steatosis and metabolic syndrome, as reported by older and smaller studies. We aimed to clarify the relation between CHE and the metabolic syndrome as well as with precisely quantified liver fat content.

Materials and Methods: CHE activity was measured photometrically (Butyrylthiocholin 5-Thio-2-nitrobenzoat) in lithium-heparin plasma in 844 humans (554 women) of the cross-sectional Tübingen Diabetes Family Study with a wide BMI range (17.6 – 55.1 kg/m2) and without severe diseases (including liver diseases). It was furthermore retrospectively measured in 108 participants of the Tübingen Lifestyle Intervention Program (TULIP) before and after a 9-month lifestyle intervention. Liver fat content was quantified with MR-spectroscopy. All participants underwent detailed metabolic phenotyping including a 2-h 75 g oGTT with glucose, insulin and C-peptide measurements at every 30 minutes. From that, insulin sensitivity was assessed using the Matsuda formula and insulin secretion was estimated as the AUC0-30 of insulin / AUC0-30 of glucose.

Results: CHE was positively associated with liver fat content, independent of sex, age and BMI (p < 0.0001). CHE activity was higher in participants fulfilling the IDF-criteria for the metabolic syndrome (p < 0.0001).

CHE was also positively associated with the fasting plasma glucose and glucose during the OGTT (AUCglucose), independent of sex, age and BMI (both p< 0.0001). While CHE was not associated with insulin secretion (p=0.7, adj. sex, age, insulin sensitivity), it was negatively associated with insulin sensitivity, independent of sex, age and BMI (p < 0.0001), as well as after additional adjustment for liver fat content (p < 0.0001).

The reduction of liver fat content during lifestyle intervention was associated with a reduction in CHE, independent of body weight loss (p < 0.0001). The change in CHE was furthermore associated with the improvement in insulin sensitivity (p < 0.0001). This remained significant even after adjustment for sex, age, BMI and liver fat (p=0.01).

Conclusion: Our cross-sectional and longitudinal results using state-of-the-art approaches for metabolic phenotyping confirm that higher CHE is a marker for liver fat accumulation and is present in patients with metabolic syndrome. We furthermore detected links to glucose tolerance and insulin sensitivity. Of note, these were independent of liver fat content. This suggests that CHE could be not just a marker for liver steatosis but could be indicative of processes in hepatocytes that contribute to metabolic health. Further investigations are needed to clarify the mechanistic contribution and potential diagnostic value of elevated CHE in hepatic steatosis and metabolic diseases.

P-03-01

Sera of Syphilis patients contain Antiphospholipid Antibodies with procoagulant properties

Nadine Müller-Calleja[1]; Anne Hollerbach[2]; Karl J. Lackner[2]

[1]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Institut für klinische Chemie und Laboratoriumsmedizin, Mainz, Germany; [2]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Institut für klinische Chemie & Laboratoriumsmedizin, Mainz, Germany

Objectives: Antiphospholipid antibodies (aPL) are causally involved in the development of the antiphospholipid syndrome (APS), an autoimmune disease characterized by recurrent thrombosis and fetal loss. Beside APS, many infections have been found to be associated with elevated aPL titers. Syphilis was the first infection to be linked to the aPL. Since Syphilis patients show a higher risk for stroke, we want to analyze whether this was due to the procoagulant properties of anti-cardiolipin(aCL) aPL that are found in these patients.

Methods: IgG fractions were prepared from those Syphilis patient's sera with a positive aCL titer. The ability of these IgG fractions to activate Tissue factor, to induce endosomal ROS generation and to stimulate TNFα production was measured in human monocytes by a single stage clotting assay, by flow cytometry or by qRT-PCR, respectively.

Results: aPL isolated from Syphilis patients could be characterized as cofactor-independent aCL since they bind to cardiolipin in the absence of any cofactors as shown by a self-made ELISA. In contrast, these Syphilis aCL (s-aCL) could not bind to b2GPI. S-aCL were able to activate the same signaling pathway that has recently been reported for autoimmune-type lipid-reactive aPL: lysobisphosphatidic acid (LBPA) presented by the CD1d-like endothelial protein C receptor (EPCR) EPCR serves as the cell surface receptor for s-aCL. Binding of s-aCL to this LBPA-EPCR complex mediates Tissue factor activation on monocytes, s-aCL internalization and subsequent sensitization of immune cells to toll-like receptor 7 agonists. Moreover, this signaling complex specifically participates in interferon responses in monocytes and dendritic cells. However, compared to aCL isolated from APS patients, s-aCL must be used in a 3-4 fold higher concentration than classical aPL to achieve the same effects.

Conclusion: s-aCL have procoagulalant and proinflammatory properties. Therefore they can increase the risk for stroke. However, compared to aPL isolated from APS patients, s-aCL have a lower affinity for cardiolipin. This may be the reason why thromboembolic events do not occur more frequently in syphilis patients.

P-03-02

Platelets and neutrophil extracellular traps promote thrombo-inflammation and glomerular endothelial dysfunction in diabetic kidney disease

Anubhuti Gupta[1]; Kunal Singh[2]; Ruaa Younis[2]; Sameen Fatima[2]; Saira Ambreen[2]; Silke Zimmermann[2]; Shruthi Krishnan[3]; Ronald Biemann[4]; Khurrum Shahzad[2]; Berend Isermann[2]; Shrey Kohli[2]

[1]Universitätsklinikum Leipzig, Institute of laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, 04103, Germany; [2]Universitätsklinikum Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, 04103, Germany; [3]Otto-von-Guericke Universität Magdeburg, Institute of Experimental Internal Medicine, 39120, Germany; [4]Universitätsmedizin Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, 04103, Germany

Aim: Diabetic kidney disease (DKD) is a major cause of end-stage renal failure contributing to morbidity and mortality worldwide. Therapeutic options to prevent or reverse DKD progression are limited or lacking, respectively. Endothelial dysfunction, platelet-hyperactivity, immune cell infiltration and glomerular filtration barrier (GFB) disruption are associated with DKD. We aim to scrutinize the mechanistic interplay between platelets and neutrophil extracellular traps (NETs) and ensuing renal thrombo-inflammation.

Method: A type 1 diabetes mouse model (streptozotocin) was used to evaluate the role of platelet activation and NET formation in DKD. Therapeutic interventions (ASA, Anakinra, Solulin, GSK484) were performed in sub-groups of mice between week 16 to 24 of diabetes to study disease reversal. In vitro studies were performed using glomerular endothelial cells (GENC), platelets and neutrophils exposed to high glucose (HG) in static and flow conditions.

Result: Experimental DKD in C57Bl6 mice resulted in albuminuria, increased fractional mesangial area, activated platelets (CD62P) and neutrophil extracellular traps (NETs; H3Cit, NE, PAD4) within glomeruli. In parallel, increased expression of inflammasome markers (NLRP3, IL1β) and reduced expression of coagulation regulator thrombomodulin (TM) was observed. In vitro, platelets and NETs exacerbate inflammasome markers (IL1β, NLRP3), reduce endothelial function markers (p-eNOS, KLF2, KLF4 and TM) in GENC and disrupted the glomerular filtration barrier (enhanced FITC-dextran leakage, disoriented VE cadherin) in HG conditions. Under flow condition, platelets enhanced NET formation on GENC monolayers exposed to HG. Inhibition of platelet activation (ASA), amelioration of NETs by inhibition of histone citrullination PAD4 inhibition (GSK484), IL-1 receptor inhibition (anakinra) or restoring TM expression (solulin) ameliorated these effects in vitro and in vivo. Further experiments targeting P-selectin mediated platelet-neutrophil interactions and evaluating the clinical relevance in patient cohorts are under-progress.

Conclusion: Taken together, hyperglycemia promotes platelet-neutrophil interactions resulting in intraglomerular NET formation, sterile inflammation, glomerular endothelial dysfunction, and barrier disruption. This results in aggravated disease course and impaired renal health in DKD. Inhibition of platelets or NETs is a promising therapeutic strategy for DKD.

P-03-03

Bariticinib reduces STAT3 activation and partly inflammatory processes in dermal fibroblasts from patients with Pseudoxanthoma elasticum

Christopher Lindenkamp; Ricarda Plümers; Michel Robin Osterhage; Isabel Faust-Hinse; Cornelius Knabbe; Doris Hendig

Herz- und Diabeteszentrum NRW, Institut für Laboratoriums- und Transfusionsmedizin , Bad Oeynhausen, Germany

Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder which is manly caused by diverse mutations in the gene encoding the ATP-binding cassette sub-family C member 6 (ABCC6). Clinical, PXE shows some characteristics of the elderly, like arteriosclerosis, loss of skin elasticity and visual impairment. As described in further studies, there are similarities between PXE and premature aging which could be seen by high activity of senescence associated ß-galacto-sidase (SA ßGal) or expression of proinflammatory factors like interleukin-6 (IL6) or monocyte chemoattractant protein-1 (MCP1), in ABCC6-deficient fibroblasts. On molecular level PXE shares some features of premature aging diseases such as Hutchinson-Gilford progeria syndrome (HGPS). Both diseases are characterized by decreased levels of adenosine-triphosphate (ATP) and pyrophosphate (PPi). Further studies revealed a permanent activation of JAK-STAT3 signaling pathway in dermal fibroblasts from HGPS patients. It was shown that treatment with JAK-inhibitor bariticinib reduces inflammation in HGPS fibroblasts. Thus, the aim of this study was to evaluate the activation level of JAK-STAT3 and the effect of JAK-inhibitor bariticinib in dermal fibroblasts of PXE patients.

Normal human dermal fibroblasts (NHDF) (n=3) and fibroblasts from PXE patients (n=3) were seeded with a final density of 177 cells/mm2. Medium was changed after 24 h to medium with lipoprotein-deficient serum (LPDS) and bariticinib. The level of activated pSTAT3 was measured after additional 72 h incubation, by immunofluorescence and western blot. Also, quantitative real-time polymerase chain reaction for analyzing mRNA expression of IL6 and MCP1, as well as IL6 protein concentration from cell culture supernatants was analyzed.

Immunofluorescence and western blot analysis showed increased pSTAT3 levels in untreated PXE fibroblasts, compared to the NHDF. Treatment with bariticinib reduced activated pSTAT3 in PXE fibroblasts to the level of NHDF. Expression of IL6, MCP-1, as well as IL6 concentration in supernatants were increased in untreated and treated PXE fibroblasts compared to NHDF. There were no significant changes in IL6 expression and IL6 concentration in treated PXE fibroblasts in contrast to untreated PXE fibroblasts. The expression of MCP-1 was significantly reduced in bariticinib treated PXE fibroblasts and NHDF in comparison to the respective untreated fibroblasts.

Our data indicate that JAK-STAT3 signaling pathway is activated in PXE fibroblasts. Treatment with JAK-inhibitor bariticinib, only reduces MCP-1 gene expression but had no effect on IL6 gene expression and IL6 protein concentration in supernatant of PXE fibroblasts. In conclusion, JAK-STAT3 signaling pathway seems to induce MCP-1 gene expression but does not seem the dominant driving force for enhanced IL6 secretion in PXE. Further studies are needed to solve the question by which mechanisms IL6 is activated in PXE.

P-03-04

Anti-DFS70: prevalence and diagnostic significance in antinuclear antibody (ANA)-positive patients

Ivana Markovic; Andreas Fischer; Gry Helene Dihazi

Universitätsmedizin Göttingen, Interdisziplinäres UMG-Labor, Göttingen, Germany

Aims: Laboratory screening of anti-nuclear antibodies (ANAs) by indirect immunofluorescence method (IIF) is often positive in patients without proven autoimmune pathologies. The most frequent pattern, which is detected is a « dense fine speckled » (DFS) pattern (AC-2), characterized by the fine-granular fluorescence of the nuclei in the chromatin interphase and metaphase, and could occur in 2-22% of healthy individuals, infection, cancer and inflammatory conditions. However, there is still need for information about its clinical significance. This study aimed to investigate the performance of available routine screening methods for detection of ANAs and anti-DFS70 antibodies, and the clinical significance of anti-DFS70 autoantibodies using remaining patient samples, which were sent to laboratory for ANA detection.

Material and Methods: 31 serum samples routinely requested for ANAs screening were analyzed using IIF on HEp-2 cell substrates (Euroimmun, Germany). The semi-quantitative determination of the anti-histone autoantibodies in the patient's serum was carried out using an enzyme immunoassay (Euroimmun, Germany). For detecting autoantibodies against dsDNA, U1RNP, Sm, Ro/SSA, La/SSB, Scl-70, Pm-scl, Jo-1 and CENP a quantitative fluorescence enzyme immunoassay for extractable nuclear antigen screen was performed (Thermo Fisher Scientific, Germany). Immunoblot (Euroimmun, Germany) enabled the detection of 14 autoantibodies against EJ, Jo1, Ku, MDA5, Mi-2α, Mi-2β, NXP2, OJ, PL-7, PL-12, PMScl100, PMScl75, Ro-52, SAE1, SRP and TIF1γ. The quantitative in-vitro measurement of antibodies of the IgG class against DFS70 in serum was performed using a quantitative fluorescence enzyme immunoassay (Thermo Fisher Scientific, Germany). Demographic and clinical data were analyzed from the medical records.

Results: Among the 31 samples, which were tested for ANA, 20 (64.5%) were ANA positive by IIF. The frequency of AC-2 immunofluorescence pattern by ANA-IIF was 16.2% (5/31), of these only four samples contained antibodies against DFS70 in serum. No significant differences were observed between anti-DFS70 positive and anti-DFS70 negative patients concerning age, gender, symptoms, clinical signs or other disease-specific antibodies. 75% of the patients with positive DFS70 antibody was without proven autoimmune pathologies. However, of the four anti-DFS70 positive patients, only one patient had accompanying autoantibodies (anti-histone and anti-dsDNA).

Conclusion: Autoantibodies against DFS70 are less prevalent in patients with proven autoimmune pathologies. Monospecific anti-DFS70 antibodies are significant in excluding ANA-associated rheumatic disease in patients presented with an AC-2 pattern. It has been observed that anti-DFS70 autoantibodies may be associated with non-ANA-associated rheumatic diseases and in many diseases related to other systems. Therefore, it is essential to evaluate these pathologies in patients positive for anti-DFS70 antibodies.

P-03-05

Investigation of the presence of immature platelets in COVID-19

Monika Wolny; Tobias Flieder; Katharina von Bargen; Anne-Kathrin Vollmer; Cornelius Knabbe; Ingvild Birschmann

Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2), which first emerged in late 2019, has infected about $520 \cdot 10^6$ people worldwide to date and led to more than $6.2 \cdot 10^6$ deaths. Infection can lead to a spectrum of COVID-19 (coronavirus disease 2019), ranging from mild cold-like symptoms to severe courses with acute respiratory distress syndrome and organ failure. The latter is often associated with pathological coagulation parameters and thromboembolic complications. In particular, immature platelets appear, which are hyperreactive and show prothrombotic activity. Immature or reticulated platelets are the platelets newly formed by megakaryocytes and released from the bone marrow into the bloodstream. IPF (immature platelet fraction) refers to the proportion of immature platelets among the total number of platelets. The fraction is between 0 and 6% in healthy individuals. The aim of this study was to determine the course of IPF during severe COVID-19.

From December 2020 to July 2021, hospitalized patients with a predominantly severe COVID-19 course were included in this study. Daily determination of IPF was performed using the Sysmex XN-1000 hematology analyzer. In case of platelet transfusion, the readings of the following three days were not included in the analysis. Because of the fluctuating number of readings, the first 40 days after intubation or hospitalization were analyzed.

A total of 83 patients were enrolled in this study. 47 patients with a severe COVID-19 course (intubation and ECMO), 21 patients with a moderate course (intubation, no ECMO), and 15 patients with a mild COVID-19 course (no intubation, no ECMO) were included. The patients with a mild course showed no thrombocytopenia and a short-term increase in median IPF above the upper norm 14 days after hospitalization (max. 8.7%). The moderately ill patients also showed no median thrombocytopenia but increased IPF (max. 9.0%) with few exceptions in the first 20 days after intubation. The severely ill

patients had the lowest median platelet count (90 - 171 • 10^6/mL) and the highest median percentage of IPF (max. 14.1%, day 39) from the second day after intubation to day 40. Comparison of IPF between surviving and deceased patients in the moderate and severe groups revealed higher than average IPF in the first 40 days after intubation (days 0 and 1 not included) for both groups. However, there was a clear difference between deceased (median 10.2-18.4%) and surviving (5.4-11.6%) patients.

In this study, it was observed that in a severe course of COVID-19, the number of platelets decreases and the number of newly formed immature platelets increases. This increase in IPF appears to be more pronounced in patients who die than in surviving patients. However, it should be explicitly noted that this is a purely descriptive observation, as the measured values vary widely. Further studies are needed to predict disease progression based on IPF.

P-03-06

1 year follow up study after mild COVID-19 still shows decent t-cell response in patients with weak antibody formation

Tobias Flieder; Monika Wolny; Katharina von Bargen; Bastian Fischer; Cornelius Knabbe; Ingvild Birschmann

Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklink der Ruhr-Universität Bochum, Bad Oeynhausen, Germany, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

Introduction: More than 2 years ago, there were the first reports from China of infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19). The two systems the body has available to protect itself against pathogens are the humoral and cellular immune responses. The humoral immune response provides for the formation of antibodies against the corresponding pathogen, whereas in the cellular immune response, immune cells such as T cells directly attack the virus or secrete interferons. It is already known that the humoral immune response after vaccination or infection with SARS-CoV-2 is maintained for only a few months. Over which period the cellular immune response remains active and whether there is a correlation between humoral and cellular immune response after SARS-CoV-2 vaccination/infection is less investigated.

Methods: In our study, we included 50 individuals from a collective (426 individuals) that we screened for antibodies to SARS-CoV-2 between March 8 and April 8, 2020. These 50 individuals had mild infection with SARS-CoV-2, confirmed by viral detection by RT-PCR, but did not develop antibodies to SARS-CoV-2 or did so for a short period of time. Approximately one year after infection (291 - 380 days), we retested these individuals for SARS-CoV-2 antibodies using six different assays. We also tested the individuals for interferon-gamma release by T cells upon exposure to SARS-CoV-2 peptides.

Results: The mean age of the participants was 49 years (interquartile range (IQR): 16.35) and 52% of participants were female. The mean duration of COVID-19 was 12.5 days (standard deviation (sd): 7.6). No participant requiring hospitalization. The time between symptom onset and readmission for the 1-year follow-up was 321.5 days (IQR: 49.3). Measurement of IFN-γ release by T-cells induced with SARS-CoV-2 peptides showed increased IFN-γ release in 76% of patients. This cut-off value for this assay was determined from measurements of individuals who had no history of infection with SARS-CoV-2.
In the determination of antibodies, we were able to show that there were very large differences between the assays used. The proportion of positive results varied between 8% and 66%.

Conclusion: We were able to show in our study that the majority of individuals still have a cellular immune response approximately 1 year after SARS-CoV-2 infection, although they showed only a mild course and no/weak humoral immune response. This is encouraging finding in terms of long-term immunity to SARS-CoV-2.

P-03-07

Comparison of two procalcitonin reagents in SARS COV 2 patients

Borros Arneth[1]; Samr Mkhlof[2]; Janina Trauth[3]; Susanne Herold[3]; Thomas Voitz[4]; Chrysanthi Skevaki[2]; Harald Renz[2]

[1]Justus Liebig University Giessen, Institute for Laboratory Medicine, Giessen, Germany; [2]Justus Liebig University Giessen, Institute of Laboratory Medicine, Giessen, Germany; [3]Justus Liebig University Giessen, Internal Medicine Infectious Diseases, Giessen, Germany; [4]Synlab Trier, Labor, Trier, Germany

Borros Arneth[1], Samr Mkhlof[1], Janina Trauth[2], Susanne Herold[2], Thomas Voitz[3], Chrysanthi Skevaki[1] and Harald Renz

[1]Institute of Laboratory Medicine and Pathobiochemistry, Justus Liebig University Giessen; [2]Clinic of Internal Medicine, Division for Infectious Diseases, University Hospital Giessen; [3]Synlab MVZ Trier

Introduction: Plasma procalcitonin is extensively used in clinical laboratories for the early detection of bacterial infections and sepsis. Differences in sensitivity and specificity between commercially available reagents and automated platforms have been previously described. The aim of the current study was to determine and compare the diagnostic performance of reagents of Diazyme laboratories on the ADVIA (Siemens) and the Thermofisher Scientific / Brahms reagent on the Centaur (Siemens) among COVID-19 patients with a clinical suspicion for bacterial superinfection.

Method: PCT reagents from Diazyme Laboratories for the ADVIA (Siemens) and from Thermofisher Scientific /Brahms for the Centaur (Siemens) have been compared using SARS COV2 positive patient samples. For the comparison to a gold standard the collected samples were additionally measured with Thermofisher/ Brahms reagent on a Kryptor (Brahms) in Trier.

Results: We were able to determine significant differences between the two reagents in about every second patient sample. Thereby the level of the Brahms/Thermofisher measurement always was significantly lower compared to the PCT level measured using the Diazyme reagent. The level of the Diazyme values often did not match with the clinical picture of the patient.

Conclusion: There are significant differences between the two reagents from Brahms/Thermofisher and Diazyme, not only with regard to the sensitivity of the reagents, but also with regard to the specificity of the two reagents.

P-03-08

Myeloid Activation Marker by Flowcytometry

Borros Arneth[1]; Samr Mkhlof[2]; Silva Herzog[2]; David Krieger[3]; Chrysanthi Skevaki[2]; Harald Renz[2]

[1]Justus Liebig University Giessen, Institute for Laboratory Medicine, Giessen, Germany; [2]Justus Liebig University Giessen, Institute of Laboratory Medicine, Giessen, Germany; [3]Justus Liebig University Giessen, Emergency Medicine, Giessen, Germany

Samr Mkhlof[1], Borros Arneth[1], Silva Herzog[1,2], David Krieger[2], Chrysanthi Skevaki[1] and Harald Renz

[1]Institute of Laboratory Medicine and Pathobiochemistry, Justus Liebig University Giessen; [2]Department of Emergency Medicine, University Hospital Giessen

Introduction: The expression of specific markers (CD64 on neutrophils and CD169 on monocytes) occurs on leukocytes due to viral and bacterial infections. We here want to use these novel markers to identify the cause (viral, bacterial) of an infectious disease in early stages and additionally we want to compare them to the established markers.

Methods: In addition to the usual laboratory routine, we took an EDTA whole blood sample from about 100 patients at admission to the emergency room with the new onset of fever. Exclusion criteria were patients with already known infections, myocardial infarction, lung emboli, and stroke. In this EDTA whole blood we examined the markers (CD64, CD169 and HLA-DR) on leukocytes by means of flow cytometry additionally to conventional lab routine (Complete blood count, CRP, PCT, microbiology).

Results: Bacterial infections can reliably be detected by using the overexpression of the marker CD64 on neutrophils. Most patients we detected with viral expression type (CD169 positive on monocytes) had SARS COV2 disease. The expression of markers on leukocytes is compared to the classical diagnostic tools as leukocytes, differential blood count, CRP, PCT and microbiology.

Conclusion: Flow cytometry is excellent for distinguishing bacterial from viral infectious diseases. In addition, if both marker profiles are present at the same time, it is also possible to identify patients with a viral disease and a bacterial superinfection.

P-03-09

Continuous monitoring of SARS-CoV-2 seroprevalence in children using residual blood samples from routine clinical chemistry

Felix Wachter[1]; Adrian Regensburger[2]; Antonia Sophia Peter[3]; Ferdinand Knieling[4]; Alexandra Wagner[5]; David Simon[6]; André Hörning[4]; Joachim Wölfle[4]; Klaus Überla[3]; Antje Neubert[4]; Manfred Rauh[4]

[1]Friedrich-Alexander-Universtität Erlangen-Nürnberg, Department of Pediatrics and Adolescent Medicine, Erlangen, Germany; [2]FAU Erlangen-Nürnberg, Pädiatrie Erlangen, Erlangen, Germany; [3]Friedrich-Alexander University (FAU) Erlangen- Nürnberg, Institute of Clinical and Molecular Virology, Erlangen, Germany; [4]Friedrich-Alexander-University (FAU) Erlangen-Nürnberg, Department of Pediatrics and Adolescent Medicine, Erlangen, Germany; [5]Friedrich-Alexander-University (FAU) Erlangen-Nürnberg, Department of Pediatrics and Adolescent Medicine, 91054, Germany; [6]Friedrich-Alexander University (FAU) Erlangen-Nürnberg, Department of Internal Medicine 3, Erlangen, Germany

Aims: The assessment of SARS-CoV-2 infections in children is still challenging, but essential for appropriate political decisions. The aim of this study was to investigate whether residual blood samples can be used for SARS-CoV-2 seroprevalence monitoring in pediatrics and to calculate the seroprevalence and underreporting in the pediatric population. Infection rates of SARS-CoV-2 in children remain a widely discussed topic. In this study we aim to establish a study design to determine seroprevalence of SARS-CoV-2 antibodies in the pediatric population in a resource saving and cost effective manner.

Materials and Methods: Residual blood samples from pediatric patients were collected in six time periods (Oct.–Nov. 2020, April 2021, and June–July 2021, November 2021, January 2022 and March 2022). They were analyzed for SARS-CoV-2 Spike protein (anti-S) and nucleocapsid (anti-N) antibodies using a commercial antibody assay by Roche diagnostics. 28 samples were further analyzed for neutralizing capability. The calculated seroprevalence was then compared to the number of officially reported cases to calculate the extent of underreporting.
While analysis of the data for the collection periods 4, 5 and 6 is still ongoing, results for the first three sample periods are already available and published by Wachter et al: Seroprevalence obtained from the antibody results of 2,626 patient samples increased from 1.38% to 9.16% and to 14.59% during the three time periods. Nucleocapsid seroprevalence was lower in all three sample periods (1.26%, 6.19%, 8.56%). Seroprevalence therefore was 3.93-5.66-fold higher than the number of cases reported by the health authorities. However, a good correlation between the reported cumulative incidence of individual provinces and the assigned seroprevalence was found (r=0.74, p=0.0151). Of the 14 samples reactive for anti-S and anti-N antibodies 11 showed neutralization capabilities, while of the 14 samples only reactive for anti-S antibodies 8 showed neutralization capabilities. Antibody levels did not differ between age groups or sexes (all p>0.05).

Discussion/Conclusions: The data suggests a low concordance of anti-S and anti-N antibodies in children. This could be due to a quick waning of anti-N antibodies. Therefore, antibody assays results of patients should be interpreted accordingly and seroprevalence studies, the ones relying on anti-N antibodies in particular, can only display the infection rates of the recent past. Our findings indicate a rising seroprevalence in the pediatric population in Germany. Our results are in line with other seroprevalence studies and therefore show that using residual blood samples of patients is a resource-saving but accurate way of sampling and obtaining patient material for seroprevalence estimation. Our study design can be easily implemented into daily laboratory routines and is a useful tool for seroprevalence surveillance.

P-03-11 / PV-05

Early detection of life-threatening infections in the emergency department using metabolomics

Sophie Neugebauer[1]; Johannes Winning[2]; Johannes Winning[3]; Thomas Lehmann[4]; Michael Bauer[3]; Michael Kiehntopf[l]

[1]Jena University Hospital, Department of Clinical Chemistry and Laboratory Diagnostics, Jena, Germany; [2]University of Applied Sciences, Ernst-Abbe-Hochschule, Jena, Germany; [3]Jena University Hospital, Department of Anesthesiology and Intensive Care, Jena, Germany; [4]Jena University Hospital, Institute of Medical Statistics, Computer Sciences and Data Sciences, Jena, Germany

Aims: Despite intensive research, sepsis remains a life-threatening organ dysfunction with high mortality (25-50%) [1, 2]. Early sepsis diagnosis is complicated by primarily nonspecific symptoms. Up to 40% of patients with severe sepsis are admitted to the emergency department [3]. Early recognition in the emergency department and initiation of therapeutic interventions is necessary to increase and improve patient survival [2,4,5]. The appearance, accumulation, or persistence of metabolites during the infection-related host response could serve as a surrogate for impaired metabolic control and for monitoring disease severity. Thus, the study aims to identify the most appropriate metabolites or combinations of metabolites for early sepsis diagnosis, identification of sepsis-related organ dysfunction and risk stratification of patients with suspected sepsis.

Materials and Methods: A total of 188 metabolites comprising six analyte classes were measured using LC-MS/MS in lithium heparin plasma samples from 400 patients on admission to the emergency department of the university hospital Jena. 160 and 24 patients developed sepsis and septic shock within 96 hours, respectively. Procalcitonin was used as reference laboratory parameter.

Results: The primary study endpoint was to define metabolites that can early identify patients with sepsis or septic shock. 44 and 30 metabolites were altered after correction for multiple testing in patients developing sepsis or septic shock, respectively. Mainly three metabolite classes: amino acids, lysophosphatidylcholines and phosphatidylcholines were associated with sepsis or septic shock and mostly decreased in their concentration during infection. Promising metabolites were selected and combined with a LASSO-regression with a 20-fold cross-validation. ROC-analysis of these models showed a sensitivity of 80.6% or 91.7% and a specificity of 79.6% or 90.2% for early detection of sepsis or septic shock, respectively.
Furthermore, amino acids, biogenic amines and lysophosphatidylcholines showed high potential for providing information of organ dysfunction or poor patients' outcome. Significant altered metabolites were combined for prognosis of unfavorable outcome of patients or the need of interventions at the intermediate care unit.

Conclusion: The present targeted metabolomics approach allowed to achieve the two study endpoints: a) to indicate metabolite patterns that are of early diagnostic value for sepsis or septic shock; b) to identify some metabolites that can early provide information about risk stratification of patients. These findings will lead to the development of improved novel diagnostic tools for early diagnosis and prognosis of sepsis and septic shock.

P-04-01

A novel automated DiaSys procalcitonin immunoassay compared with four different BRAHMS-partnered immunoassays

Abass Eidizadeh; Mechthild Wiederhold; Moritz Schnelle; Andreas Fischer; Lutz Binder

Universitätsmedizin Göttingen, Institut für Klinische Chemie, Göttingen, Germany

Introduction: Procalcitonin (PCT) is an important biomarker of sepsis and respiratory infections. Various automated immunoassays for measuring PCT in patient plasma are available in medical laboratories. However, due to a lack of international reference material for PCT, the assays are not always comparable.

Methods: In this study, we compared a new turbidimetric immunoassay from DiaSys, measured on the Abbott Architect c16000 and Alinity c, with four BRAHMS-associated chemiluminescence immunoassays (Abbott Architect i2000SR, Alinity i, Roche Cobas e411 and DiaSorin Liaison XL) using 120 random patient plasma samples from the clinical laboratory routine at the University Medical Center Goettingen.

Results: The DiaSys assay showed clear differences as compared to the BRAHMS-associated assays when measured on Architect c: i.e. 58% positive mean bias vs. Architect i, 67% vs. Cobas and 23% vs. Liaison. As a result, additional 19% our patients would have a suspected bacterial infection, when using PCT values from the DiaSys assay and commonly accepted decision limits. A crosscheck of the DiaSys calibrator on the BRAHMS-associated systems showed a low recovery of the calibrator material (approx. 50%).

Conclusions: Overall, this study shows significant differences between the DiaSys and BRAHMS-associated assays. This could be attributed to a potential DiaSys calibrator problem. This highlights the need for an international reference material for harmonization of the PCT assays.

P-04-02

Control of neutrophil effector function by the actin-regulatory protein Coronin-1a

Anton Shaverskyi; Lee Kyeong-Hee; Niko Föger

Medizinische Hochschule Hannover, Institut für Klinische Chemie, Hannover, Germany

Background: Neutrophils are innate immune cells that as key players of acute inflammation play fundamental roles in antimicrobial responses but can also contribute to inflammation-related tissue damage. To encounter pathogenic challenge, neutrophils have developed powerful defense mechanisms, such as the release of bioactive mediators from secretory granules and the generation of reactive oxygen species (ROS) to kill phagocytosed microbes. Neutrophil activation and execution of effector function involves dynamic reorganization of the actin cytoskeleton. The regulatory pathways and functional links between neutrophil activation/function and actin cytoskeletal regulation are, however, still only poorly understood.

Objective: We here explored the role of the evolutionary highly conserved actin-regulatory protein Coronin-1a (Coro1a) for neutrophil effector function.

Methods: Employing neutrophils from Coro1a-deficient and wild type mice we analyzed different neutrophil-mediated defense reactions that were induced by various stimuli. Additional experiments were aimed at uncovering the mechanism underlying Coronin-1a function in neutrophils.

Results: Gene and protein expression analysis confirmed high expression of Coronin-1a in neutrophils. Analysis of developing neutrophil subsets in the bone marrow indicated normal neutrophil development in Coro1a-deficient mice and allowed us to examine the effects of Coro1a-deficiency on effector function of mature neutrophils. Consistent with a negative regulatory function of Coronin-1a on actin polymerization, Coro1a-deficient neutrophils had increased F-actin levels, indicating altered actin cytoskeletal organization. Importantly, evaluation of ROS generation (also called oxidative burst reaction), a key antimicrobial defense mechanisms of neutrophils, revealed impaired oxidative burst formation in Coro1a-deficient neutrophils in response to a wide range of different stimuli. Similarly, the release of prestored mediators from neutrophil granules, termed neutrophil degranulation, was also significantly reduced in Coro1a-deficient neutrophils, despite normal levels of intragranular compounds in resting cells. Initial mechanistic studies indicate an involvement of Coronin-1a in cellular signaling pathways during neutrophil activation.

Conclusion: In summary, our data revealed alterations in actin cytoskeletal regulation in Coro1a-deficient neutrophils that were associated with impaired oxidative burst formation and decreased neutrophil degranulation (mediator release), thus indicating a critical involvement of Coronin-1a in neutrophil-mediated defense mechanisms. Ongoing experiments are aimed at further identifying the underlying mechanism and its physiological relevance.

P-04-03

2 years external quality assessment for the detection of anti-SARS-CoV-2 antibodies - a critical retrospective

Maximilian Kittel[1]; Anna Bode[2]; Romy Eichner[1]; Sihem Aida[1]; Volker Ast[3]; Michael Neumaier[3]; Verena Haselmann[1]

[1]Universitätsmedizin Mannheim, Institut für klinische Chemie, Mannheim, Germany; [2]Referenzinstitut für Bioanalytik, RfB, Bonn, Germany; [3]Medical Faculty Mannheim of Heidelberg University, Institut für klinische Chemie, Mannheim, Germany

In the last year, a large number of assays for the serological detection of antibodies to the new SARS-CoV-2 virus have been brought to market and are being widely used in laboratories. These new developments have highlighted the importance of controlling the analytical methods currently in use to ensure patient safety. External quality assessment (EQA) is an important tool for both standardization of test results and their harmonization, and thus for ensuring high-quality diagnostic procedures. As these results are essential to estimate the prevalence of SARS-CoV-2 infections, the effect of immunizations, and post-infection immunity, this level of quality is mandatory. The Reference Institute for Bioanalytics (RfB) was the first provider to offer a proficiency test, for the detection of anti-SARS CoV-2 antibodies.

In the CoVimm EQA-schemes blinded panels of pre-characterized human serum samples with variable anti-SARS-CoV-2 antibody titers for detection of different anti-SARS-CoV-2-antibodies (IgG, IgA, IgM, total, nucleocapsid, and spike-protein-specific).

In this study, the 4 rounds of the CoVimm EQA were evaluated and compared in an aggregated format with the goal to gain insight into the quality and development of diagnostics for the detection of anti-SARS-CoV-2 antibodies.

In the four distribution rounds from 2020 to 2021, a total of 296 laboratories from 25 countries reported a total of 5,020 results for anti-SARS-CoV-2 antibody detection using more than 26 different assays. In terms of diagnostic sensitivity and specificity, significant differences were found between the various assays used and also between certified and lab-developed tests. Moreover, it could be observed that with the progress of the pandemic and the availability of vaccines, different requirements were imposed on the methods of antibody detection. The evaluation of the EQA-results also revealed, that there are still considerable deficits in the application of the test procedures on the part of the users. In particular, the use of obviously unsuitable assays concerning their intended use illustrates these application errors.

In summary, the EQA highlighted various aspects of the diagnostic situation. First, it should be emphasized that the testing landscape remains heterogeneous, but is increasingly concentrated among large providers.

Regrettably, there have been no noticeable improvements in the standardization of methods during the last year.

Assuming that the lessons learned from the current pandemic prove true, the number of cases, and thus the workload of laboratories will decrease significantly during the summer season. This break should be used urgently to refocus on and improve the quality of diagnostics offered. Current deficiencies should be addressed and laboratories should be aware of their responsibility for reported results.

P-04-04

The First Infection Wave: Clinical And Laboratory Characteristics Of COVID-19 Patients At The University Hospital Schleswig-Holstein In Kiel

Viktoria Backes; Frank Leypoldt; Jeanette Franzenburg; Justina Dargvainiene; Daniela Esser; Klaus-Peter Wandinger; Robert Markewitz; Ralf Junker

Universitätsklinikum Schleswig-Holstein, Institut für Klinische Chemie, Campus Kiel, Kiel, Germany

Aims: After the outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan in December 2019, the novel virus spread quickly throughout the world, causing a pandemic. Although most people develop mild symptoms, especially old and multimorbid patients have an increased risk for an adverse outcome. The aim of this study was to analyse the cohort of coronavirus disease 2019 (COVID-19) patients at the University Hospital Schleswig-Holstein Campus Kiel during the first infection wave and derive a laboratory-value-based prediction model for severe COVID-19 in hospitalized patients.

Methods: This retrospective cohort study was conducted on 43 consecutive hospitalized patients with positive SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction, admitted to the University Hospital Schleswig-Holstein in Kiel from March to August 2020. Non-hospitalized patients were excluded. All patients were categorized according to the Ordinal Scale for Clinical Improvement (WHO) into two groups: mild to moderate disease versus severe disease. Clinical and laboratory parameters were acquired from patient files and compared between the groups.

Results: 31 patients were categorized as mild to moderate disease and 12 patients as severe disease. Age over 60 years (p=0.0479), chronical heart failure (p=0.0321) and oxygen supplementation at the day of admission (p < 0.0001) were associated with severe disease. Main complications of COVID-19 were acute renal failure, cardiac arrhythmia, and septic shock. 18.6 % of patients died during the evaluation period. Most common cause of death was septic shock. C-reactive protein (day 0+1 of hospitalisation: p=0.0007, day 4+5 of hospitalisation: p=0.0035), interleukine-6 (day 0+1: p=0.002, day 4+5: p < 0.0001), neutrophil-to-lymphocyte ratio (day 0+1: p=0.0002, day 4+5: p=0.0105) and procalcitonin concentrations (day 0+1: p=0.0002, day 4+5: p < 0.0001) were significantly higher in patients with severe disease. Furthermore, a low prothrombin time at the day of admission was associated with severe disease (p=0.0002). Sodium (day 0+1: p=0.1435, day 4+5: p=0.0006) and creatine kinase (day 0+1: p=0.0547, day 4+5: p < 0.0001) were significantly higher in patients with severe COVID-19 than in mild-to-moderate disease during the course of hospitalization but not at admission.

Conclusion: We could identify several inflammatory and acute-phase parameters which were significantly associated with a severe course of COVID-19 in hospitalized patients. Our results support the hypothesis that worse outcomes are mainly associated with hyperinflammation leading to multi-organ failure, including kidney damage and altered coagulation. A prediction model of risk factors for severe course of COVID-19 in hospitalized patients did not yield sufficient power due to the low incidence of COVID-19 in Schleswig-Holstein during the first wave resulting in small patient numbers.

P-04-05

Potential of integrative diagnostics predicting ICU demand in COVID-19 patients

Catharina Gerhards[1]; Verena Haselmann[1]; Samuel Schaible[2]; Volker Ast[1]; Maximilian Kittel[1]; Matthias Ebert[3]; Andreas Teufel[3]; Manfred Thiel[4]; Alexander Hertel[2]; Stefan Schönberg[2]; Matthias Frölich[2]; Michael Neumaier[1]

[1]Medical Faculty Mannheim of the University of Heidelberg, Institute for Clinical Chemistry, Mannheim, Germany; [2]Medical Faculty Mannheim of the University of Heidelberg, Department of Radiology and Nuclear Medicine, Mannheim, Germany; [3]Medical Faculty Mannheim of the University of Heidelberg, Department of Medicine II, Mannheim, Germany; [4]Medical Faculty Mannheim of the University of Heidelberg, Department of Anaesthesiology and Surgical Intensive Care Medicine, Mannheim, Germany

Introduction: The establishment of integrative diagnostic models combining quantitative imaging and laboratory findings may support the identification of vulnerable COVID-19 patients and aid assessments regarding required intensive care unit (ICU) treatment. We have investigated laboratory biomarkers including cell-free deoxyribonucleic acid (cfDNA) and radiomics for their synergistic integrated diagnostics potential.

Methods: Hospitalized SARS-CoV-2 infected patientes (n=52) were enrolled between May 2020 and September 2021. Retrospective image segmentation analyses of chest computed tomography (CT) and analysis of routine laboratory biomarkers together with prospectively obtained quantitative cfDNA concentrations were performed using separate feature selection and application of a minimal redundancy algorithm for both diagnostic modalities. The algorithm was established using cross-wise validation and subsequent verification in subset of algorithm-naïve patients. The clinical decision endpoint "ICU stay likely/unlikely" was optimized based on the prediction by the algorithm.

Results: The integrated model comprises six radiomics and seven laboratory biomarkers. Root mean square of the deviations between actual and predicted ICU-days was 5.3 days in cross-validation set and 12.3 days in test-cohort. Radiomic model accuracy was 0.54, cfDNA model accuracy was 0.47, routine laboratory model accuracy was 0.74 and combined model accuracy was 0.87 with an AUC of 0.91. The combined model performed superior to the individual radiological and laboratory models to predicting ICU requirement (adjusted R2 = 0.896).

Conclusion: The integration of radiomics and laboratory data shows synergistic potential to improve clinical decision making of COVID-19 patients. Based on the results of our routine patient cohort, this model may contribute to stratification of ICU capacities.

P-04-06

CD248 induces maladaptive unfolded protein response in diabetic kidney disease

Ronald Biemann[1]; Shruthi Krishnan[2]; Jayakumar Manoharan[1]; Dheerendra Gupta[1]; Akash Mathew[1]; Shrey Kohli[1]; Khurrum Shahzad[1]; Michael Naumann[2]; Berend Isermann[1]

[1]Universitätsklinikum Leipzig AöR, Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Leipzig, Germany; [2]Otto-von-Guericke University Magdeburg, Institute of Experimental Internal Medicine, Magdeburg, Germany

Aims: Dysfunction of mesangial cells plays a major role in the pathogenesis of diabetic kidney disease (DKD), the leading cause of end-stage renal disease. The underlying molecular mechanisms, however, are incompletely understood.

Methods and Results: By unbiased gene expression analysis of glucose-exposed mesangial cells, we identified the transmembrane receptor CD248 as the most upregulated gene and maladaptive unfolded protein response (UPR) as one of the most upregulated pathways. Upregulation of CD248 was confirmed in glucose-stressed mesangial cells in vitro, in renal glomeruli isolated from diabetic mice (STZ and db/db models, representing type 1 and type 2 diabetes mellitus, respectively) in vivo, and in glomerular kidney sections from patients with DKD. Time course analysis revealed that glomerular CD248 induction precedes the onset of albuminuria, mesangial matrix expansion and maladaptive UPR activation (hallmarked by C/EBP homologous protein, CHOP, induction) but is paralleled by loss of the adaptive UPR regulator spliced X box binding protein (sXBP1). Mechanistically, CD248 induces the assembly of a multiprotein UPRosome comprising heat shock protein 90 (HSP90), BH3 interacting domain death agonist (BID) and inositol requiring enzyme 1 (IRE1α), in which BID impedes IRE1α-mediated XBP1 splicing and sXBP1-dependent gene expression. Overexpression of HSP90 or BID in vitro or genetic reduction of XBP1 in vivo abrogates the protective effects of CD248-deficiency.

Conclusion: In the current study, we identified CD248 as a regulator of the adaptive UPR mediator XBP1 that induces maladaptive UPR signaling in renal glomeruli under diabetic conditions and in mesangial cells exposed to high glucose conditions in vitro. This research is expected to provide new mechanistic insights and identify the transmembrane receptor CD248 as a potential biomarker and a new druggable target in DKD.

P-04-07

Evaluation of a laboratory-based high-throughput SARS-CoV-2 antigen assay

Sebastian Hörber[1]; Christoph Drees[1]; Tina Ganzenmueller[2]; Dirk Biskup[3]; Andreas Peter[1]

[1]Universitätsklinikum Tübingen, Institut für Klinische Chemie und Pathobiochemie, Tübingen, Germany; [2]Universitätsklinikum Tübingen, Institut für Medizinische Virologie, Tübingen, Germany; [3]CeGat GmbH, CeGat GmbH, Tübingen, Germany

Objectives: Antigen tests are an essential part of SARS-CoV-2 testing strategies. Rapid antigen tests are easy to use but less sensitive compared to nucleic acid amplification tests (NAT) and less suitable for large-scale testing. In contrast, laboratory-based antigen tests are suitable for high-throughput immunoanalyzers. Here we evaluated the diagnostic performance of the laboratory-based Siemens Healthineers SARS-CoV-2 Antigen (CoV2Ag) assay.

Methods: In a public test center, from 447 individuals anterior nasal swab specimens as well as nasopharyngeal swab specimens were collected. The nasal swab specimens were collected in sample inactivation medium and measured using the CoV2Ag assay. The nasopharyngeal swab specimens were measured by RT-PCR. Additionally, 9046 swab specimens obtained for screening purposes in a tertiary care hospital were analyzed and positive CoV2Ag results confirmed by NAT.

Results: In total, 234/447 (52.3%) participants of the public test center were positive for SARS-CoV-2-RNA. Viral lineage B1.1.529 was dominant during the study. Sensitivity and specificity of the CoV2Ag assay were 88.5% (95%CI: 83.7%-91.9%) and 99.5% (97.4%-99.9%), respectively. Sensitivity increased to 93.7% (97.4%-99.9%) and 98.7% (97.4%-99.9%) for swab specimens with cycle threshold values < 30 and < 25, respectively. Out of 9046 CoV2Ag screening tests from hospitalized patients, 21 (0.2%) swab specimens were determined as false-positive by confirmatory NAT.

Conclusions: Using sample tubes containing inactivation medium the laboratory-based high-throughput CoV2Ag assay is a very specific and highly sensitive assay for detection of SARS-CoV-2 antigen in nasal swab specimens including the B1.1.529 variant. In low prevalence settings confirmation of positive CoV2Ag results by SARS-CoV-2-RNA testing is recommended.

P-04-08

Alternative polyadenylation regulates the SARS-CoV-2 entry factor NRP1

Sophie Hartleb; Essak Khan; Jamie Nourse; Sven Danckwardt

Universitätsmedizin Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany

Background: Neuropilin-1 (NRP1) has recently been identified as neuronal SARS-CoV-2 (co)receptor controlling host cell entry and infectivity. Apart from membrane bound NRP1 a truncated isoform lacking the transmembrane domain exists, which acts as functional antagonist to full-length NRP1. Recently, diversification of the transcriptome at the 3'end by alternative polyadenylation (APA) has emerged as a pervasive and evolutionarily conserved layer of gene regulation. APA is, for example, involved in the IgM heavy chain class switch in activated B-cells resulting in the conversion from a membrane bound to a soluble IgM. Here we set out to explore if and how APA affects the expression of soluble and membrane bound NRP1.

Methods: The existence and location of several poly-A-sites in the human NRP1 gene was confirmed by 3' RACE PCR. To measure the influence of different APA factors on the expression of NRP1, we transfected BE2C cells with silencing RNAs to knockdown central components that regulate APA (PCF11, CPSF6 and NUDT21). The knockdown efficiency was controlled by western blot. The transfected cells were harvested to isolate RNA and proteins. RT-qPCR was performed to measure expression changes of NRP1 isoforms on mRNA level.

Results: We confirmed different poly-A-site usage in the human NRP1 gene, which leads to the expression of different NRP1 RNA isoforms. We also demonstrate that CPSF6, a key determinant regulating APA, controls poly-A-site usage, with knockdown of CPSF6 resulting in upregulation of the soluble NRP1 isoform.

Conclusion: We show that APA regulates the expression of NRP1, the neuronal SARS-CoV-2 entry (co)receptor. Downregulation of CPSF6 results in the expression of a truncated NRP1 mRNA isoform, encoding a soluble NRP1 protein that lacks the transmembrane domain. Truncated NRP1 thereby functionally competes with full-length membrane bound NRP1 and acts as a soluble decoy receptor. Based on these findings is tempting to speculate that APA evolved as a regulatory mechanism controlling SARS-CoV-2 cell entry and infectivity.

P-04-09

Performance evaluation of the EUROArray assay for molecular detection of dermatomycosis

Michal Krolik[1]; Susanna Bigler[2]; Thomas Bodmer[3]; Martin Risch[4]; Lorenz Risch[4]; Nadia Wohlwend[1]

[1]Dr. Risch, Mikrobiologie, Buchs, Switzerland; [2]Dr. Risch, Klinische Chemie , Liebefeld, Switzerland; [3]Dr. Risch, Mikrobiologie, Liebefeld, Switzerland; [4]Dr. Risch, Allgemeine Innere Medizin, Buchs, Switzerland

Introduction: Superficial and cutaneous dermatomycosis is one of the most common fungal infection and remains a global concern. Increased mobility facilitates spreading and manifestation of originally rare dermatophyte species. While conventional culture is considered the gold standard for diagnosis of dermatomycosis, it is laborious, lengthy and requires a high level of expertise. In contrast, novel molecular approaches promise rapid yet sensitive and specific detection of dermatophytes. Here, we evaluate the performance of a novel PCR-based microarray compared to culture as reference.

Materials & Methods: A total of 272 KOH positive clinical samples were prospectively analysed in parallel by culture and the EUROArray Dermatomycosis (EUROImmun, Kriens, Switzerland). For cultural analysis, sample material was plated and incubated on Sabouraud, Dermatophyte and Candida Agar (BioMérieux, Petit-Lancy Switzerland). Primary material was digested by Proteinase K followed by automated extraction via easyMAG (BioMérieux, Petit-Lancy Switzerland) and subsequently used for the identification of 23 dermatophyte, 3 yeast and 3 mould species by the EUROArray.

Results: Dermathophytes were detected in 233/272 (85%) of the KOH positive clinical samples by EUROArray with Trichophyton rubrum (n=194) and Trichophyton interdigitale (n=36) clearly dominating over Microsporum canis (n=2) and Nannizzia gypsea (n=1). Whereas culturally only 93/272 (34%) of the obligate pathogenic agents could be identified. Results from culture were available after a mean of 25 days, while results from the EUROArray were obtained on the same day that the samples were processed.

Conclusion: Our data illustrate the increased sensitivity and shortened time to result compared to cultural analysis. We demonstrate that clinically relevant and human pathogenic dermatophytes, which did not yield a corresponding result in the time-consuming cultural rearing, are identified by molecular biological detection. Overgrowth by mould and inhomogeneous distribution of dermatophytes in the primary material are major obstacles. These can be overcome by the EUROArray enabling targeted therapy within a short period of time.

P-04-10

Sensitivity and Specificity of the Roche SARS-Cov-2 Antigen Assay in a hospital setting

Boris Rolinski[1]; Julian Gebauer[1]; Anja Prantz[1]; Dusan Krnac[1]; Christoph Kutschker[2]

[1]Elblandkliniken, Elblab, Meißen, Germany; [2]Elblandkliniken, Krankenhaushygiene, Meißen, Germany

Aim: We evaluated the performance of the Roche SARS-CoV-2 Antigen Assay with respect to calibration stability and robustness as well as sensitivity and specificity.

Methods: We run the Roche SARS-CoV-2 Antigen Assay in three 400 bed hospitals on every patient at admission in conjunction with a SARS-CoV-2 rt-PCR (Seegene). Antigen tests were performed on two Cobas 6000 and one Elecsys e411. Data were evaluated from 10.3.2021 to 23.04.2022. A total of 35090 data sets were available for analysis.
Results: Antigen results ranged from 0,07 to 34149 U/ml and ct values ranged from 9 to 39,89. A total of 32642 samples were negative both by PCR and Antigen Assay, whereas 1421 samples where positive in both assay. 151 samples were false positive and 522 false negative resulting in a sensitivity of 0,7313 and a specificity of 0,9954.

Conclusion: The Roche SARS-CoV-2 Antigen assay performed very stable over more than one year and on three different instruments. The assay proved to be sufficient sensitive and highly specific for the detection of SARS-CoV-2 infection.

P-04-11 / PV-04

CRP in gout: Not just a biomarker?

Konstantin Neumann; Korbinian Brand; Anika Alberts; Anne Kathrin Wessig

Medizinische Hochschule Hannover, Institut für Klinische Chemie - Zentrallabor, Hannover, Germany

Introduction: Gout is a common form of arthritis caused by crystallization of uric acid. The uric acid crystals induce a strong inflammatory response that is hardly distinguishable from inflammation induced by bacterial infection and includes increases in serum levels of C-reactive protein (CRP). It is unclear, if the innate immune system recognizes the crystals or merely reacts to the damage they cause.

Aim: We aimed to identify proteins of the immune system that interact with gout crystals.

Methods: We purified proteins from different body fluids including synovial fluid and identified the proteins by liquid chromatography-coupled mass spectrometry. Recombinant proteins of the identified proteins were used to test their binding to uric acid crystals and their impact on inflammatory responses induced by the crystals.

Results: Besides strong binding of apolipoprotein B, we found specific binding of several proteins of the immune system capable of inducing inflammatory responses. Strongest binding was found for CRP. Binding was strong enough to deplete CRP from human serum by addition of uric acid crystals. Binding of CRP induced complement activation on the surface of uric acid crystals. In addition to CRP, we found immunoglobulin M (IgM) to bind to the crystals, in all donors tested. Antibodies usually arise in response to foreign antigens. However, the antibodies recognizing uric acid crystals were also found in cord blood serum, which means they are formed before birth, suggesting they are natural/innate antibodies. Both CRP and IgM were capable of activating the complement system, which alerts the immune system by releasing inflammatory peptides, C3a and C5a. In the absence of CRP and IgM, no complement activation was induced by uric acid crystals, indication that we have found the main complement sensors for uric acid crystals. However, while IgM was only able to induce release of C3a but not C5a, CRP was able to propagate the complement cascade to the very end and induce release of the more inflammatory C5a.

Conclusion: We have identified the main innate immune sensor proteins that link uric acid crystals to complement activation. The unique ability of CRP to drive production of the more inflammatory C5a suggests elevated CRP may enhance the ability of the immune system to detect the crystals and therefore favor the initiation of gout flares.

P-04-12

Antimicrobial Activity of CLEC3A: Potential in the Prevention and Treatment of Septic Arthritis

Dzemal Elezagic

Universitätsklinikum Köln, Institut für klinische Chemie

Introduction: The dramatic increase in antibiotic resistance has caused bacterial infections to once again become a serious global health threat. Moreover, the stagnating development of novel antibiotics urges for alternative antimicrobial agents. Particularly promising alternatives to conventional antibiotics are antimicrobial peptides (AMPs), which are part of the innate immune system. The cartilage-specific C-type lectin domain family 3 member A (CLEC3A) exhibits structural similarities to AMPs, which prompted us to investigate its antimicrobial activity.

Methods: We performed immunoblot to detect CLEC3A peptides in human cartilage extracts. To investigate their antimicrobial activity, we designed peptides and recombinantly expressed CLEC3A domains and used them to perform viable count assays using E.coli, P.aeruginosa and S.aureus. We investigated the mechanism of their antimicrobial activity by fluorescence and scanning electron microscopy. In addition, we coated CLEC3A peptides on titanium, a commonly used prosthetic material, and performed fluorescence microscopy to quantify bacterial adhesion. Moreover, we assessed the peptides' cytotoxicity against murine fibroblasts (NIH3T3) using MTT cell viability assays. To enhance the peptides´ performance, we altered the native peptides' sequences, generating 6 modified peptides.

Results: CLEC3A fragments were indeed detected in human cartilage extracts. Moreover, bacterial supernatants lead to fragmentation of recombinant and cartilage-derived CLEC3A. CLEC3A-derived peptides killed E.coli, P.aeruginosa and S.aureus. The modified peptides exhibited even more efficient bacterial killing (including that of a Methicillin-resistant S.aureus strain). The antimicrobial activity of the native peptides occurs by permeabilizing bacterial membranes. Coating CLEC3A-derived AMPs on titanium lead to significantly reduced bacterial adhesion to the material. Additionally, modifying the peptides considerably reduces cytotoxicity levels against NIH3T3 cells.

Conclusions: We first identify cartilage-specific AMPs originating from CLEC3A and resolve the mechanism of their antimicrobial activity. In a translational approach, through modifying peptides´ sequences, we pinpoint CLEC3A-derived AMPs with enhanced antimicrobial activity and reduced cytotoxicity. In addition, by coating prosthetic material with the peptides, we point to a novel approach in the prevention and treatment of septic arthritis. In vivo experiments involving mouse infection models are currently ongoing and are expected to shed light on the practical use of native and modified CLEC3A-derived AMPs in fighting infection.

P-05-01

Naturally occuring non-radioactive Calcium isotope ratios in the human body as a biomarker for bone metastasis of prostate cancer -a pilot study-

Burkhard Brandt[1]; Axel Semjonow[2]; Katrin Schlack[3]; Alexander Heuser[4]; Ana Kolevica[5]; Anton Eisenhauer[6]

[1]UKSH, Institut für Klinische Chemie, Kiel, Germany; [2]UKM, Prostatazentrum - Uroonkologie, Klinik für Urologie, Münster, Germany; [3]UKM, Münster, Prostatazentrum - Uroonkologie, Klinik für Urologie , Münster, Germany; [4]Geomar, Helmholtz-Zentrum für Ozeanforschung, Kiel, Germany; [5]Geomar/OsteolabsGmbH, Helmholtz-Zentrum für Ozeanforschung und Osteolabs, Kiel, Germany; [6]Geomar/Osteolabs GmbH, Helmholtz-Zentrum für Ozeanforschung und Osteolabs, Kiel, Germany

Aims: Prostate cancer (PCa) is the second most frequent malignancy in men worldwide. Although 5-year survival in patients with organ-confined PCa is nearly 100%, metastasis to the bones still remains incurable. Therefore, there is an urgent need for markers able to predict bone metastasis (BM), in order to personalize patients' (pts) treatment. The ratios of Calcium isotopes like 44Ca and 42Ca (δ44/42Ca), can be used to identify unbalanced bone mineral disorders, e.g. osteoporosis. This is based on the kinetic isotope fractionation factor between blood and bones being constant in humans. In this study we tested how sensitive δ44/42Ca changes in serum reflect PCa bone metastasis.

Materials and Methods: Sera from 20 pts were measured by plasma-mass spectroscopy for δ44/42Ca-values. For QC all data were compared and calibrated to international standards. The long-term standard reproducibility was in the order of 0.06 ‰. Nine pts (6 with PCa, 3 without) had no signs of bone metastasis. Eleven pts had moderate to multiple BM. BM were evaluated by PSMA-PET/CT or bone scintigraphy. BM pts received various androgen deprivation therapies, and, 4 received also taxane therapy. For osteoprotection Denosumab , Zoledronic acid and Vit D3 was given in 2, 1 and 1 pt., resp.

Results: Six of 9 pts w/o BM had normal age adjusted δ44/42Ca values, 2 showed Ca resorption and 1 Ca absorption ratios. Ca resorption occurred in 3 BM pts with moderate numbers of BM and low PSA levels (< 0.05 – 8 ng/ml). Eight pts with multiple BM and high PSA levels (31 – 1443 ng/ml) had Ca absorption. In this high δ44/42Ca subgroup 3 pts had Denosumab/ Zoledronic acid as bone protection. Unexpectedly, Vitamin D3 supplementation made no δ44/42Ca increase in 1 BM pt.

Discussion (Conclusion): We applied for the first time Ca isotope ratios as a biomarker for PCa bone metastasis. Overall, 2/3 of non-metastatic pts showed balanced δ44/42Ca, whereas BM pts were found in both groups representing Ca resorption and absorption as well. From published reports it might be hypothesized that Ca resorption show earlier phases of metastases which is in agreement with the lower number of BM and PSA values of the pts. In contrary, pts with multiple metastases and high PSA levels where found in the Ca absorption group indicating the final osteoblastic/ sclerotic phase of PCa bone metastases. This hypothesis might be substantiated by additional δ44/42Ca measurements from pts only displaying BM in PET-PSMA scan. The δ44/42Ca values in the absorptive range for pts with osteoprotective treatment by Denosumab and Zoledronic acid might also support this assumption. Nevertheless, an extended study is needed to control preanalytical influencing factors which may cause Ca disequilibrium. Those are benign gastrointestinal, kidney, endocrine and bone diseases and Ca supplementation. Possible influencing factors might explain why some patients in the study are misclassified by their δ44/42Ca measurements.

P-05-02

Influence of coagulation proteases and signalling on cognitive function

Silke Zimmermann; Gyulten Mangova; Akash Mathew; Shihai Jiang; Berend Isermann

Universitätsklinikum Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany

Aims: Since effects of thrombin on the blood brain barrier (BBB) have been proposed, we want to define the relevance of the thrombomodulin (TM) - activated protein C (aPC) system for BBB integrity and neuronal cell function. In Germany, one million patients are treated with lifelong anticoagulant therapy (anticoagulants). We speculate that anticoagulants, via influencing coagulation factors, may interfere with neuronal function, impair cognition or convey other CNS-related side-effects. Clinical reports suggested that anticoagulation affects cognition. We want to define (i) the impact of thrombin on neuronal function and cognitive performance in mice and (ii) whether the changed behavior of the animals is a consequence of the increased coagulation activation.

Material and Methods: We performed snRNA sequencing of wildtype (WT) versus TMPro/Pro (mutation leads to endothelial dysfunction due to reduced function of the TM-protein C pathway murine brains. We use mice with genetically altered activity of the TM-PC system to study its impact on cognitive function in mice, investigating locomotor activity, fear-related exploratory behavior, sensorimotor gating and learning patterns. Evans Blue (EB) extravasation test will show us, how (i) genotypes are affected regarding BBB integrity and (ii) whether anticoagulation affects integrity. Using CRISPR/Cas9 mediated cell-specific deletion of EPCR or PARs (PAR1-4) we want to identify the receptor through which aPC and thrombin modulate the function of brain cells, given the available data on IIa`s and aPC`s effect on endothelial barrier.

Results: We observed reduced EB extravasation in APChigh (aPC overexpression) mice in a chronic kidney disease mouse model as compared to WT or TMPro/Pro mice. Single nuclei RNA-sequencing of wildtype and TMPro/Pro murine brains revealed striking differences in various cell type clusters, comprising neurons, microglia cells, glia cells, pericytes and endothelial cells. Functional annotation revealed downregulation of genes related to "learning" in the TMPro/Pro mice. Indeed, we saw impairment in behavioral testing of TMPro/Pro mice.

Discussion: Our data establish that coagulation proteases differentially regulate the BBB in vivo. Altered coagulation activation is associated with structural defects in the CNS and with altered cognition. Recent data suggest that different anticoagulants differentially regulated coagulation protease dependent signaling, which may affect disease outcome. A prime example is activated protein C (APC), a blood protease with anticoagulant activity and cell-signaling activities. Of note, receptors for coagulation proteases are widely expressed at the blood brain barrier (BBB) and by various cells in the central nervous system (CNS). APC variants have shown benefits in preclinical models of ischemic stroke, brain trauma, multiple sclerosis and amyotrophic lateral sclerosis. Our data may show, for the first time, how coagulation may affect cognition.

P-05-03

Using quantitative morphometry data, the impaired formation of lamellipodia in Glanzmann thrombasthenia patients could be evaluated

Katharina von Bargen[1]; Max-Joseph Kraus[2]; Marcus Dittrich[3]; Christina Brandenburger[1]; Monika Wolny[1]; Tobias Flieder[1]; Johannes Rheinlaender[4]; Tilman E. Schäffer[4]; Günther Kappert[5]; Susan Halimeh[5]; Cornelius Knabbe[1]; Erwin Strasser[6]; Heiko Neeb[2]; Ingvild B

[1]Herz- und Diabeteszentrum Nordrhein-Westfalen, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany; [2]Universität Koblenz-Landau, Institut für Medizintechnik und Informationsverarbeitung, Koblenz, Germany; [3] Universität Würzburg, Institut für Humangenetik und Bioinformatik, Würzburg, Germany; [4]Universität Tübingen, Institute of Applied Physics, Tübingen, Germany; [5]Gerinnungszentrum Rhein-Ruhr, GZRR, Duisburg, Germany; [6]Klinikum der Universität München, LMU München, Abteilung für Transfusionsmedizin, Zelltherapeutika und Hämostaseologie, München, Germany

Introduction: After vascular injury, platelet adhesion to the extracellular matrix leads to platelet activation, which in turn induces platelet shape change. Thereby the reorganization of the cytoskeleton is mediated by a variety of signaling pathways. Among other things, the fibrinogen receptor (GPIIb/IIIa complex) is also involved. This complex plays a crucial role in primary hemostasis by mediating platelet aggregation. Therefore, the study aimed to investigate the shape change (spreading) in dependence of the GPIIb/IIIa complex. For this purpose, platelets from Glanzmann thrombasthenia patients (GT-patients) were studied. This is a platelet dysfunction with a quantitative or qualitative defect of the GPIIb/IIIa complex.

Methods: In the present study, platelet shape change was investigated in a total of five healthy donors and six GT patients. For this purpose, platelets were allowed to spread on fibrinogen under different conditions (without activator, with ADP, or with TRAP), the actin cytoskeleton was stained with phalloidin, and then 40 immunofluorescence images were acquired per condition and time point. The immunofluorescence images were then evaluated using an algorithm (automated quantitative morphometry analysis) by determining various parameters such as area, fractal dimension, number of pseudopodia, etc. In addition, the morphometry of the platelets was examined by electron microscopy.

Results: Analysis of the immunofluorescence images shows that the GT platelets have a spreading defect, which is particularly characterized by the absence of lamellipodia formation. Whereas the healthy platelets have mostly a fully spread shape after 45 min, the GT platelets persist in the early phase of spreading, which is characterized by a large number of long pseudopodia. Overall, the results of the algorithm show that the individual parameters (such as number of pseudopodia, FD, circularity, and area) describing the morphometry differ significantly between the studied collectives (healthy and GT). Here, the differences are particularly distinct at the late spreading time points. Thus, the GT platelets are much smaller due to the large number of pseudopodia, and the FD is increased. Electron microscopy also shows the altered morphometry of the GT platelets.

Conclusion: Spreading analysis show that platelet shape change is impaired in GT platelets. Both immunofluorescence microscopy and electron microscopy could show an absence of lamellipodia formation. Quantitative morphometry analysis was used to better describe cytoskeletal reorganization and to show the differences between the two collectives. Overall, quantitative morphometry analysis is a useful tool to better describe the different stages of platelet shape change in patients with thrombocytopathy.

P-05-04

Predictors of hypercoagulability in prediabetes

Sebastian Hörber[1]; Karina Althaus[2]; Tamam Bakchoul[2]; Jürgen Machann[3]; Norbert Stefan[4]; Andreas Birkenfeld[4]; Andreas Fritsche[4]; Andreas Peter[1]

[1]Universitätsklinikum Tübingen, Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, Tübingen, Germany; [2]Universitätsklinikum Tübingen, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany; [3]Universitätsklinikum Tübingen, Section on Experimental Radiology, Department of Radiology, Tübingen, Germany; [4]Universitätsklinikum Tübingen, Department of Internal Medicine IV, Division of Diabetology, Endocrinology and Nephrology, Tübingen, Germany

Background: Obesity and insulin resistance predispose for arterial and venous thrombosis that can be explained by a hypercoagulable state. However, data about underlying associations of the hypercoagulability with metabolic alterations is limited. Therefore, the aim of the present study was to identify metabolic predictors of hypercoagulability in prediabetes.

Methods: Endogenous thrombin potential (ETP) was determined in 141 subjects with impaired glucose tolerance and/or impaired fasting glucose using a commercially available thrombin generation assay. All subjects were metabolically characterized including an oral glucose tolerance test. Furthermore determination of body fat distribution and liver fat content was performed using magnetic resonance imaging and spectroscopy, respectively.

Results: ETP was significantly associated with fasting plasma glucose, insulin sensitivity and body fat distribution. In particular, increased amounts of total adipose tissue, visceral adipose tissue and subcutaneous adipose tissue were significantly associated with an increase in ETP. Increased liver fat content was also related to higher ETP. Subjects with fatty liver had higher levels of ETP compared to subjects without fatty liver. Adjusting for insulin sensitivity, fasting plasma glucose and body fat compartments ETP remained significantly and independently elevated in subjects with fatty liver compared to controls.

Conclusion: ETP is closely linked to metabolic alterations in prediabetes. Body fat distribution, particular increased liver fat content is significantly and independently associated with hypercoagulability in prediabetes and may therefore contribute to the increased risk for arterial and venous thrombosis. Further analysis will focus on the underlying mechanisms including molecular associations of hypercoagulability and liver fat content in prediabetes.

P-05-05

Tumour derived prothrombin interacts with tumour PAR1 receptors

Yaman Mouhish[1]; Jamie Nourse[2]; Essak S. Khan[2]; Sven Danckwardt[2]

[1]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz , Germany; [2]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany

Background: Thrombin is a liver-derived serine protease involved in hemostasis, acting through catalytic activation of soluble substrates (fibrinogen) and circulating cells (platelets). In addition, thrombin has a host of actions on cells with functions in development, angiogenesis, wound healing, inflammation, atherosclerosis, brain disorders, and tumour biology through activation of membrane-bound G-protein coupled protease-activated receptors (PARs). Previously, we uncovered extrahepatic prothrombin expression in emerging fibrosarcoma tumours, which drives tumour proliferation and invasiveness (Nourse et al.,bioxiv 2021). Here we investigated the interaction between endogenous tumour-derived (pro)thrombin and PAR1 on the surface of these cells by using bioluminescence resonance energy transfer (BRET).

Methods: To establish the BRET reporter assay system, we produced a prothrombin-luciferase (emission max 535 nm) and a PAR1-turbo fluorescent protein (emission at 635 nm) fusion construct, which were then used in transient transfection experiments of HEK cells. A bioluminogenic substrate (coelenterazine) was added to the co-transfected cells to elicit the energy transfer between the prothrombin-luciferase and the PAR1 turbo FP, resulting in a specific red fluorescent signal upon the interaction of (pro)thrombin with PAR1.
Subsequently, we transfected endogenously prothro-mbin-luciferase expressing fibrosarcoma cells (obtained from a newly generated transgenic reporter mouse model after chemical tumor induction with methylcholanthrene (Nourse et al.,bioxiv 2021)) with the PAR1 turbo FP construct to study the interaction between endogenous (pro)thrombin and PAR1 using the established BRET assay system.

Results: First, we confirmed the production of a functional prothrombin-luciferase fusion protein and the presence of PAR1-turbo FP on the membrane of the cells. After establishing optimal BRET assay conditions, we were able to observe red-shifted signal in transfected cells, which could be modulated with ecarin (increase in red signal) and hirudin (reduced red signal), respectively. These findings confirm a successful function of the established BRET assay principle. Furthermore, we could show an emission stroke shift in endogenously prothrombin expressing cancer cells, suggesting that prothrombin binds to PAR1 receptors located on the membrane of these cells.

Conclusion: We successfully established a BRET reporter assay system to monitor (pro)thrombin-PAR1 interaction. We demonstrate that tumor-derived-prothrombin binds to PAR1 receptors expressed on the membrane of the tumor cells. Regarding the wide-spread clinical use of thrombin-targeting by direct oral anticoagulants, determination of the role and underlying mechanisms of thrombin in tumour growth may reveal previously unidentified benefits of selective therapeutic targeting of the hemostatic system in cancer.

P-05-06

Automated 24/7 screening and quantification of DOACs in plasma in a single run on CLAM2030 – LCMS8050

Anna Abratis[1]; Moritz Schnelle[1]; Frank Streit[1]; Manuel Wallbach[2]; Nils Kunze-Szikszay[3]; Sebastian Uwe Schnitzler[3]; Julie Schanz[1]; Andreas Fischer[1]; Ivana Markovic[1]

[1]Universitätsmedizin Göttingen, Interdisziplinäres UMG Labor, Göttingen, Germany; [2]Universitätsmedizin Göttingen, Department of Nephrology and Rheumatology, Göttingen, Germany; [3]Universitätsmedizin Göttingen, Department of Anesthesiology, Göttingen, Germany

Aims: In recent years, therapeutic drug monitoring (TDM) as part of DOAC therapy has gained in importance, since the outcome can be improved through individual dose adjustment, especially when treating critically ill emergency patients. At present, the effects of various DOACs are usually assessed indirectly and insufficiently (e.g. by determining the thromboplastin time or the activated partial thromboplastin time). Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is an appropriate system for simultaneous measurement of multiple drugs. Until today, however, the use of LC-MS/MS Systems in emergency labs were not established.
Therefore, this study aims to develop and to validate a new LCMS-based method to screen and quantify different DOACs simultaneously in a single run. Similar methods have so far been restricted to research purposes, usually presupposing trained staff and long running times. Connecting the LCMS to an automated sample preparation module, we assessed its

suitability for DOAC screening on a routine 24/7 basis. We compared the method for three of the available DOACs using the LCMS-8050 system coupled to CLAM-2030 (both Shimadzu) versus commercially available chromogenic tests.

Methods: The plasma samples from 56 anesthesiologically and nephrologically supervised patients were collected. DOAC plasma concentrations of Apixaban, Dabigatran and Rivaroxaban were measured on a LCMS system through an automated sample preparation module. Sample protein precipitation and chromatographic separation with a sharp linear gradient on a fused core column at 45°C were performed automatically by CLAM-2030. The target compounds were identified by parent ions and optimized MRM transitions. Quantification was performed by using deuterated internal standards. Quality controls were checked twice a day. In parallel, the quantitative determination of DOACs were assessed using conventional automated chromogenic tests (DTI-Assay (Dabigadran), Anti-Xa-Assay (Apixaban, Rivaroxaban), HemosIL (Werfen Company) on a IL Coagulation System (ACL TOP 750). Patient plasma samples were placed on the devices according to their arrival in the lab.

Results: The Screening results have shown a good correspondence with the patients' data. Passing–Bablok regression analysis revealed good comparability between the methods (Apixaban r=0.984, y=1.019*x−1.354; Rivaroxaban r=0.986, y=1.063*x−1.663; Dabigadran r=0.988, y=0.856*x−0.362). The precision of calibrations (range about 10 to 500ng/ml) and controls was within the manufacturer limit. The automated system was straightforward and proved easy to handle after short training periods. Running time including sample preparation was approx. six minutes.

Conclusion: The presented method is convincing in its easy handling and is conceivable for (24/7) routine measurements. In contrast to previously used methods, particularly the contemporaneous assignment and quantification of different DOACs is innovative.

P-05-07

Review: The Peripheral Blood Smear

Evadne Parulan Holzhueter

Achterwehr, Germany

Review: Peripheral Smear

Case Report: This is a case of a 74 year old female on a yearly medial check.up on the 25th of October 2021. Except for a mild to moderate hypochromic anemia the diferential blood picture showed the following cellular percentage distribution as follows:
- Neutrophil — 52%
- Eosinophil — 3.5%
- Basophil — 1.2%
- Monocyte — 12.3%
- Lymphocyte – 31%

Examination of the peripheral blood smear through the microscope revealed presence of plasma cells of varying stages of maturation. Furthermore a request for Immune Fixation test confirmed the diagnosis of Monoclonal Gammopathie, IgM Lambda. The increased percentage of Monocytes done through automation gave me the impression that the plasma cells are recognized as such. Therefore i like to present the following points of view relevant to consider as follows:
1. The examination of the peripheral blood smear through the microscope remains a standard operating procedure to detect early morphological, behavioral cellular changes in the human blood cells
2. To develop our skills and competence to recognize the presence of normal and abnormal cells as early as possible.
3. To emphasize the importance of laboratory diagnosis in health care and clinical decision making.

P-05-08

Procoagulant platelets as a diagnostic tool for heparin-induced thrombocytopenia using platelet-rich plasma by flow cytometer

Lisann Pelzl[1]; Aleyna Karakuyu[1]; Hisham Jaffal[1]; Anurag Singh[1]; Irene Marini[1]; Jan Zlamal[1]; Stefanie Hammer[2]; Karina Althaus[3]; Tamam Bakchoul[3]

[1]Universitätsklinikum Tübingen, Institute for Clinical and Experimental Transfusion Medicine, Medical Faculty of Tuebingen, Tuebingen, Germany; [2]Universitätsklinikum Tübingen, Centre for Clinical Transfusion Medicine, University Hospital of Tuebingen, Tuebingen, Germany; [3]Universitätsklinikum Tübingen, Universitätsklinikum Tübingen, Institute for Clinical and Experimental Transfusion Medicine, Medical Faculty of Tuebingen; Centre for Clinical Transfusion Medicine, University Hospital of Tuebingen, Tuebingen, Germany

Heparin-induced thrombocytopenia (HIT) is caused by anti- PF4/heparin IgG antibodies, which activate the platelets and lead to thrombocytopenia and thrombosis. The diagnosis of HIT is usually confirmed by using functional assays such as the Heparin-Induced Platelet Activation assay (HIPA assay). However, functional assays are time consuming and routinely available only in specialized laboratories.

The aim of the current study was to establish a flow cytometer-based assay to determine procoagulant platelets in platelet-rich plasma (PRP) for the diagnosis of HIT. Sera samples from patients with HIT (HIT group) were incubated with PRP from healthy donors for different durations with (30, 60, 90 minutes). Procoagulant platelets were determined by analyzing double expression of P-selectin (CD62p) and phosphatidylserine (PS) externalization by flow cytometry. CD32a-mediated cross-link and platelet stimulation with ionomycin were used as positive controls.

Sera from HIT-diagnosed patients but not from the control-group induced a significant increase in the procoagulant platelet subpopulation in the presence of 0.2 U/mL heparin (% double positive CD62/PS: 1.2 ± 1.1 vs 18.5 ± 8.1, p=0.0021). The optimal incubation time was 60 minutes. A donor dependency of the flow cytometric method was not observed (control vs HIPA+: 0.7 ± 0.59 vs 19.0 ± 3.2, p=0.0129). In addition, the use of washed platelets and PRP with HIT-sera led to the same results in this flow cytometric method (34.2 ± 6.3 vs 30.1 ± 2.2, ns).

Our data reflects towards suitability of aPRP-based protocol that can be used to detect the ability of HIT antibodies to induce procoagulant platelets by flow cytometry. In our ongoing studies, we are currently investigating the potential clinical implementation of this protocol in the diagnostic work up for HIT.

P-05-09

Calcium, calcium-sensing receptor and its role in leukaemia progression

Daniela S. Krause

Uniklinik Marburg und Georg-Speyer-Haus Frankfurt, Laboratoriumsmedizin, Marburg, Germany

Introduction: 99% of the body's calcium is stored in bone, and calcium is released during bone remodeling. The calcium sensing receptor (CaSR) plays a role in the localization of normal haematopoietic stem cells in the BM microenvironment (BMM). However, the role of this receptor and its associated pathways for leukaemia development, therapy success and whether modulation of this receptor may be beneficial therapeutically, is not known.

Methods: Hypothesizing that the CaSR contributes to development, progression and response to therapy in leukaemia, we employed various in vitro assays, in vivo microscopy, leukaemia induction and in vivo treatment assays to test this question.

Results: The local calcium concentration forms a gradient in the BMM with highest calcium concentrations close to the endosteum. The calcium concentration in the BMM, CaSR expression on leukaemia cells, CaSR sensitivity to extracellular calcium, adhesion and migration in various concentrations of calcium differ between leukaemia types. CaSR acts as tumor suppressor or an oncogene in different leukaemias. In acute myeloid leukaemia (AML), limiting dilution transplantation of CaSR-deficient AML-initiating cells revealed a 7-fold reduction of leukaemic stem cells. Downstream of CaSR, we implicated filamin A and other proteins as important signaling molecules. Treatment of mice with a CaSR agonist or antagonist differentially impacted myeloid leukaemias.

Conclusion: In summary, our results suggest that the CaSR and possibly calcium ions from the BMM strongly and differentially influence leukaemia progression, with filamin A playing an essential role in AML. As an adjunct to existing treatment strategies, targeting of CaSR with specific pharmacologic agents may be beneficial in different leukaemias.

P-05-10

Liquid profiling of circulating tumor DNA in colorectal cancer: steps needed to achieve its full clinical value as standard care

Maren Hedtke[1]; Matthias Frölich[2]; Angelika Duda[1]; Laura Mirbach[1]; Volker Ast[1]; Victor Costina[1]; Uwe Martens[3]; Ralf Hofheinz[4]; Michael Neumaier[1]; Verena Haselmann[1]

[1]Universitätsmedizin Mannheim, Institut für Klinische Chemie, Mannheim, Germany; [2]Universitätsmedizin Mannheim, Klinik für Radiologie und Nuklearmedizin, Mannheim, Germany; [3]SLK Kliniken Heilbronn, Medizinische Klinik III, Heilbronn, Germany; [4]Universitätsmedizin Mannheim, III. Medizinische Klinik, Tagestherapiezentrum, Mannheim, Germany

Introduction: The analysis of circulating tumor DNA (ctDNA) is at the threshold of implementation into standard care for colorectal cancer (CRC) patients. However, data about the clinical utility of liquid profiling (LP), its acceptance by clinicians, and its integration into clinical workflows in real-world settings remain limited.

Methods and Results: In total, 243 LP tests for 168 CRC patients were performed as part of standard care for RAS using beads, emulsification, amplification, and magnetics (BEAMing), for BRAF V600 by digital droplet PCR (ddPCR), or for both molecular targets. LP tests requested as part of routine care since 2016 were retrospectively evaluated. Results show restrained request behavior that improved moderately over time, as well as reliable diagnostic performance comparable to translational studies, with an overall agreement of 91.7%. Extremely low ctDNA levels at < 0.1% in over 20% of cases, a high frequency of concomitant driver mutations (in up to 14% of cases), and ctDNA levels reflecting the clinical course of disease were revealed. However, certain limitations hampering successful translation of ctDNA into clinical practice were uncovered, including the lack of clinically relevant ctDNA thresholds, appropriate time points of LP requests, and integrative evaluation of ctDNA, imaging, and clinical findings.

Conclusion: These results highlight the potential clinical value of LP for CRC patient management and demonstrate issues that need to be addressed for successful long-term implementation in clinical workflows.

P-06-01

Quantity or quality? Deciphering the diagnostic relevance of anti-drug antibody analysis by surface plasmon resonance

Melina Grasmeier; Susanne Weber; Markus Thaler; Peter Luppa

Klinikum rechts der Isar der TU München, Institut für Klinische Chemie und Pathobiochemie, München, Germany

Aims: Inflammatory bowel disease (IBD) affects up to 1.3% of the western population and is characterized by chronic or relapsing intestinal inflammation. Biologic tumor necrosis factor (TNF) blockers, pioneered by the therapeutic antibody infliximab (IFX), opened up the possibility of achieving disease remission. Yet, all biologics can provoke formation of anti-drug antibodies (ADA) that may cause therapy failure. 65% of patients on IFX develop ADA and thus, monitoring of both IFX and ADA is recommended. Currently used assays for TNF blocker and ADA assessment lack harmonization and could so far not sufficiently elucidate the impact that ADA quantity and quality have on patient-individual disease progress. Addressing the need for more powerful analytics, we present a surface plasmon resonance (SPR)-based biosensor.

Materials and Methods: An assay for rapid ADA purification from serum via magnetic IFX beads followed by SPR analysis was developed. We present the dissociation ratio (DissR) as an indicator of ADA:IFX binding kinetics, which is assessed within the ADA quantification run. 45 sera from IBD patients under IFX therapy were analyzed by SPR and a diagnostics-approved ELISA. The individual ADA kinetics (only accessible by SPR) were evaluated and correlated with therapeutic outcomes.

Results: The SPR assay exhibited robust drug-tolerance in presence of up to 10-fold molar excess of IFX. LOD and LQO of ADA quantification were determined as 0.15 µgEq/mL and 0.40 µgEq/mL. Semi-quantitative ADA assessment was possible between LOD and LOQ. The ADA detection rate in the patient sera was similar for SPR and ELISA (52% and 55%). The moderate correlation of ADA concentrations between SPR and ELISA ($R^2=0.69$) is owed to the methodological differences.

DissR was shown to be independent of ADA concentration and unbiased by serum-individual matrix effects. Interestingly, ADA concentrations determined by ELISA showed stronger positive correlation with DissR ($R^2=0.66$) compared with SPR quantification ($R^2=0.57$), i.e., high-affinity ADA were observed in higher concentrations. This affinity bias may be associated with determination of falsely low ADA titers in patients with lower-affinity ADA. Monitoring of larger patient cohorts with the less biased SPR assay will show if this finding is clinically relevant.

[Association studies of DissR with therapy success are in progress. At the congress, a larger cohort size and accordingly adjusted results will be presented.]

Conclusion: Enabling parallel ADA quantification and binding kinetic analysis, SPR biosensors deliver more information about ADA than currently used assays do. Larger studies could contribute to a better understanding of ADA occurrence in IBD patients treated with biologics. If future studies suggest clinical relevance, ADA kinetics may aid to re-evaluate the diagnostic suitability of currently used methods for ADA quantification.

P-06-02

Comparison of two LC-MS/MS methods for quantification of mycophenolic acid in the sera of patients

Joachim Kuhn; Cornelius Knabbe

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklink der Ruhr-Universität Bochum

Aims: Measurement of mycophenolic acid (MPA) concentration in human blood is an important feature within the framework of therapeutic drug monitoring (TDM) of heart transplantation patients.

Methods: A total of 100 clinical samples from heart transplanted patients which were treated with mycophenolate mofetil (MMF) were used to compare a fully validated MPA LC-MS/MS method with a new faster one. The MPA measurements of the serum samples were performed after sample preparation using liquid/liquid extraction on a Quattro micro tandem mass spectrometer within a run time of 3.5 min on the one hand and, on the other, on a Xevo TQD tandem mass spectrometer with a total run time of only 1.6 min. MPA-D3 was used as internal standard.

Results: Excellent correlation between the two LC-MS/MS methods could be found. The Passing-Bablok regression analysis revealed an intercept of -0.02 mg/L (95% confidential interval (95% CI), -0.07 to 0.01 mg/L) and a slope of 1.00 mg/L (95% CI, 0.97 to 1.02 mg/L). Cusum test for linearity shows that there was no significant deviation from linearity (P = 0.89). The correlation coefficient was 0.99. Furthermore, comparing measurements were performed between two Xevo TQD tandem mass spectrometers using the faster MPA LC-MS/MS method. Very good correlation between these comparing measurements could also be found. The Passing-Bablok regression analysis revealed an intercept of -0.06 mg/L (95% CI, -0.08 to 0.04 mg/L) and a slope of 1.02 mg/L (95% CI, 1.01 to 1.03 mg/L). Cusum test for linearity shows that there was no significant deviation from linearity (P = 0.98). The correlation coefficient was 0.99.

Conclusion: In summary, we could show that within the framework of a change control a significantly faster LC-MS/MS measurement of MPA without loss of quality was possible. The new method was more than twice as fast as the fully validated old method and the correlation between the two LC-MS/MS methods was excellent, respectively.

P-06-03

Fully automated application of the LCMS-8060NX system coupled to CLAM-2030 in 24/7 routine using HL-7 interface (beta-version) standards

Frank Streit; Andreas Fischer; Ivana Markovic

Universitätsmedizin Göttingen, Interdisziplinäres UMG Labor, Göttingen, Germany

Background and Aims: Liquid chromatography coupled with mass spectrometry (LC-MS/MS) offers exceptional sensitivity and specificity and is widely used for therapeutic drug monitoring and toxicological screening. The development of preanalytical and analytical sample management improved the efficiency of sample processing, the precision and quality of manually work, as well as automated validation and interpretation of data. Recent advancements in LC-MS/MS development offer innovative analytical platforms, however routine applications are still limited. In our laboratory, there are several laboratory developed tests (LDT) using automated LC-MS/MS on Clam2030-LCMS8060 NX established (i.e. the measurement of antibiotics, anticonvulsive drugs and direct oral anticoagulants concentrations (DOACs)). Therefore, we aimed to develop and validate a fully automated application of the LCMS-8060NX system coupled to CLAM-2030 in 24/7 routine by using the HL-7 interface (beta-version, for research only) standards.

Materials and Methods: The clinical laboratory automated module CLAM2030 combined with an LCMS8060NX (both Shimadzu) was tested for efficiency of preanalytical and analytical sample management for three different LDT-procedures (sample preparation, analyzing, calibration identification and calculation of quality controls (Alsachim and Recipe)). HL-7 interface (beta-version) standards were used for bidirectional communication between the laboratory information system (OSM, Germany) and Clam2030-LCMS8060 NX.

Results: Patient samples and quality control samples for three different LDT-procedures (TDM of antibiotics, anticonvulsive drugs and DOACs) were measured 5 times in a randomized manner. Variation of results and retention times are presented. Different acceptance criteria for analytes, as well as stable isotopic labelled internal standards were used to verify analytical processes in terms of identification, calculation and result transmission. We demonstrate an effective work flow from requesting a laboratory test until transmission of the data using a bidirectional HL-7 interface (beta-version) protocol between the laboratory information system and the Clam2030-LCMS8060 NX instrument.

Conclusion: Simultaneous measurement of different LDT-procedures makes CLAM-2030-LCMS8060 NX an attractive device for 24/7 use in clinical and toxicological diagnostics. Automated randomized measurements of patient samples with the LCMS-8060NX system coupled to CLAM-2030 requesting different LDT procedures were successfully transferred to the laboratory information system (LIS) using HL-7 Interface (beta-version) standards, enabling its applications in 24/7 routine.

P-06-04

Simplified preanalytical handling of plasma samples in patients treated with High-dose Methotrexate therapy and Glucarpidase rescue

Amir Jahic[1]; Franz Knörnschild[2]; Sven Liebig[2]; Immanuel Merz[2]; Peggy Kießling[3]; Monika Prpic[1]; Ulrich Keller[2]; Stefan Schwartz[2]; Kai Kappert[1]

[1]Charité-Universitätsmedizin Berlin, Institut für Laboratoriumsmedizin, Klinische Chemie und Pathobiochemie, Berlin, Germany; [2]Charité-Universitätsmedizin Berlin, Department of Hematology, Oncology and Cancer Immunology, Berlin, Germany; [3]Labor Berlin - Charité Vivantes GmbH, Laboratoriumsmedizin & Toxikologie, Berlin, Germany

Aims: High-dose methotrexate (HD-MTX) therapy is toxic and therefore requires an obligatory rescue approach combining leucovorin and, depending on individual patient history, glucarpidase administration. The exogenous folate derivative leucovorin provides a source of intracellular tetrahydrofolates and counters the toxic effect of the folate antagonist methotrexate (MTX). The recombinant bacterial enzyme glucarpidase is a rescue agent that specifically cleaves MTX and to a minor extent its active metabolite 7-OH-MTX extracellularly, forming the inactive metabolites 2,4-diamino-N10-methylpetroic acid (DAMPA) and OH-DAMPA in vivo. Glucarpidase theoretically might continue to metabolize MTX and 7-OH-MTX after blood withdrawal ex vivo. However, no consensus about preanalytical sampling and processing of blood collecting tubes intended for laboratory MTX and 7-OH-MTX testing after HD-MTX has been established.

Material and Methods: One hundred ten plasma specimens from hematooncological patients (count: four, age: 74-86 years) that received HD-MTX, leucovorin rescue and glucarpidase, are selected as samples without (i.e. native) and with supplements (sodium chloride, NaCl; hydrogen chloride, HCl). Patient samples are further stored at various temperature conditions, prior to measuring MTX and MTX metabolites by liquid chromatography (LC) - tandem mass spectrometry (MS), LC-MS/MS. LC-MS/MS (MTX, 7-OH-MTX, DAMPA) measurements are compared to immunoassay (MTX) for selected conditions.

Results: We will systematically evaluate different preanalytical conditions, including the effect of acid (HCl) addition, aiming at presumed glucarpidase inactivation ex vivo. MTX measurements determined by an immunoassay and the LC-MS/MS are expected to be different. MTX immunoassay (ARK diagnostics, Inc.) is expected to show a positive bias compared to the gold standard approach (LC-MS/MS). LC-MS/MS based quantification of MTX and MTX metabolites in acid pretreated, i.e. inactivated specimens is expected to exhibit significant differences when compared to measurements obtained from their non-inactivated comparative samples. Moreover, specific processing conditions are suspected to affect the stability of MTX and MTX metabolites in plasma samples when stored at different temperatures.

Discussion and Conclusions: Preanalytical laboratory specimen management is still challenging in routine diagnostics. Even for well-established laboratory parameters in hematooncological diagnostic settings, the storage temperature and the pretreatment of blood collecting tubes could be of high relevance for further clinical and therapeutic decisions.

P-06-05

The impact of the EMA DPYD variant analysis recommendations from 2020 on requesting behavior and number of analyses

Maren Hedtke; Romy Eichner; Laura Mirbach; Michael Neumaier; Verena Haselmann

Universitätsmedizin Mannheim, Institut für klinische Chemie, Mannheim, Germany

Introduction: A major cause of toxic or potentially lethal side effects of fluoropyrimidine therapy (like 5 fluorouracil or capecitabine) is a deficiency or a complete lack of the dihydropyrimidine dehydrogenase (DPD) activity. DPD is the main enzyme for the degradation of the aforementioned drugs. Mutations that lead to a decreased enzyme activity are found in up to 9% of the population, 0.1 – 0.5% show a complete lack of DPD activity. Therefore, since 2020 the European Medicines Agency (EMA), the Federal Institute for Drugs and Medical Devices (BfArM) and the German society for hematology and medical oncology (DGHO) recommend testing for four DPYD mutations that affect enzyme activity before the first administration of fluoropyrimidines.

Methods: In a retrospective data evaluation 386 patient results (PCR and subsequent pyrosequencing) were analyzed for the frequency and the distribution of the variants *2A, *13, haplotype B3 (or c.1129-5923C>G) and c.2846 A>T (or p. D949V) of DPYD. Furthermore, the number of requests from clinicians between 2015 and 2021 is evaluated with regard to the change of requesting behavior with the introduction of mandatory DPYD testing.

Results: The most frequently found variant was a heterozygous mutation for haplotype B3 that was found in 4.6% of the patients. Heterozygosity for *2A and c.2846A>T was found in 0.78% and 1.3% respectively, whereas *13 was not detected at all. Homozygous mutations were not found for any of the four variants. Identified variant frequencies are in accordance with previously published data. In 2019 we had 8 requests for DPYD analysis while in 2021 there were 233.

Conclusion: We want to emphasize the value of DPYD genotyping for patients before therapy with fluoropyrimidines. The drastic increase of test requests shows how many patients were not analyzed before 2020 and how many side effects or even fatal outcomes could have been prevented.

P-06-06

An European IVD conform LC-MS-based method for monitoring of Azathioprine metabolites in whole blood

Verena Horneffer-van der Sluis

Universitätsklinikum Hamburg-Eppendorf (UKE), Institut für Klinische Chemie und Laboratoriumsmedizin, Hamburg, Germany

Introduction: Azathioprine is a widely used immunosuppressant drug with broad indications, including treatment of inflammatory bowel disease and organ rejection in transplanted patients. Its metabolites are cytotoxic and metabolic rates differ highly among individuals. TDM is needed to assess treatment efficacy and dosage regime. However, there is a lack in commercially available CE marked tests in the EU and laboratory developed tests (LDT) remain to be established. Here, we present the implementation of a liquid chromatography mass spectrometry based assay for quantifying Azathioprine metabolites 6-methylmercaptopurine (6-MMP) and 6-thioguanine nucleotides (6-TGN) in whole blood samples according to European 2017/746 on In-Vitro-Diagnostics regulation (IVDR).

Materials and Methods: EDTA samples were prepared as described previously [1]. A seven point calibration curve and two quality control samples of different concentration ratios are processed in parallel to the samples before injected into a Waters Acquity UPLC-system hyphenised with a Waters TQS triple quadrupole mass spectrometry instrument equipped with electrospray ionisation. Compound specific multiple reaction monitoring transitions are acquired during a 6 min Reversed Phased chromatographic gradient. Method documentations are obtained and maintained as required by the European IDVR guidelines.

Results: Validation of the method with regards to sensitivity, linearity, accuracy, bias, and precision according the CLSI recommendation given in EP05, 06, 09, and 17 based on more than 100 sample injections revealed following: a LLOQ of 50 pmol/0.2mL (4 samples in duplicate at 2 days) and a linearity of > 0.995 up to a ULOQ of 10,000 pmol/0.2mL (5 replicates per point for 2 operators). An accuracy and precision of better than 15% is achieved for within laboratory performance including within-run, between days, and inter-operator comparison (5 samples in 5 replicates at 3 days and 2 operators). The bias is determined to be within 3%. The method is applied to routine sample analysis of more than 40 samples.

Discussion and Conclusion: In conclusion, we describe an IVDR consistent UPLC–MS/MS based method that is sensitive, linear, and reproducible for the simultaneous quantification of 6-MMP and 6-TGN in whole blood samples.

P-06-07

A simple LC-MS/MS method for iohexol quantification in serum

Jonathan Stefi; Katharina Habler; Michael Vogeser; Michael Paal

Klinikum der Universität München, LMU München, Institut für Laboratoriumsmedizin, München, Germany

Introduction: The measurement of glomerular filtration rate (GFR) is a very important tool for the assessment of renal function and an indispensable indicator for the progression of chronic kidney disease. Measuring GFR by iohexol clearance is increasingly applied in clinical practice (e.g. critically ill patients) given that estimations of the GFR with creatinine and cystatine do not necessarily reflect the actual GFR. The aim of the current study was to develop a highly selective LC-MS/MS method for the quantification of iohexol in human serum to allow accurate GFR measurements with this nonionic contrast medium.

Methods: After cleanup of 50 μL serum by protein precipitation, iohexol was chromatographically separated within 3.5 minutes on a Acquity BEH-C18 column using water-formic acid and methanol-formic acid as mobile phases for gradient elution. Iohexol was quantified within the range of 5 – 500 mg/L using iohexol-D5 as internal standard. The method was comprehensively validated according to the European Medicines Agency (EMA) bioanalytical method validation protocol.

Results: Inaccuracy and the imprecision coefficient of variation were ≤ 7.2 % and ≤ 4.6 % for all quality controls. The internal standard consistently compensated for matrix effects. Analysis of external quality control (EQA) samples gave deviations of ≤ 1.9 % from expected nominal concentrations.

Conclusion: We hereby present a robust and accurate LC-MS/MS method that is suitable for iohexol quantification in human serum. Due to the simple sample preparation protocol and chromatography setup, the method is readily implementable in clinical LC-MS/MS laboratories.

P-06-08

Working Group: Digital Competence - Strengthening digital competence in laboratory medicine

Jakob Adler[1]; Alexander Tolios[2]; Ronald Biemann[3]

[1]Medical Laboratory for Clinical Chemistry, Microbiology, Infectious Disease and Genetics "Prof. Schenk/Dr. Ansorge & Colleagues", Clinical Chemistry, Endocrinology, Therapeutic Drug Monitoring, Magdeburg, Germany; [2]Medical University of Vienna, Department of Blood Group Serology and Transfusion Medicine, Wien, Austria; [3]University of Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany

Aims: More and more digital applications are finding their way into everyday medical practice. In recent years tech giants such as Google are developing Deep Learning-based algorithms for diagnosing and classifying diseases such as diabetes mellitus through retinal image analysis and are even running ahead of the universities in some fields of medical research. Since digital competence among physicians and professionals in laborory medicine is neither taught during medical graduate training nor part of the already existing range of competences, the Young Laboratory Section (Sektion "Junges Labor") of the German Society of Laboratory Medicine ("DGKL") created a working group to provide support in that field.

Work content, platform and methods: The main goal of this working group is to teach its members the basic principles of being able to efficiently perfom workflow-oriented tasks using programming languages. Colleagues get trained to independently analyze laboratory data.
To this end, our main medium-term goal is to learn the programming language R, which specializes in statistics and data analytics. In addition, simple "supporting" tools such as writing Markdown text for e.g. static analyses documents or websites as well as version control (Git and GitHub) in order to be able to work together on projects are tought.

In addition, well-founded statistical knowledge will be required, which is to be imparted through the participation of external lecturers in the regularly held online sessions. As a working environment, the working group uses the platform "GitHub", a website on which a graphical interface for the version management software "Git" can be used free of charge.

For this purpose, the organization "DGKL Junges Labor - AG: Digitale Kompetenz" was founded on GitHub. Additionally, in order to keep track of the jungle of "IT buzzwords" (e.g. deep learning, blockchain, cryptography, etc.), current "trending words" are presented in an easy-to-access format of an "IT buzzword bingo" (5 - 8-minute presentations) and then discussed.

Outlook: The working group is initially planned to run for about two years. In addition to the training of working group members, further training offers are to be created in order to facilitate the introduction of the programming language R for the members of the DGKL. After the two-year initial phase, it is planned to create a multi-day "Digital Competence" course that could be offered by the DGKL.

P-06-09

Development of a photonic microring resonator sensor system for the fast detection of biomolecules in point-of-care testing devices

Christina Hoffmann[1]; Peter Hoffmann[1]; Jakob Reck[2]; Moritz Kleinert[2]; Klara Mihov[2]; Norbert Keil[2]; Vera Froese[3]; Ulrich Kertzscher[3]; Kristina Mykhailiuk[4]; Julia Michaelis[4]; Hans-Jürgen Heupke[5]; Sören Scholand[5]

[1]Charité - Universitätsmedizin Berlin, Institut für Laboratoriumsmedizin, Klinische Chemie und Pathobiochemie, Berlin, Germany; [2]Fraunhofer Heinrich-Hertz-Institut, Photonic Component Department, Berlin, Germany; [3]Charité - Universitätsmedizin Berlin, Institut für Kardiovaskuläre Computer-assistierte Medizin, Berlin, Germany; [4]SCIENION GmbH, SCIENION GmbH, Berlin, Germany; [5]Eschweiler GmbH & Co. KG, Eschweiler GmbH & Co. KG, Kiel, Germany

Aims: Microring resonators are round optical waveguides which resonate with a characteristic wavelength. If an analyte attaches to the surface, the optical properties change, and a wavelength shift can be observed. This shift is proportional to the mass (amount) of the attached analyte. The aim of the optION sensor project is to research and develop photonic microring resonators as analytic tools for in vitro diagnostics.

Materials and Methods: The photonic sensor chips were fabricated from silicon nitride wafers using photolithography. The ring resonators were functionalized with sodium ionophore as capture molecule. As a reference, one ring resonator remained without functionalization. Aqueous solutions with NaCl concentrations between 500 mM and 125 mM were applied to the optION sensor. Between measurements, the chip was rinsed with Triton X-405 and ddH2O.

Results: The maximal wavelength shift occurred after about 10 seconds. It is possible to observe the binding curve of the analyte. The repeated usage of the same sensor after washing was successful. Sodium ions were detected in the investigated concentration range between 500 mM and 125 mM, which reaches physiologically relevant concentrations.

Conclusion: The photonic microring resonators are a promising technology for fast and label-free point-of-care measurements. Funding: Federal Ministry of Education and Research, grant 13GW0243F

P-06-10

Large-scale comparison of central laboratory SLS and POCT co-oximetry hemoglobin determination at the emergency department of the University Hospital Bonn, Germany

Ramona Christina Dolscheid-Pommerich[1]; Sarah Verlage[2]; Birgit Stoffel-Wagner[1]; Ingo Gräff[3]

[1]University Hospital Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, Bonn, Germany; [2]University of Cologne, Department of Rehabilitation and Special Education, Köln, Germany; [3]University Hospital Bonn, Emergency Department, Bonn, Germany

Introduction: Determination of hemoglobin (Hb) concentration with either POCT co-oximetry or central laboratory SLS detection is critical regarding diagnosis of anemia or critical transfusion limits. At the emergency department of the University Hospital Bonn (UKB), blood gas analysis, including co-oximetry, is performed during a patient's first medical examination. At the same time, venous blood is taken and immediately sent via pneumatic tube for further analysis, including blood cell count, to the central laboratory (CL). Given the importance of properly determining hemoglobin concentrations for diagnosing anemia, it is essential to know whether the two methods yield comparable results and whether both are equally appropriate for different age groups. In the present study, we examined this question using a large-scale sample.

Methods: N = 19182 patients were included (10439 male, 8743 female, mean age: 57.4 years, min 18, max 102 years). The cohort of patients was divided into the following three subgroups: 1 (age > 18-64 years, n = 11201), 2 (65-84 years, n = 6500) and 3 (age > 85 years, n = 1481). For each patient, POCT Hb concentration (RapidLab 1265, Siemens Healthineers) and CL concentration (XN1000, Sysmex) were compared. The whole collective as well as the subgroups were analyzed regarding diagnosis of anemia, according to the WHO definition, and critical anemia (Hb < 8 g/dl).

Results: Overall, a significant correlation was found for Hb concentrations between both methods, r = .96, p < 0.001. There were significant differences between the two methods, with POCT yielding higher Hb concentrations (average value: 13.43 g/dl, SD ± 2.41) than CL (average value, 13.1 g/dl SD ± 2.35), p < 0.001. For the whole sample, both methods differed significantly in their classification of anemia according to WHO (male p < 0.001, female p < 0.001) and critical anemia (p < 0.001). In the three subgroups, classification of anemia differed for all age groups (1: male p < 0.001, female p < 0.001, 2: male p < 0.001, female p < 0.001, 3: male p < 0.001, female p < 0.001). Regarding classification of critical anemia, the two methods differed depending on age group. For subgroups 1 and 2, POCT and CL Hb concentrations differed significantly in their classification of critical anemia (1: p = 0.025, 2: p = 0.025), while for subgroup 3, there was no significant difference between both methods used (3: p = 0.054).

Conclusion: Overall, POCT and CL values were highly correlated, however, both methods also yielded relevant differences concerning diagnosis of anemia. Hemoglobin concentrations determined with SLS CL are more likely to yield a diagnosis of anemia compared to Hb determined with POCT co-oximetry at UKB.

P-07-01

Short chain fatty acids and bile acids in human faeces are associated with the intestinal cholesterol conversion status

Silke Matysik

Regensburg, Germany

Silke Matysik[1*], Sabrina Krautbauer[1], Gerhard Liebisch[1], Hans-Frieder Schött[2]

[1]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany; [2]Singapore Lipidomics Incubator (SLING), Life Sciences Institute, National University of Singapore, Singapore

Introduction: The analysis of human faecal metabolites can provide an insight into metabolic interactions between gut microbiota and host organism. The creation of metabolic profiles in faeces has received little attention until now and reference values, especially in the context of dietary and therapeutic interventions, are missing. The aim of the present study is to give concomitant concentration ranges of faecal sterol species, bile acids and short chain fatty acids based on a large cohort.

Methods: Sterol species, bile acids and short chain fatty acids in human faeces from 165 study participants were quantified by LC-MS/MS. For standardization, we refered all values to dry weight of faeces.

Results and Discussion: Based on the individual intestinal sterol conversion we classified participants into low and high converters according to their coprostanol/cholesterol ratio. Based on a large number of study participants we give a general quantitative overview of several metabolites in human faeces that can be used as reference values. The intestinal cholesterol conversion is a distinctive feature to evaluate SCFA and bile acid concentrations. Patient stratification into high or low sterol converter groups is associated with significant differences in faecal metabolites with biological activities. Such stratification should then allow assessing faecal metabolites better before therapeutic interventions. Low converters excrete significantly more straight chain fatty acids and bile acids than high converters. 5th, 95th percentile and median of bile acids and short chain fatty acids were calculated for both groups.
The strength of our calculation is that our data base on i) a large cohort, ii) an uncontrolled diet which should reflect the behaviour of the normal population and iii) a comprehensive data set from various countries in Europe.

P-07-02

Preanalytical considerations for clinical lipidomics

Qingqing Wang[1]; Miriam Hoene[2]; Chunxiu Hu[1]; Andreas Peter[2]; Guowang Xu[1]; Rainer Lehmann[2]

[1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian 116023, China, CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian, China; [2]University Hospital Tübingen, Institute for Clinical Chemistry and Pathobiochemistry, Tübingen, Germany

Introduction: The interest in clinical lipidomics increased continuously during recent years. Most commonly big cohort studies are performed with plasma or serum biobank samples collected years ago. Those blood samples were transported, handled and processed at that time according to various preanalytical protocols or standard operating procedures. In view of the fact that clinical lipidomics cover lipid species of various stabilities we investigated alterations of the lipidome in whole blood after drawing based on pre-analytical variabilities. This is the first report performed in a considerable number of samples from different individuals.

Methods: More than 400 lipid species of 14 classes were profiled in > 800 EDTA plasma samples by UHPLC-MS. Before plasma separation whole blood samples were exposed to various conditions (three different temperatures (4°C, 21°C, 30°C) and six time-intervals from 30 min up to 24h). These lipidomes were compared to lipidomes of at once after blood collection prepared plasma samples. Significant alteration of a lipid was defined as >10% changes in the lipid level (p < 0.05, FDR < 0.05).

Results: Summer time conditions, meaning sample handling or transportation at 30°C, led within 60 min to significant changes in > 6% of lipid species. Most instable lipid classes are (O-acyl)-hydroxy fatty acids (FA), free FA, and LPCs. In at once cooled blood samples only 0.7% of all lipid levels were altered after 4h and transportation for 24h is possible (< 6% changes in lipid levels at 4°C). Even at 30°C for 24h several lipid classes were stable. Detailed information for distinct lipid classes and species will be provided.

Conclusion: In clinical lipidomics a time span of 4h at 4°C between blood drawing and separation of plasma for transportation and handling is acceptable. We detected a high variability between and within distinct lipids and classes and recommend therefore careful assessment of the sample history before performing high resolution clinical lipidomics investigations.

P-07-03

High-Throughput Sample Preparation in NMR-Spectroscopy

Marc Fenzlaff[1]; Ann-Kristin Henning[1]; Kathrin Budde[1]; Friederike Gauß[1]; Daniel Rosenkranz[2]; Astrid Petersmann[2]; Matthias Nauck[1]

[1]University Medicine Greifswald, Institute of Clinical Chemistry und Laboratory Medicine, Greifswald, Germany; [2]University Medicine Oldenburg, Institute of Clinical Chemistry und Laboratory Medicine, Oldenburg, Germany

Background and objectives: Sample preparation in nuclear magnetic resonance (NMR)-spectroscopy can be time-consuming, especially if a huge amount of samples should be measured (e.g. in epidemiological studies). Furthermore, the inter-observer variability has an impact on the quality of the prepared samples and consequently on the spectra quality. This study aimed to compare the manual and the automated sample preparation for NMR measurements in terms of time, reference concentration and congruence of the spectra.

Study design and methods: To achieve comparable results, we used a plasma pool for both manual and automated sample preparation. Manual sample preparation consists of mixing 300 μL of phosphate buffer [prepared with D2O and contained sodium 3-trimethylsilyl-(2,2,3,3-D4)-1-propionate (TSP) as reference, (pH 7.4)] with 300 μL plasma directly in a 5 mm NMR-tube. Automated sample preparation was realised by a pipetting robot (Tecan Freedom Evo) which is equipped with four needles. Spectra were recorded on Bruker Avance Neo 600 MHz NMR-spectrometer at 310 K as a standard one-dimensional 1H-NMR pulse sequence including water suppression. Whenever four samples were completely prepared with both preparation methods, the time of sample preparation was monitored and compared. The TSP signals of both preparation methods were overlapped to have a visual impression on spectra quality. In addition to the visual evaluation, the means of the TSP concentrations were calculated and compared to the reference concentration.

Results: Manual pipetting of 60 plasma samples took about 49 minutes compared to the automated pipetting which only took about 37 minutes in average. Thus, automated pipetting resulted in an average time saving of 25 %. In addition, the hands-on time dropped from 49 to 37 minutes. With regard to the quality of the spectra, we observed that the overlapped TSP-signals in both methods were almost congruent. The measured TSP-concentrations of both sample preparations were nearly identical after measurement of 16 plasma samples (mean manual preparation = 35.10 mmol/L, relative standard deviation RSD = 0.54 %; mean automatic preparation = 35.05 mmol/L, RSD = 0.79 %). The reference value of TSP is 35.90 mmol/L.

Conclusion: The present study has shown that high-throughput sample preparation provides a convenient tool in NMR-spectroscopy. It minimizes the time-consuming work for the staff and affords a comparable spectra quality compared to the manual preparation. Therefore, an automated sample preparation in NMR-spectroscopy should be considered, especially for the analysis of huge amounts of samples.

P-07-04

Cognitive impairment coincides with increased circulating levels of endocannabinoids.

Alexander Gaudl; Uta Ceglarek

Universitätsklinikum Leipzig, Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Leipzig, Germany

Introduction: Impairment of cognitive functioning is a concomitant in most neurodegenerative disorders (NDD). Still, their cause and genesis remain mostly undefined. Several mechanisms depending on cell type, stage of development, and brain region have been proposed. The endocannabinoid system (ECS), including at least the cannabinoid receptors type 1 and type 2 as well as endogenous cannabinoids (eCB) and enzymes facilitating their synthesis and degradation, has been shown to contribute to the development of NDDs in animal experiments. The aim of this study was to associate the eCBs 2-arachidonoyl glycerol (2-AG), arachidonoyl ethanolamide (AEA), oleoyl ethanolamide (OEA), and palmitoyl ethanolamide (PEA) with cognitive functioning in a case/control cohort within the LIFE Adult study.

Methods: An LC-MS/MS method was established to simultaneously quantify 2-AG, AEA, OEA, and PEA in human plasma within a total run time of 10 minutes. From the LIFE Adult study 500 probands were selected by the inclusion criteria of an age $\geq$ 60 years and the participation in a neuro-psychological test battery to address cognitive performance in a surrounding of Alzheimer's disease or dementia. Hereby, the SISCO score is determined, which derives from summation of distinct items from the unit „Strukturiertes Interview für die Diagnose einer Demenz vom Alzheimer-Typ, der Multiinfarkt- (oder vaskulären) Demenz und Demenzen anderer Ätiologie nach DSM-III-R, DSM-IV und ICD-10" (SIDAM), and caps at 55 points. Subcohorts depicting high cognitive performance (SISCO $\geq$ 53) as well as slight cognitive impairment (SISCO $\leq$ 45) were used for association analysis.

Results: Statistically significant concentration differences between the two subcohorts were found for 2 AG, AEA, and OEA. Influencing factors were found to be sex (for AEA, OEA) and BMI (for 2 AG, AEA). After adjusting for these confounders AEA and OEA remained significantly different and showed both a negative association with cognitive performance (odds ratio per standard deviation [OR] 1.33, 95 % CI 1,17-1,46), OEA (OR 1.23, 95 % CI 1.06-1.37).

Conclusion: The negative association of AEA with cognitive performance opposes to former results from nimal experiments, which showed for example increased memory performance during increased half-life of AEA in the synaptic cleft. This might indicate an inverse relation of total concentration and residence time and, therefore, a much more complex role of eCBs in cognitive functioning.

P-07-05

Determination of internal circadian time with metabolites

Sophie Neugebauer[1]; Wolfgang Schmidt-Heck[2]; Mirko Peitzsch[3]; Gianni Panagiotou[2]; Achim Kramer[4]; Michael Kiehntopf[1]

[1]Jena University Hospital, Department of Clinical Chemistry and Laboratory Diagnostics, Jena, Germany; [2]Hans-Knoell-Institute, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany; [3]University Hospital and Medical Faculty Carl Gustav Carus, Technical University Dresden, Institute of Clinical Chemistry and Laboratory Medicine, Dresden, Germany; [4]Charité Universitätsmedizin Berlin, Laboratory of Chronobiology, Institute for Medical immunology, Berlin, Germany

Aims: The circadian clock is a fundamental biological program that coordinates 24-hour rhythms in physiology, metabolism, and behavior, and has a major impact on health. The benefits of therapy adapted to the time of day (chronotherapy) have been demonstrated in many studies. Since internal circadian time is different for each individual, the basis of any personalized chronotherapy is to determine the individual chronotype. In this regard, internal time is not a stable characteristic, but is influenced by several factors (e.g., age, gender, ambient light, season). Currently, a simple diagnostic tool for determining individual internal circadian time is lacking. In this project, mass spectrometric methods will be used to determine selected small molecules and metabolites to establish a chronotool that can be used to determine internal circadian time.

Materials and Methods: Serum samples from eleven healthy men were obtained from the 4-day laboratory BOTI (BOdy TIme) study, which was conducted at Charité Universitätsmedizin Berlin. The study consisted of an adaption night, a baseline night, a 40-hour episode of sleep deprivation and an 8-hour recovery sleep episode. Before the baseline night current gold standard for internal circadian time, the dim-light melatonin onset (DMLO), was determined in saliva samples. The sleep deprivation period was carried out under constant routine conditions. The concentration of up to 630 metabolites from 26 biochemical classes (e.g., acylcarnitines, amino acids, bile acids, ceramides, cholesteryl esters, lipids) were measured using LC MS/MS. To create and evaluate predictive models of time-based metabolomics data four different methods of machine learning (Random Forest, Cubist, ZeitZeiger, least absolute shrinkage and selection operator (LASSO)) were applied.

Results: After data processing concentrations of 494 out of 630 metabolites were used for building a model for determination of the internal time. The predictor determination was performed with two different predicted variables (external and internal time) and four metabolite data formats (1-sample: single measurement in the time series or 2-sample: ratio of two measurements 6 hours apart in the time series and scaled or scaled and denoised data). All types of predictors performed comparably within one method of machine learning: Random Forest, Cubist, ZeitZeiger and LASSO. Comparing the different machine learning methods LASSO and LASSO with maximum 10 features showed the best performance with 77.27-93.51% and 67.53-90.26% of predictions showing an error ≤ 2 hours, respectively.

Conclusion: The study showed a highly accurate model to estimate the internal circadian time from a single blood sample. Only a few metabolite concentrations are needed, and the model is as comparable with the current gold standard. Thus, the described metabolite model provides an accurate tool for personalization of health care according to the patient's circadian clock.

P-07-06

Sterol characterization of lipid rafts by targeted mass spectrometry

Ilijana Begcevic Brkovic[1]; Madlen Reinicke[1]; Soroth Chey[1]; Ingo Bechmann[2]; Uta Ceglarek[1]

[1]Leipzig University, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany; [2]Leipzig University, Institute of Anatomy, Leipzig, Germany

Introduction: Cholesterol is an essential component of the mammalian plasma membrane and is highly concentrated in specialized microdomains of the plasma membrane termed as lipid rafts. Non-cholesterol sterols such as lanosterol (LA), desmosterol (DE) and 7-dehydrocholesterol (7-DHC), and nutritional derived plant sterols such as campesterol (CA), sitosterol (SI) and stigmasterol (ST) are structurally and functionally similar to cholesterol (CH) and are therefore incorporated in cell membranes, too. There is some evidence that membrane fluidity and signaling may be affected by the non-cholesterol content. The aim of this study was to develop targeted liquid chromatography mass spectrometry (LC-MS/MS) assay for analysis of sterol compounds in lipid rafts of microglia cell membranes.

Methods: Human EDTA-plasma (pooled and individual) was used for method development and validation purpose. Samples were prepared according to the established protocol, involving precipitation with methanol/isopropanol (50/50, v/v) solution, containing relevant internal standards. Following, samples were separated and analyzed over the 14-min LC gradient, utilizing a Shimadzu Nexera XR HPLC system (Duisburg, Germany), online coupled to a QTRAP 6500 (Sciex, Framingham, MA, USA) mass spectrometer. Lipid rafts were isolated from mouse microglia SIM A9 cells applying the OptiPrep™ discontinuous density gradient with 20-hour ultracentrifugation. Extracted lipids were analyzed with developed MS-assay, while lipid rafts marker flotillin-1 was determined by Western blot.

Results: All sterol compounds had good linear response with R2 ≥ 0.9982. The estimated LLOQs were: 0.05 mg/L (CA, SI and LA), 0.1 mg/L (BR and ST) and 2.5 mg/L (CH). Recovery was in the range of ± 15% and within-day/between-day precision of native and spiked plasma was below 10% and 7%, respectively. Plasma sterols were stable at 10 °C for at least 48 hours and were resistant to repeated freeze and thaw cycles. Method comparison between LC-MS/MS and gas chromatography-MS method showed good agreement (Spearman correlation r=0.8912, p < 0.001). Similar abundance profile was observed for sterols and flotili-1 in the isolated lipid rafts fractions. DE and LA had the highest concentration in the lipid rafts, followed by the SI, ST and CA.

Conclusion: Overall, this study demonstrates successful development, validation and implementation of the quantitative, multiplex LC-MS/MS assay for sterol analysis. The method has wide application e.g. in human plasma, lipid rafts, but also other biological samples, using simple preparation protocol and small sample volumes (10 μL). Future work will be focused to reveal functional importance of sterol compounds in the lipid rafts fractions.

P-07-07

Targeting ABCC6 in Mesenchymal Stem Cells: Impairment of Differentiated Adipocyte Lipid Homeostasis

Ricarda Plümers; Michel Robin Osterhage; Christopher Lindenkamp; Isabel Faust-Hinse; Cornelius Knabbe; Doris Hendig

Herz- und Diabeteszentrum NRW, Institut für Laboratorium- und Transfusionsmedizin, Bad Oeynhausen, Germany

Introduction: Ectopic calcification of connective tissue, especially elastic fibers, is the main characteristic of pseudoxanthoma elasticum (PXE, OMIM 264800), a rare genetic multisystem disorder. Mutations in ABCC6, an ATP-binding cassette (ABC) transporter with so far unknown function were found to cause PXE. The lipid transporting properties of a variety of ABC transporters as well as metabolic studies suggest an involvement of ABCC6 in lipid homeostasis. To bring light onto the participation of ABCC6 in adipocyte metabolism, our aim was to generate an ABCC6 knockout by using genome editing in human mesenchymal stem cells (hMSCs) and perform adipogenic differentiation experiments with this cell line.

Methods: Successful adipogenic differentiation was confirmed via fluorescent staining for lipids with Bodipy and gene expression analysis via quantitative real-time PCR (qRT-PCR) of adipocyte master regulator PPARg. ABCC6 gene expression was evaluated during differentiation. An ABCC6 deficient cell culture model was generated using clustered regulatory interspaced short palindromic repeat (CRISPR-Cas9) system. Adipocyte lipid homeostasis was analyzed via triglyceride measurement in cell culture supernants and mRNA expression of low density lipoprotein receptor (LDLR), lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL; also known as palatin-like phospholipase domain containing protein 2, PNPLA2), fatty acid elongase 3 (ELOVL3) and ABC transporter C 1 (ABCA1).

Results: Induction of adipogenic differentiation goes along with significantly elevated ABCC6 gene expression reaching highest level in mature adipocytes. To further clarify the role of ABCC6 in lipid homeostasis, CRISPR-Cas9 mediated ABCC6 knockout was performed and resulted in significantly reduced ABCC6 mRNA expression. ABCC6 deficiency in MSCs did not disturb lipid droplet formation and did not influence gene expression of differentiation markers of adipogenesis but resulted in decreased triglyceride content in cell culture supernants. Results of gene expression analysis in mature adipocytes revealed diminished gene expression levels of LDLR, LPL, ATGL, ELOVL3 and ABCA1 indicating impaired lipid uptake, lipolysis, and cholesterol efflux.

Conclusion: Although ABCC6-deficiency does not disturb adipogenic differentiation, lipid homeostasis in mature adipocytes in dysregulated. Quantifications of triglycerides in cell culture supernants and gene expression analysis indicate an impaired lipolysis and lipid trafficking in adipocytes.

P-07-08

Evaluating the mRNA-Expression of ABCC6 in human mesenchymal stem cells during induced oxidative stress

Michel Robin Osterhage; Ricarda Plümers; Christopher Lindenkamp; Isabel Faust-Hinse; Cornelius Knabbe; Doris Hendig

Herz- und Diabeteszentrum NRW, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

Background: The autosomal-recessive disorder Pseudoxanthoma elasticum (PXE, OMIM #264800), characterized by calcification and fragmentation of elastic fibres in the skin, retina and vessel walls, can be caused by mutations in the ABCC6 gene. This gene encodes for ATP-Binding Cassette subfamily C member 6 (ABCC6), a transporter mainly localized in die basolateral membrane of hepatocytes and kidney cells.

It has been shown that fibroblasts from PXE patients exhibit a senescence-like phenotype with elevated β-galactosidase activity and increased mRNA expression of the cell cycle inhibitor p21 and interleukin (IL) 6. Furthermore, signs of oxidative stress have been reported in the serum of PXE patients and in PXE fibroblasts. Due to the possible role of p21 in the aging process of stem cells, followed by a loss of regenerative potential, human mesenchymal stem cells (hMSCs) were chosen for these investigations.

Methods: To induce cellular aging, hMSCs were incubated with 1 mM H2O2 for 1 h following 72 h of incubation. Removal of apoptotic cells was performed by reseeding the hMSCs in a density of 8.000 cells/cm2. Senescence was validated by evaluating the mRNA expression of senescence markers using qRT-PCR, measuring the β-galactosidase activity and immunofluorescence staining of p21. In addition, the mRNA expression of ABCC6 was determined by qRT-PCR.

Results: The β-galactosidase activity of the hMSCs increased by 50% following treatment with H2O2 with elevated mRNA expression of p21 and senescence-associated cytokines IL1β and IL8 further confirming the senescent phenotype. The immunostaining of p21 revealed an increase in the portion of p21-positive cells to 100%. The mRNA expression of ABCC6 increased up to 10-fold.

Conclusion: The elevated mRNA expression of ABCC6 in hMSCs following treatment with H2O2 indicates an association of ABCC6 with the process of cellular senescence and oxidative stress. This pathomechanistic link should be further investigated by knockout of ABCC6 in hMSCs and evaluation of markers of senescence and oxidative stress.

P-07-09

Suppression of Human Xylosyltransferase I in H2O2-Induced Acute Senescent Myofibroblasts

Vanessa Schmidt; Justus Maximilian Ohmes; Thanh-Diep Ly; Bastian Fischer; Anika Kleine; Cornelius Knabbe; Isabel Faust-Hinse

Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklink der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

Introduction: Fibrosis represents a dysregulated wound healing process in which extracellular matrix (ECM)-producing myofibroblasts, after completion of the repair process, do not induce apoptosis or cellular senescence but persist in the tissue. Cellular senescence of myofibroblasts during the physiologic wound healing process is referred to as acute senescence and serves to protect against fibrosis. Cellular senescence, by definition, is an irreversible form of cell cycle arrest preventing damaged cells from undergoing proliferation and precluding potential neoplastic transformation. The xylosyltransferase isoform XT-I represents a biomarker of myofibroblast differentiation. The isoforms XT-I and XT-II catalyze the initial rate-limiting step of proteoglycan (PG) biosynthesis by transferring xylose from UDP-xylose to specific serine residues of the PG core protein. Known mediators of XT-I regulation in context to fibrotic processes are transforming growth factor β1 (TGF-β1), activin A or interleukin 1β. Although the regulation of XT- I in profibrotic processes is mainly understood, little is known about the XT-I regulation during the transition of myofibroblasts into an acute senescent cell state. The aim of this study was to determine whether XT-I is regulated during this transition and, consequently, has an impact on ECM remodeling at the end of the physiological wound healing process.

Methods: To investigate XT-I regulation in acute senescent myofibroblasts, primary normal human dermal fibroblasts (NHDF) were treated with H2O2 and cultured in a myofibroblast cell culture model. XT-I regulation was examined at gene, protein and enzyme activity levels by quantitative real-time PCR, western blot analysis and a XT-I selective mass spectrometric activity assay. The induction of an acute senescence state was verified by a senescence-associated (SA) β-galactosidase activity assay and the determination of p21 and p16 protein expressions by immunofluorescence microscopy.

Results: Treatment of NHDF with H2O2 significantly reduced XYLT1 and XYLT2 mRNA expression in comparison to control. Based on the results of the mass spectrometry activity assay, the significantly decreased XT protein expression determined by western blot, was assigned to the XT-I isoform. Characterization of the SA secretory phenotype revealed significantly increased gene expressions of interleukin 1β and matrix metalloproteinase 1. In contrast, collagen I, fibronectin and decorin expressions were significantly reduced. However, on TGF-β1 gene expression senescence induction had no physiologically relevant effect.

Conclusion: In summary, our study demonstrated an anti-fibrotic effect in acute senescent myofibroblasts leading to a suppressed XYLT1 and XT-I expression. It can be concluded that XT-I plays an important role in the switch between physiological and pathological wound healing process. Nevertheless, further studies are needed to elucidate the underlying signaling pathways.

P-07-10

Impact of manufacturer-dependent differences on in vitro specific IgE results in low concentrated serum samples

Nathalie Wojtalewicz[1]; Laura Vierbaum[1]; Kathrin Kabrodt[2]; Ingo Schellenberg[3]

[1]INSTAND e.V., wiss. Mitarbeiter, Düsseldorf, Germany; [2]Hochschule Anhalt, Institute of Bioanalytical Science, Bernburg, Germany; [3]INSTAND e.V., Vorstand, Düsseldorf, Germany

Aims: The in vitro detection of specific IgE (sIgE) against different allergen sources in serum is a widely used method to identify possible sensitizations to this source which could manifest in allergic disease. In the past, INSTAND repeatedly reported huge manufacturer-dependent differences for important allergen sources like hymenopteran venoms, food allergens and inhalant allergens. The aim of this study was the evaluation of the current status quo of manufacturer-dependent differences in the in vitro detection of sIgE for allergy diagnostics observed in external quality assessment (EQA) schemes.

Materials and Methods: We evaluated, manufacturer-dependent, the semiquantitative EQA results for sIgE for the allergen sources European house dust mite, cow's milk protein and wasp venom obtained between 2018 and 2022. For this analyzes, samples with low sIgE concentrations were chosen, especially when at least one manufacturer collective showed sIgE levels around the cut-off value of 0.35 kU/L. We analyzed the differences in mean for the three biggest manufacturer collectives, including 95 % confidence intervals.

Results: For all reviewed allergen sources, we observed samples where at least one manufacturer collective reported a negative result (< 0.35 kU/L), while others reported a clear positive result (> 0.35 kU/L). The differences were higher in case of house dust mite and cow's milk protein, while the results for wasp venom show a trend to harmonization. In case of house dust mite allergens, the three biggest manufacturer collectives showed mean-differences up to 3.8-fold.

Discussion (Conclusion): While the manufacturer-dependent differences in low concentrated samples have declined for the allergen source wasp venom, they diverge in case of the allergen sources house dust mites and cow's milk protein. Unfortunately, there is no positive trend of harmonization for both allergens, for these differences have been observed since 1995 and were reconfirmed in 2017. As currently lyophylized sample material is used for this EQA, the introduction of liquid samples from individual donors in future EQA surveys is planned with the intention to eliminate the possibility of matrix effects influencing the EQA results. Without a proper harmonization, clinicians are dependent on a thorough clinical anamnesis to accompany the in vitro diagnostics, especially when a positive sIgE value is close to the cut-off value of 0.35 kU/L.

P-07-11

Maintaining automatic 24/7 post-requests and proper workflows on the laboratory automation system in the event of recurring delivery shortages for separation tubes

Bozena Zechmeister; Andreas Fischer; Gry Helene Dihazi

Universitätsmedizin Göttingen, Interdisziplinäres UMG Labor, Göttingen, Germany

Aims: Due to recently recurring delivery bottlenecks for laboratory mateials (lithium heparin plasma separation tubes) and the impact on laboratory results of additional requests in an automated 24-hour operation, the effects of sample storage of tubes with and without separation material were examined.

Materials and Methods: Blood was collected from 20 healthy volunteers using gel (routine) and non-gel (spare) plasma separation tubes. A total of 23 clinical-chemical parameters were determined at 0 h, 4 h, 8 h, 24 h, 48 h and 72 h. The samples were processed on the laboratory automation system (LAS), centrifuged but not separated by a filter. Between the measurements, the samples were automatically stored at 4°C in the connected archive. Statistical differences were evaluated using a generalized estimating equation regression model.

Results: For most parameters, there was no significant change in the measurement results within the tested time, regardless of the collection tube. Significant differences in the comparison of the measurements over time could be detected for some parameters, but there were no significant differences in the results in the first 8 h and there were hardly any differences between the different tubes. Parameters known to be sensitive to interference from erythrocyte or platelet contamination were stable for at least 8 hours after the first measurement.

Discussion (Conclusion): These data indicate that sample storage in the primary tube, both with and without the plasma separation tube, might cause relevant changes for certain parameters. However, there was little difference between using tubes with or without plasma separation. To maintain an effective workflow in the routine laboratory and to ensure the possibility of additional requests within 24 hours in the event of supply shortages, the use of tubes without separation material is sufficient for most parameters.

P-07-12 / FV-03

Establishing reference intervals for NMR spectra in human plasma

Daniel Rosenkranz[1]; Kathrin Budde[2]; Ann-Kristin Henning[2]; Gunnar Brandhorst[1]; Sarah Schäfer[1]; Friederike Gauß[2]; Nele Friedrich[2]; Astrid Petersmann[1]; Matthias Nauck[2]

[1]Carl-von-Ossietzky University, Institute for clinical chemistry and laboratory medicine, Oldenburg, Germany; [2]University Medicine Greifswald, Institute for clinical chemistry and laboratory medicine, Greifswald, Germany

Background: Reference intervals are an important tool for the clinical interpretation of laboratory results. The usual approach for establishing reference intervals implies the calculation of designated percentiles after the measurement of a single measurand in a defined "healthy" reference population. For multi-measurand determinations within one analytical run, e.g. from NMR spectra, the calculation of reference intervals becomes more complex and is not well established.

Material and Methods: In this work, 600 MHz NMR spectra from approximately 400 human plasma samples out of the study of health in Pomerania (SHIP) serve as the data base for the calculation of a reference spectrum. The NMR-measurement provides 4000 measuring points per spectrum, which refers to a spectral resolution of 0.0025 ppm (1.5 Hz). Percentiles of intensities are calculated at each measuring point and the reference spectrum is depicted as color-coded intensities. At this time point, no disease-specific exclusions were applied, so that the results are preliminary.

Results and Discussion: The established reference spectrum represents the distribution of the intensity of a given ppm across a large population. The intensity percentiles at a given ppm are color-coded, so that we are able to describe regions at the spectra which are uniform up to regions, which are very variable between participants.

Conclusion: According to the high standardization and comparability of the NMR spectra in human plasma samples, reference spectra are needed, to improve the medical value of this technology in the health care setting. Here we show a first approach to calculate a reference spectrum out of a large epidemiological study for plasma, measured by NMR spectroscopy.

P-07-13 / PV-01

CRISPR/Cas9 induced homozygous Xylosyltransferase-II deficiency in neonatal human dermal fibroblasts results in altered proteoglycanbiosynthesis

Anika Kleine; Phil Lange; Vanessa Schmidt; Thanh-Diep Ly; Isabel Faust-Hinse; Cornelius Knabbe; Bastian Fischer

Herz- und Diabeteszentrum Nordrhein-Westfalen, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

Background: The xylosyltransferase isoforms XT-I and XT-II catalyse the initial step of the proteoglycan (PG) tetra-saccharide linker region to bind glycosaminoglycans (GAG) to the PG-core protein. Through this essential step, the XT isoforms crucially contribute to the homeostasis of ECM remodelling.
Spondylo-ocular syndrome is a rare autosomal recessive inherited disease with features such as skeletal fragility, short stature, developmental delay as well as cardiac and ocular manifestations. Various XYLT2 mutations have been identified to be causative concerning disease manifestation. Due to the low availability of patient cells, the aim of this work was to generate an isolated CRISPR/Cas9-mediated XYLT2 knockout in neonatal human dermal fibroblasts to analyse the effects of XT-II deficiency on the cells. In addition, this approach could help to better understand the hitherto unexplained relevance of two different XT-isoforms in all higher organisms.

Methods: CRISPR/Cas9 based XYLT2 knockout: normal human dermal fibroblasts (NHDF) were revers transfected with 10 nM of a ribonucleoprotein (RNP)-complex targeting the XYLT2 gene. After 24 h the cells were sorted using FACS technology, genomic DNA was isolated and afterwards analysed using sanger sequencing.
Analysis of relative mRNA expression and XT activity: relative mRNA expression levels were determined using quantitative real-time PCR. Intra- (cell culture lysates) and extracellular (cell culture supernatants) XT-activity was measured using an in-house UPLC-MS-assay by specifically detecting the xylosylated peptide.

Migration assay: An artificial scratch within a confluent monolayer of control and XYLT2-deficient fibroblasts was created. The migration of cells into the area was observed for 96 h.

Senescence assay: NHDF and XYLT2-deficient cells were cultivated for 72 h and subsequently harvested. The senescence-associated-β-galactosidase-activity was observed photometrically.

Results: CRISPR/Cas9-based genome editing was successful due to a single thymine base deletion, resulting in a shortened amino acid sequence (p.Val95Glyfs*3). Compared to controls, XT-II deficient cells showed reduced mRNA expression levels of different glycosyltransferases, as well as a significantly reduced XT activity. In addition, the XYLT2-deficient cells closed the artificial gap more slowly and showed a significantly increased senescence-associated-β-galactosidase activity in comparison to the control fibroblasts.

Conclusion: Using the CRISPR/Cas9 system, a XYLT2-deficient fibroblast culture was successfully obtained. The lack of XT activity also lowers the mRNA expression of the remaining transferases involved in the tetrasaccharide linker synthesis. The migration capacity of the cells is impaired and there is a marked increase in cellular senescence. Our data reveal an impact of XYLT2 expression on the cellular metabolism. Further effects need to be analysed in the future.

P-08-01 / FV-04

RESULTS OF THE EXTERNAL QUALITY ASSESSMENT SCHEME (EQA) FOR ISOLATION AND ANALYSIS OF CIRCULATING TUMOR DNA (ctDNA)

Romy Eichner[1]; Parviz Ahmad-Nejad[2]; Sascha Neumann[3]; Wolf J. Geilenkeuser[3]; Laura Mirbach[1]; Angelika Duda[1]; Verena Haselmann[1]

[1]Universitätsmedizin Mannheim, Institut für Klinische Chemie, Mannheim, Germany; [2]HELIOS Klinikum Wuppertal, Witten/Herdecke University, Institut für Medizinische Labordiagnostik, Wuppertal, Germany; [3]Stiftung für Patho-biochemie und Molekulare Diagnostik (SPMD), Referenzinstitut für Bioanalytik (RfB), Bonn, Germany

Background: Circulating tumor DNA (ctDNA) analysis, commonly referred to as liquid biopsy, has the potential to revolutionize oncologic diagnostics by helping to stratify targeted therapies, serving as a personalized tumor marker for treatment monitoring, and ultimately facilitating the detection of emerging resistance mechanisms. However, several obstacles hamper successful translation into standard care, with the lack of harmonized preanalytical and analytical issues being the most significant concern. This external quality assessment (EQA) scheme aimed to address issues of analytical quality as prerequisite for an urgently needed standardization of laboratory workflows.

Methods: In each EQA schemes offered, three samples containing 2-3 mL EDTA-plasma spiked with fragmented genomic DNA isolated from tumor cell lines were provided for analysis of sequence variations in KRAS p.G12/p.G13, BRAF p.V600E and EGFR p.T790M. The variant allele frequency (VAF) ranged from 0% to 10%. Laboratories were asked to use their routine procedures and report following: 1) time elapsed for processing of samples, 2) storage temperatures, 3) method used for extraction and quantification, 4) genotyping methods and results.

Results: A total of 184 laboratories from 13 European countries participated in these EQAs. Neither the median shipment time to participants of 2.8 days nor the total time elapsed before analysis of 7 days affected the overall diagnostic performance. 73.5% reported to isolate cfDNA manually, and the most commonly used isolation kit was the QIAamp circulating nucleic acid kit (52.9%). On average, 2.6mL plasma were used for cfDNA isolation, with 83.3% of laboratories using buffer for cfDNA elution. Isolated cfDNA was quantified by Qubit in 57.8% of cases, followed by 9.8% using ddPCR and 9.3% using NanoDrop. Importantly, cfDNA equivalent to 640µL plasma was used on average for ctDNA analysis, representing only 21.3% of the maximum possible input. For analysis, ddPCR (45.2%) was used most frequently, followed by qPCR (16.5%), and MassArray (12.1%). Overall, 95/1152 genotypes were determined inaccurately, resulting in an overall error rate of 8.3%. The false-positive rate was 4.2% and increased with time. The false-negative rate was 8.9%, with the error rate increasing with decreasing VAF, e.g., exceeding 30% at a VAF of 0.1%. Noteworthy, the error rate varied significantly depending on the method used, with the lowest error rate observed for ddPCR and BEAMing.

Conclusion: This EQA schemes illustrate the current variability in multiple stages of cfDNA processing and analysis of ctDNA, resulting in an overall error rate of 8.3%. Importantly, the error rate clearly depends on the method used and the VAF of the target gene. Of note, the false-negative rate has improved over time, suggesting that laboratories are using more sensitive techniques, while the false-positive rate is increasing, indicating problems in accurately determining the detection limit.

P-08-02

NMR-based quantification of amino acids – a method comparison

Friederike Gauß[1]; Kathrin Budde[1]; Ann-Kristin Henning[1]; Angelika Hafke[2]; Ralph Krätzner[3]; Matthias Nauck[1]; Astrid Petersmann[4]

[1]Universitätsmedizin Greifswald, Institut für Klinische Chemie und Laboratoriumsmedizin, Greifswald, Germany; [2]Universitätsmedizin Göttingen, Institut für Klinische Chemie, Göttingen, Germany; [3]Universitätsmedizin Göttingen,

Klinik für Kinder- und Jugendmedizin, Göttingen, Germany; [4]Universitätsmedizin Oldenburg, Institut für Klinische Chemie und Laboratoriumsmedizin, Oldenburg, Germany

Aims: Multiple studies show the potential of amino acids (AA) to serve as biomarkers in prognosis and diagnosis of various diseases, e.g. diabetes. 1H-NMR-platforms are increasingly used in metabolic profiling studies due to their inherent advantages like robust and reproducible measurements. Still, NMR methods are not used in clinical settings. In order to evaluate the use of NMR methods for AA measurement in research and routine patient care, we compared levels of AA measured on a 1H-NMR platform with those measured on a well established HPLC platform.

Materials and Methods: Eleven AA were measured in 90 EDTA-plasma samples from patients of the University Medicine Greifswald. Measurements were conducted on a HPLC platform (Biochrom 30+ system, Biochrom, Cambridge, UK) and on a 600 MHz 1H-NMR platform (Bruker AVANCE-III, Bruker Biospin, Ettlingen, Germany). All measurements were performed in duplicates. For evaluation of precision of the two methods, coefficients of variation (CV%) were calculated from duplicates and Passing-Bablok regression analysis was conducted. For the comparison of NMR and HPLC measurements, we determined Pearson correlation coefficients and performed Passing-Bablok regression.

Results: CV based on duplicates were lower on average for HPLC than for NMR measurements: median CV were 2.79% (interquartile range IR: 2.75% − 3.25%) and 10.22% (IR: 6.73% − 13.77%) for HPLC and NMR, respectively. Further, Passing-Bablok analysis revealed no or only small bias between the first and second measurement with very few exceptions for NMR-measured AA. The comparison of NMR and HPLC measured levels showed a median correlation coefficient of r=0.82 (IR 0.76 − 0.91). Highest correlation was found for phenylalanine (r=0.99), tyrosine (r= 0.98), glycine (r= 0.92) and alanine (r = 0.89). In Passing-Bablok regression, no or only very small bias was found for phenylalanine and alanine.

Discussion: In the comparison of AA levels measured on a NMR and a HPLC platform, two AA, namely phenylalanine and alanine, were highly comparable in terms of high correlation and no or only small bias. Thus, NMR methods have the potential to be applied in clinical context. In research context, NMR measurements of AA, which correlate well with HPLC levels, may be used in profiling studies, e.g. in the epidemiological field, to detect significant associations to clinical outcomes despite some bias between the exact AA levels.

Generally, precision of NMR measurements and correlation between HPLC and NMR measured levels were higher for those AA with the highest measured concentration levels. Still, also AA with low concentrations, such as tyrosine, showed a good performance, which indicates, that improvement of quantification algorithms or measurement time may lead to a higher analytical quality of NMR methods.

P-08-03 / FV-05

AI in smart laboratory processes - medicalvalues and smartLIS

Detlef Pechermeyer[1]; Jan Kirchhoff[2]

[1]labdock GmbH, Geschäftsführung, Hamburg, Germany; [2]medicalvalues GmbH, Geschäftsführung, Karlsruhe, Germany

Introduction and Objectives: The power of modern laboratory medicine allows a wide range of analytes and measurements. In the short time available, it is not always possible for the sender to exhaust the complete diagnostic potential and also to consider new, innovative analytes. The focus is on targeted and personalized diagnostics for patients.

We want to show how networking of controlled requisition/CDS in the laboratory order system with processing in the laboratory system and guided validation in the practice can function, where added value is created and content can be managed.

The special feature here is the connection of an AI system with the process in the LIS.

The following 3 areas should be covered:
– Initial request: supporting the physician in selecting correct parameters in an intuitive interface.

– Reporting in the laboratory: support of the laboratory physician in the reporting of findings
– Reporting at the physician's site: information and possibilities for targeted reflex testing

Change management in the laboratory and at the sender will also be addressed.

Results: Using the example of different areas such as endocrinology, coagulation and infectiology, it will be shown how on the one hand
– Medical guidelines
– Requirement in accordance with guidelines
– Formal principles (EBM rules)

can be taken into account in the request, thus preventing or avoiding inquiries and incorrect requests, and on the other hand how the laboratory physician can be supported in the validation of the results.

Discussion and Conclusion: For a sustainable implementation, international standards and interfaces such as FHIR and LOINC were used. This is supported by a modern software architecture, but also by a cooperative approach / mindset among all project participants. The presentation will also critically examine and discuss the medical device requirements.

P-08-04

Evaluation of hemolysis index thresholds in seven routine biochemistry assays

Nicole Hoffmeier[1]; Martin Mühlbauer[2]; Angelika Carl[1]; Gunnar Brandhorst[1]; Astrid Petersmann[1]

[1]Klinikum Oldenburg AöR, Universitätsinstitut für Klinische Chemie und Laboratoriumsmedizin, Oldenburg, Germany; [2]MVZ Medizinisches Labor Oldenburg GmbH, MVZ Medizinisches Labor Oldenburg GmbH, Oldenburg, Germany

Background: In vitro hemolysis in laboratory samples is one of the most frequent preanalytical errors and bears the risk of clinical misinterpretation of test results and consequently false patient treatment. Modern laboratory analysers are able to assess the degree of hemolysis to prevent reporting of impaired results. Manufacturers provide hemolysis index (HI) thresholds for each measurand. High reliability of these thresholds is important for patient safety by two means: 1. too forgiving thresholds may cause erroneous results to be released; 2. too strict thresholds may cause unnecessary suppression of test results which in turn may put the patient at risk as well. Therefore, the aim of this study was to evaluate the reliability of the manufacturer's thresholds in view of patient safety.

Methods: Samples were collected from anonymized residual patient blood (n=15). A dilution series was then prepared with defined degrees of haemolysis from 4 mg/dl up to 167 mg/dl and seven measurands susceptible to haemolysis namely potassium, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), haptoglobin, total and direct bilirubin and high-sensitive troponin T were measured in each dilution on the cobas pro system (Roche Diagnostics, Mannheim, Germany). For each measurand distribution of results was depicted as boxplots. Relative changes in measurement results across the dilution series were compared to various relative limits, for both, increase and decrease of measurement results with increasing hemolysis.

Results: Relative result increase and decrease of the investigated measurands was evaluated for 5, 10, 15, and 20% and compared to different HI levels in the dilutions. A 5% increase or decrease – depending on the measurand - was found at HI levels of 92 for potassium (20), 34 for AST (20), 19 for LDH (15), 577 for haptoglobin (10), 179 for total bilirubin (800), 8 for direct bilirubin (10) and 3305 for troponin T (100), respectively (manufacturer's thresholds are given in parentheses). For some measurands like potassium, troponin T and haptoglobin the effect of hemolysis was found to be considerably smaller compared to the manufacturer's recommendations. For direct and total bilirubin effects of hemolysis were more severe than recommendations of the manufacturer reflected.

Conclusion: Hemolysis levels impacting measuring results deviated considerably from the manufacturer's recommendations. For five of the investigated measurands the given HI appeared too strict, for two measurands too forgiving. Further studies can help to confirm these findings and assess their clinical significance.

P-08-05 / FV-06

Eight months of experiences in newborn screening for sickle cell disease (SCD) and spinal muscular atrophy (SMA)

Friederike Hörster; Ulrike Mütze; Joachim Janda; Rafael Tesorero; Patrik Feyh; Georg F. Hoffmann; Jürgen G. Okun

Universitätsklinikum Heidelberg, Dietmar-Hopp-Stoffwechselzentrum Neugeborenen Screening, Heidelberg, Germany

Introduction: Since October 2021, SCD and SMA are included in the German national newborn screening (NBS) panel. Our center started a 3-months NBS pilot study in July 2021. For SCD screening, blood transfusion and prematurity (< 34 weeks of gestation) have been announced as major diagnostic pitfalls.

Aims: To report first experiences on prevalence, disease confirmation and diagnostic pitfalls.

Materials and Methods: Real-time quantitative PCR (RT-qPCR) investigation of SMA, SCD and TRECs is performed using a common platform to facilitate high-throughput analysis. For SMA screening, the SMN1 gene is used as a target, while beta-actin is used as an internal control for successful amplification. For SCD screening, the common mutation is targeted in RT-PCR. In a second-tier approach for SCD, all samples revealing a suspicious first tier result undergo ESI-MS/MS analysis of characteristic hemoglobin fragments using a commercial kit (SpOtOn Clinical Diagnostics Limited).
Results: 84 769 newborns (26 764 within the pilot phase and 58 005 within the regular screening program) have already been investigated. Due to low beta-actin levels in 24 cases a control card was requested.

SMA: We identified 13 newborns affected by SMA (birth prevalence about 1: 6 500), 12 of them already confirmed by MLPA (multiplex ligation dependent probe amplification) revealing 0 SMN1 copies and 1-4 SMN2 copy numbers, NBS revealed no false positive results.

SCD: We identified 21 newborns with SCD (birth prevalence about 1:4 000): n=15 homozygous Hb S/S, n= 4 compound heterozygotes Hb S/Hb C, n=2 compound heterozygotes HbS/ beta thalassemia major- confirmation was mostly done by molecular genetics. NBS revealed no false positive results. One recall card needed to be requested due to blood transfusion and three recall cards due to gestational age below 34 weeks (total number of newborns < 34 weeks of gestation in the observed period was 3 502, number of transfusions estimated 298).

Conclusion: NBS for SMA and SCD has been successfully established as a Multiplex-qPCR in parallel with the detection of TRECs. In SCD screening, a second-tier approach based on RT-qPCR for the common SCD mutation and subsequent ESI-MS/MS analysis of hemoglobin fragments is feasible and facilitates dealing with the two major pit-falls bloodtransfusion and prematurity.

P-08-06

Evaluation of a new PTS prototype for automatic unloading

Juliane Schöpfel; Friederike Gauß; Matthias Nauck

University Medicine Greifswald, Germany, Institute of Clinical Chemistry and Laboratory Medicine, Greifswald, Germany

Introduction: A new carrier for pneumatic tube systems (PTS) was developed by Aerocom®, which supports the automated unloading of blood samples. This carrier is a further development in a line of various prototypes. It is equipped with eight transparent tubes containing a fixing system for blood sample tubes. Up to 24 blood sample tubes can be transported at the same time in one carrier. There is no need for special packing or unpacking of the transported samples – neither on the ward nor in the laboratory. The carrier has been developed to work together with an automated unloading station. After unloading a direct transfer of the sample tubes into a bulk loader is feasible. In this study we examine the influence of the transport and unloading process using the new carrier on blood sam-ple quality and compare it to courier transport.

Methods: Duplicate blood sample sets (1 EDTA, 1 Lithium Heparin, 1 Citrate) of 20 volunteers were split among the different transportation methods: 1. courier and 2. new PTS carrier. After transport, 51 measurands from clinical chemistry, hematology and coagulation were measured and compared. We determined the median concentration (1st - 3rd quartile) for each measurand for courier and PTS transportation, respectively. In addition, Spearman correlation coefficients and conducted Wil-coxon rank sum tests were determined. Statistical significance was assumed at FDR-adjusted p-value < 0.05.

Results: All investigated measurands showed a good concordance between the two transport methods: median correlation coefficient was 0.95 (interquartile range 0.88 – 0.97). In Wilcoxon rank sum tests no significant differences between courier and PTS transport were found. Especially the hemolysis sensitive measurands potassium, lactate dehydrogenase (LDH) and free hemoglobin (fHb) showed no statistically significant difference, however a trend toward higher levels after PTS transport was observed for fHb and LDH.

Conclusion: Our results show, that the new carrier by Aerocom® is suitable for transporting diagnostic blood samples. Sample transport and sample processing can be carried out faster and measurement re-sults are more rapidly available for patient treatment. However, we see further improvement po-tential for the prototypes. The mechanical components of the carriers are exposed to physical stress and tend to wear out quickly. Also the weight of the new carriers is clearly higher compared to conventional PTS carriers. This may be one reason for the mentioned trend of higher LDH and fHb levels in the new carriers.

P-08-07 / FV-07

Large Performance Differences in Glucose Measurement Devices used in Patient Self-Monitoring

Marla Hiepler[1]; Friederike Gauß[2]; Daniel Rosenkranz[1]; Angelika Carl[1]; Astrid Petersmann[1]

[1]Universitätsmedizin Oldenburg, Institut für klinische Chemie und Laboratoriumsmedizin, Oldenburg, Germany; [2]Universitätsmedizin Greifswald, Institut für klinische Chemie und Laboratoriumsmedizin, Greifswald, Germany

Background: Blood glucose systems for patient self-monitoring are not covered by the guideline of the German Medical Association for the quality assurance of laboratory tests. Analytical quality is assessed as part of the approval process but is not mandatory thereafter. Consequently, patients often do not apply and monitor internal quality controls on a regular basis e.g. like in medical laboratories.

Materials and Methods: In our study we investigate the analytical performance of 13 glucose measurement devices from 7 different manufacturers for patient self-monitoring and compare them with glucose concentrations obtained from a system used in hospital care (Cobas pro, Roche Diagnostics, Mannheim). Two approaches were chosen: a method comparison and an imprecision study. For method comparison, 15 EDTA-whole blood from left over samples were measured on the patient self-monitoring systems. Within 15 min these whole blood samples were centrifuged and the EDTA plasma was used for the determination of the comparison glucose concentration at the Cobas pro platform. Thereafter, the plasma-referenced values of the patient self-monitoring systems were compared to the glucose concentrations from the hospital care platform. For comparison, we calculated correlation coefficients and conducted linear regression analysis.

For determination of imprecision, EDTA plasma pools were prepared from left-over material. The pools encompassed six different glucose concentrations which were measured in duplicates for five consecutive days. Coefficients of variation were calculated for each method.

Results: The values of the investigated patient self-monitoring systems show good agreement with the laboratory method. Correlation coefficients were ≥0.959. The systematic measurement deviation was below 5 % for eight of the systems examined but above 5 % and up to 14% for the remaining five systems. Nine systems for patient self-monitoring were also comparable with the laboratory method in terms of imprecision < 5%, as recommended by the "Kommission für Labordiagnostik in der Diabetologie (KLD)". However, four of the systems had a considerably higher imprecision with a coefficient of variation of up to ± 10 %.

Conclusion: The investigated systems for patient self-monitoring showed variable analytical results, which has to be confirmed in further studies. About one third of the investigated devices for patient self-monitoring showed an unacceptable high imprecision and a clinically relevant systematic measurement deviation. This should be taken into account when such systems are used for patient-self monitoring.

P-08-08

Commutability as a qualitative pre-requirement for a valid evaluation of proficiency testing schemes using the example of steroid hormones

Laura Vierbaum[1]; Nathalie Wojtalewicz[1]; Thomas Keller[2]; Stephan Weber[3]; Ingo Schellenberg[4]; Peter B. Luppa[5]

[1]INSTAND e.V., wissenschaftliche MA, Düsseldorf, Germany; [2]ACOMED statistik, Geschäftsführer, Leipzig, Germany; [3]ACOMED statistik, MA, Leipzig, Germany; [4]INSTAND e.V., Leitung der Geschäftsstelle, Vorstand, Düsseldorf, Germany; [5]Klinikum rechts der Isar der TU München, Institut für Klinische Chemie und Pathobiochemie, München, Germany

Introduction: High quality of proficiency testing (PT) samples is a basic necessity to allow conclusive statements on the quality and homogeneity of medical laboratory analysis. Sample materials for interlaboratory comparison often require artificial processing, e.g. spiking of sufficient analyte concentration or the addition of stabilizing additives. These supplements might hamper the laboratory detection of the analytes thus the commutability of such PT materials must be verified.
In past PT surveys, INSTAND notices manufacturer-dependent differences in the detection of the steroid hormones testosterone (TEST) and progesterone (PROG).

Methods: To determine whether these differences were due to artificial processing of the sample or whether they have test-specific causes, native fresh pooled sera were tested for TEST and PROG in several PT surveys, in addition to the regularly processed INSTAND PT samples. In parallel to the PT sample shipment and data collection, stability testing was performed for the fresh sample material.
As reference method procedures are available for TEST and PROG, the manufacturer-collective results were normalized to the reference method value (RMV). A comparative analysis of the relative differences was performed for any combination of fresh and stabilized samples.

Results: For the fresh serum samples, a total of 390 TEST results were reported in two surveys and 342 PROG results in three surveys. A comparison of the relative differences between the two sample types showed variations in means around 16 % to 27 % for TEST and around 16 % to 58 % for PROG in dependence of the manufacturer. Constant relative differences between the sample types indicate no problems with commutability for individual manufacturer collectives.
Conclusion: The results of this study emphasize that the deviations of the collective showing repeatedly unsatisfactory passing rates in past PTs are not induced by the sample additives but rather due to an insufficient calibration or insufficient specificity of the respective test method. After error analysis in cooperation with a manufacturer, deviations of TEST detection from the RMV have notably decreased. This positive effect could be due to recalibration effort in terms of metrological traceability. Based on this development, we recommend the initiation of error analysis for PROG detection as well.
The knowledge of specimen characteristics is critical to evaluate the causes of observed non-permissible deviations and thus provide the scientific background for initiating appropriate corrective action.

P-08-09

Product problems of In-vitro diagnostics for COVID-19 disease – Analysis of FSN published by the BfArM 2020-2021

Rüdiger Siekmeier; Jürgen Hannig

Pharmazeutisches Institut der Universität Bonn, Drug Regulatory Affairs, Bonn, Germany

Introduction: Directive 98/79/EC and Regulation 2017/746 regulate marketing and market surveillance of In-vitro diagnostics (IVD) in the European Economic Area. In cases of incidents and field safety corrective actions (FSCA) manufacturers have to inform responsible Competent Authority (D: BfArM) and public by field safety notices (FSN). FSN are published by BfArM. In December 2019 a new disease termed 2019-nCoV (now COVID-19) by the World Health Organization (WHO) on Feb. 11th 2020 was firstly described. Until April 26th 2022 at least 511 million infections/6.2 million deaths were caused worldwide. Many tests were developed for disease detection (PCR, RT-PCR, other molecular and antigen (AG) tests (laboratory tests, rapid tests (lateral flow assays, LFA))) for professional/lay users and immunological tests (antibody (AB) tests (laboratory tests, LFA)). Study aim was to analyze FSCA/FSN for COVID-10 tests/analyzers for product problems, resulting risks and type of FSCA.

Materials and Methods: All FSCA/FSN for IVD for COVID-19 diagnostics published by BfArM (http://www.bfarm.de/DE/ Medizinprodukte/riskinfo/kundeninfo/functions/kundeninfo-node.html) until 31.12.2021 were analyzed.

Results: 39 FSCA for COVID-19 IVD were found, i. e. 9.8 % of all FCSA for IVD (2020: 11 (5.9 %), 2021: 28 (13.3 %)). FSCA 2020/2021 affected molecular IVD for virus detection in nose/throat swabs (PCR, RT-PCR, Multiplex-PCR: 4/7, PCR controls: 0/2, PCR software: 2/1, racks for PCR analyzer: 0/1, saliva sampling system: 0/1), IVD for AB detection in blood/ serum (LFA: 2/0, ELISA: 1/0, tests/test specific software/reagent/calibrator for immune analyzer: 1/4, blot 1/0) and IVD for AG detection in swabs/saliva (LFA: 0/9, test for immune analyzer: 0/1, POCT with analyzer: 0/2). FSCA affected (both years, mult. entries) reagents/calibrators/controls (30), software (7), sample materials (1), analyzer racks (1) and analyzers (1). Product failures were (both years, mult. entries) errors in labeling/instructions for use (IFU; 11), production errors (12; e. g. filling, packaging, product release), software errors (7), material errors (5), fluid leaks (3), microbial contamination (1) and temperature error (1) and no information (3). Potential consequences of product failures (both years, mult. entries) were false-positive/false-negative results (19/12), invalid/inconclusive results (8), normal function (6), delayed function (1) and no information (5). Corrective measures (both years, mult. entries were) recalls (32; FSN mandatory), customer recommendations (29; of these with information for control of results: 14), software-upgrade (9), modification of labeling/IFU (5) and modification of production/quality control (4).

Conclusion: FSCA of IVD for COVID-19 diagnostics represent a relevant number of all FSCA. Product failures and corrective measures differ in respect to analytical principles and type of IVD. FSN play an important role for risk reduction in cases of product failure.

P-09-01

Determination of Age- and Sex-specific Reference Intervals for Cholinesterase and Gamma-Glutamyl Transferase Using Real-World Data of the Marienhospital in Stuttgart, Germany: Comparison of Three Different Indirect Methods

Anne Meyer[1]; Robert Müller[1]; Markus Hoffmann[1]; Jürgen Bartz[1]; Matthias Orth[2]

[1]Abbott GmbH, Core Diagnostics, Wiesbaden, Germany; [2]Vinzenz von Paul Kliniken gGmbH, Marienhospital Stuttgart, Instituts für Laboratoriumsmedizin, Stuttgart, Germany

Introduction: Accurate reference intervals are crucial for the correct interpretation of laboratory results. A fast and cost-effective alternative to direct reference interval estimation is provided by indirect methods using routine laboratory data. The objective of this study was to compare reference intervals provided by the manufacturer to estimates calculated with three indirect methods. For this comparison, we used the reference intervals stated in Abbott's instruction for use and real-world data of cholinesterase (CHE) and gamma-glutamyl transferase (GGT) provided by the Marienhospital in Stuttgart.

Methods: Routine laboratory data of CHE and GGT that had been analyzed on the Alinity c platform were used to determine refence intervals. The datasets were cleaned, and data pre-processing steps were applied to decrease the number of presumably diseased subjects in the data. This helps the indirect methods to identify and mathematically model the result distribution of the healthy population. Reference intervals were estimated with a modified Hoffmann method (RefLim), based on a quantile-quantile plot, the Truncated Maximum Likelihood method (TML), and an inverse modeling approach (refineR) for both sexes and for different age groups.

Results: For CHE, all three indirect methods showed a good agreement with comparable results. However, for women, the lower reference limits were consistently higher than the expected values provided by the manufacturer, and for men the same was observed for the upper reference limits.
For GGT, the situation was more complex due to a poor agreement between the three methods and an age-dependent increase of the upper reference limits for both sexes. In general, RefLim tended to estimate wider and refineR narrower reference intervals, while TML was in between. All three methods confirmed the lower reference limits reported by the manufacturer and obtained different age-dependent upper reference limits.

Conclusion: Our study reinforces the call of numerous guidelines and recommendations that reference intervals reported by a manufacturer should be compared with results from the laboratory's own subject population. Using CHE as an example, we showed that three different indirect methods based on completely different statistical procedures yield comparable results. The derived statistical models of the healthy population were similar, which indicates that they reflect the true reference population. The example of GGT, on the other hand, showed that these methods can result in different and possibly false reference limits if the presumably pathological results in a mixed population cannot be correctly identified and separated with the statistical techniques of the indirect methods.

P-09-02

A tool for plausibility checks of reference intervals

Sandra Klawitter[1]; Georg Hoffmann[2]; Stefan Holdenrieder[3]; Tim Kacprowski[4]; Frank Klawonn[5]

[1]Ostfalia University of Applied Sciences, Department of Computer Science, Braunschweig, Germany; [2]Trillium GmbH, Medizinischer Fachverlag, Grafrath, Germany; [3]German Heart Centre, Institute of Clinical Chemistry, München, Germany; [4]Peter L. Reichertz Institute for Medical Informatics of Technical University of Braunschweig and Hanover Medical School, Data Science in Biomedicine, Braunschweig, Germany; [5]Ostfalia University of Applied Sciences / Helmholtz Centre for Infection Research, Biostatistics, Braunschweig, Germany

Background: Laboratory information systems typically contain hundreds or even thousands of reference limits stratified by sex and age. Since under these conditions a manual plausibility check is hardly feasible, we have developed a simple algorithm that facilitates this check. A user-friendly open-source R tool is available as a Shiny application at github.com/SandraKla/Zlog_AdRI.

Methods: Based on the zlog standardization, we can possibly detect critical jumps at the transitions between age groups, regardless of the analytical method or the measuring unit. Its advantage compared to the standard z-value is that means and standard deviations are calculated from the reference limits rather than from the underlying data itself. The purpose of the tool is illustrated by the example of reference intervals of children and adolescents from the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER).

Results: The Shiny application identifies the zlog values, lists them in a colored table format and plots them additionally with the specified reference intervals. The algorithm detected several strong and rapid changes in reference intervals from the neonatal period to puberty. Remarkable jumps with absolute zlog values of more than 5 were seen for 29 out of 192 reference limits (15.1%). This might be attenuated by introducing shorter time periods or mathematical functions of reference limits over age.

Discussion: Age-partitioned reference intervals will remain the standard in laboratory routine for the foreseeable future, and as such, algorithmic approaches like our zlog approach in the presented Shiny application will remain valuable tools for testing their plausibility on a wide scale.

P-09-03

Refining Diagnosis Paths for Medical Diagnosis based on an Augmented Knowledge Graph

Jan Kirchhoff[1]; Florian Stumpe[1]; Niclas Heilig[1]; Lucie Flek[2]; Joan Plepi[2]; Heiko Paulheim[3]

[1]medicalvalues GmbH, Research, Karlsruhe, Germany; [2]Philipps Universität Marburg, Language Technologies, Marburg, Germany; [3]Universität Mannheim, Data and Web Science Group, Mannheim, Germany

Medical diagnosis is the process of making a prediction of the disease a patient is likely to have, given a set of symptoms and observations. This requires extensive expert knowledge, in particular when covering a large variety of diseases. Such knowledge can be coded in a knowledge graph – encompassing diseases, symptoms, and diagnosis paths. Since both the knowledge itself and its encoding can be incomplete, refining the knowledge graph with additional information helps physicians making better predictions. At the same time, for deployment in a hospital, the diagnosis must be explainable and transparent. In this paper, we present an approach using diagnosis paths in a medical knowledge graph. We show that those graphs can be refined using latent representations with RDF2vec, while the final diagnosis is still made in an explainable way. Using both an intrinsic as well as an expert-based evaluation, we show that the embedding-based prediction approach is beneficial for refining the graph with additional valid conditions.

In this paper, we have introduced the medicalvalues knowledge graph, which is used for medical diagnosis using so-called diagnosis paths. Those paths allow for a transparent prediction of a patient's disease. Since the paths are developed manually, they are notoriously incomplete. To tackle this incompleteness, we have introduced an approach which first enriches the medicalvalues knowledge graph into a augmented graph, connecting it to a large dataset of patient records. On that augmented graph, we have trained vector embeddings with RDF2vec, which are used to predict completions.

Both in an internal validation as well as in an expert evaluation, we have shown that the prediction of such extensions is possible with high precision. This methodology of enriching the graph and producing predictions therewith is independent of the task and domain at hand. One key limitation of the approach is the external data used, which is data gathered from intensive care units. Therefore, diseases which do rarely lead to treatments in intensive care are not well covered. In order to augment diagnosis paths for as versatile diseases as possible, other external datasets should be considered as well. Here, the connectors to clinic information systems (CIS) and laboratory information systems (LIS) may also add large-scale instance data in the future, which can also be exploited with the same methodology.

So far, drugs are not represented in the medicalvalues knowledge graph. In the future, we would like to include them, both as a part of a patient's medical history (i.e., existing medication), as well as possible treatments once a diagnosis is made. To that end, we plan to augment the graph with existing datasets on drugs and drug interactions.

Full paper: https://arxiv.org/pdf/2204.13329.pdf

P-09-04

RefLim: A Graphical and Numerical Approach to Reference Interval Verification

Georg Hoffmann[1]; Frank Klawonn[2]

[1]Trillium GmbH Medizinischer Fachverlag, Trillium Akademie, Grafrath, Germany; [2]Helmholtz Centre for Infection Research, Biostatistics, Braunschweig, Germany

Aim: There is a wide range of direct and indirect methods, by which reference limits can be determined [1]. Comparably little attention has been paid to the verification of specified reference intervals from test package inserts and other external sources. The respective guideline procedure [2] is simple, but due to the low number of just 20 values, it suffers from unacceptably wide confidence limits [3], and the criterion that ≥ 90% of the measured values must fall within the specified limits cannot detect excessively wide reference intervals [4].
We aimed to develop an alternative method that is as easy to perform, but works with a moderate number of routine laboratory data and detects all kinds of deviations between expectation and observation.

Methods: We suggest a modification of a previously published iterative boxplot method [5] to create substantially stronger truncation. We derive the quantiles q of the expected central 95% of reference values from the quartiles Q1, Q2, and Q3 according to the following algorithm:

$$\mathrm{var} = \min(Q2 - Q1, Q3 - Q2)$$
$$q(0.025, 0.0975) = Q2 \pm f \cdot \mathrm{var}$$

where f is 2.91 for the first truncation step and 3.08 for all subsequent iterations.

This algorithm is applied to log-transformed values assuming that unknown distributions of reference values can be modelled as lognormal [6]. Outliers beyond the estimated quantiles are removed and the algorithm is iterated, until no more outliers are detected. From the truncated data, zlog values are calculated [7] with reference to the specified limits, and a normal quantile-quantile plot with 39 equidistant quantiles is generated [5]. A linear regression line $y = a + b \cdot x$ is calculated from the central 33 data points, and the deviations of a and b from the expected values 0 and 1, resp., are used as measures of discordance between expectation and observation.

Results: Based on public data (archive.ics.uci.edu/ml/datasets/HCV+data) for routine liver biomarkers (e. g. transaminases, bilirubin) from 540 blood donors and 75 hepatitis C patients, we demonstrate four types of deviations, which can be quantified by our method: the intercept a signifies positive or negative shifts of the given reference interval, whereas the slope b indicates whether that interval is too narrow or too wide. We use empirical thresholds for both constants to visually represent the magnitude of the deviations by traffic light colors. Comparable results are obtained for blood donors and mixed data. Out of 16 manufacturer-derived reference intervals, nine should be rejected (red), and four should at least be given more careful consideration (yellow).

Conclusion: Our method provides an easy and robust way to verify given reference intervals using routine data and intuitive graphics. Our little study makes it likely that a substantial proportion of manufacturer-derived limits needs to be reevaluated using more sophisticated methods for reference interval determination [1].

P-09-05

Artificial Intelligence in laboratory medicine and the responsibility of laboratory information system providers

Julian Gebauer; Anja Prantz; Daniel Lasch; Boris Rolinski

Elblab GmbH, Elblandklinikum Meißen, Meißen, Germany

Aims: Artificial intelligence and machine learning have made substantial advances during the last decade in a broad field of applications ranging from fraud detection over speech recognition to self-driving cars. In healthcare machine learning approaches are state of the art in medical imaging. In laboratory science however applications are uncommon despite the fact that laboratory medicine with its numerical and structured data would be a very suitable field for machine learning. Meaningful but yet unknown correlations are expected to be discovered. First models e.g. for estimation of iron deficiency anemia, liver function parameters or low-yield repetitive laboratory tests are already described in basic research. However, their validation in prospective studies or their application in clinical routine seems to be inhibited by rigid laboratory and clinical information systems. Therefor we evaluated technical possibilities for establishing data science approaches in routine diagnostics.

Methods: The authors evaluated possible work flows for the application of data science and machine learning methods in laboratory routine or prospective study settings.

Results: Interfaces to a plethora of devices and applications are a key feature of every LIS. However most LIS providers tend to distribute these as so-called highly customized software solutions at a high price, even if the connected device is used in virtually every laboratory. One possible and cost-effective workaround could be intercepting interface communication. Another more desirable and efficient approach would be the usage of standardized resources and application programming interfaces like HL7 FIHR.

Conclusions: While granular and inconsistent interfaces resulted from the first steps of digitalization in laboratory medicine, nowadays well-structured and consistent standards for exchanging health care data like HL7 FIHR are available as open source standards. Therefor we demand that laboratory and hospital information systems provider anticipate in the evaluation and validation of innovative data science techniques by catalyzing their development through deployment of open-source interfaces or free application programming interfaces for research use or development and validation of novel data science techniques in laboratory diagnostic routine.

P-09-06

Identification of Putative Non-Substrate-Based XT-I Inhibitors by Small Molecule Library Screening

Thanh-Diep Ly; Anika Kleine; Bastian Fischer; Vanessa Schmidt; Doris Hendig; Joachim Kuhn; Cornelius Knabbe; Isabel Faust-Hinse

Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany

Instruction: Fibroproliferative disorders are characterized by excessive accumulation of extracellular matrix (ECM) components, including collagens and proteoglycans (PGs), which can cause organ dysfunction. The transition of fibroblasts to ECM-synthesizing myofibroblasts in the presence of fibrotic mediators such as transforming growth factor-β1 (TGF-β1) is a key event of this process. The rise in myofibroblast content is marked by an increase in intracellular and extracellular activity of xylosyltransferase-I (XT-I), which is the initial enzyme of PG biosynthesis. Therefore, human XT-I resembles not only a myofibroblast marker but also a serum biomarker for accessing the proteoglycan biosynthesis rate under fibrotic conditions. Accordingly, the inhibition of XT-I would be a promising treatment option for fibrosis reducing ECM accumulation.

Methods: We used a natural product-derived molecular library to identify non-substrate-based inhibitors of the human XT-I by ultra-performance liquid chromatography/electrospray ionization tandem mass spectrometry. We combined this cell-free approach with virtual and molecular biological analyses to confirm and prioritize the inhibitory potential of the identified compounds. The characterization of the compound's efficacy in TGF-β1-mediated XYLT1 transcriptional regulation in primary human dermal fibroblasts, the key cells of ECM remodeling, was investigated by gene expression analyses.

Results: Through this approach, we identified amphotericin B and celastrol as novel non-substrate-based XT-I protein inhibitors. The XT-I inhibitory effect of amphotericin B was mediated by a non-competitive inhibition mode, while that of celastrol was based on a competitive mode of inhibition. Both compounds reduced the XYLT1 mRNA-expression levels and cellular XT-I activity of the primary cells. Furthermore, we demonstrated that the cellular effects mediated by amphotericin B and celastrol were due to inhibitor-induced changes in the TGF-β and microRNA-21 signaling pathway. **Conclusion:** The results of this study provide a promising basis for the optimization and future use of the XT-I inhibitors amphotericin B and celastrol as therapeutic agents for the treatment of fibroproliferative diseases.

P-09-07

Erythropoietin determinations in proficiency tests - variation in laboratory results and method precision

Luisa Toll[1]; Folker Wenzel[1]; Nathalie Wojtalewicz[2]; Laura Vierbaum[2]; Ingo Schellenberg[2]; Mario Thevis[3]

[1]Furtwangen University, Faculty of Medical and Life Sciences, Villingen-Schwenningen, Germany; [2]INSTAND e.V., Society for Promoting Quality Assurance in Medical Laboratories, Düsseldorf, Germany; [3]German Sport University Cologne, Institute of Biochemistry/ Center for Preventive Doping Research, Cologne, Germany

Luisa Toll[1,3,*], Folker Wenzel[1,2], Nathalie Wojtalewicz[2], Laura Vierbaum[2], Ingo Schellenberg[2], Mario Thevis[3]

[1]Faculty of Medical and Life Sciences, Furtwangen University, Villingen Schwenningen, Germany; [2]INSTAND e.V., Society for Promoting Quality Assurance in Medical Laboratories, Düsseldorf, Germany; [3]Institute of Biochemistry/ Center for Preventive Doping Research, German Sport University Cologne, Cologne, Germany;

*Corresponding author. E-mail address: tolu@hs-furtwangen.de

Aims: The aim of the study was to summarize the results of the proficiency tests for Erythropoietin (EPO) determination managed by INSTAND e.V. and to evaluate the measurement quality of the specific methods used by the participating laboratories.

Materials and Methods: In each of a total of nine test series, two samples (sample a and b) with different EPO concentrations were send to the participating laboratories. The median of each round was calculated for sample a and b, respectively. Deviation from the median and the relative error-values was calculated. The criteria of acceptance were set to 20 % around the median. In consideration of the used methodology, the relative deviation of one method around the total mean as well as the mean without the respective method was calculated and the outcomes were contrasted.

Results: The first proficiency test was performed with a number of 10 participants and in the following test series it has increased up to a maximum of 85 participating laboratories in series 8. The average fraction of measurements not meeting the defined criteria of acceptance was 14.3 ± 17.4 % (sample a) and 8.2 ± 6.1 % (sample b). The relative deviation tended to be higher in a lower concentration range. Enzyme linked immunosorbent assay (ELISA) showed significantly higher values compared to chemiluminescent immunoassay (CLIA) and luminescence-enhanced enzyme immunoassay (LEIA) in all cases.

Conclusion: The results appeared to be well harmonized in most cases. However, individual proficiency tests showed higher scatterings of the measured values. This should be observed in future test series.

P-09-08

SARS-CoV-2-PCR pool testing: high efficiency without sensitivity loss

Konstantin Gröber; Katja Graul; Angela Bessert; Thomas Demant

Städtisches Klinikum Dresden, IKL - Institut für Klinische Chemie und Labormedizin, Dresden, Germany

Aims: The COVID-19 pandemic brought enormous challenges for clinical laboratories: demand for timely and precise SARS-CoV-2-PCR results increased rapidly while consumables for sampling and testing were in ever shorter supply. Laboratories had to build up adequate testing capacities in terms of machines and personnel under great time pressure. We implemented a multiple-swab pool testing method without any volume dilution [1] in a hospital lab setting, which increased efficiency dramatically, providing economic SARS-CoV-2-PCR screening without loss of sensitivity.

Materials and Methods: Up to 10 nasopharyngeal swabs were pooled directly into one 3,0 ml UTM (Universal Transport Medium) tube. Additionally, dry nasopharyngeal swabs were collected from each participant of the pool, serving as secondary samples for eventual re-testing. After RNA extraction and RT-qPCR, negative pool test results were reported. For positive pool tests, the corresponding secondary samples were tested to identify the infected individual(s) causing the positive pool test result.

Results: From April 2020 to March 2022 a total of 25.243 pool tests were analyzed, resulting in 770 positive pool samples with 840 participants found SARS-CoV-2-PCR positive with a mean difference between pool and secondary sample of 0,1 Ct values (standard deviation: 3,6 Ct values). In approx. 4 % of positive pool tests no positive participant could be identified. Overall, 7 results per one PCR pool test were produced using this protocol, which reduces the price per result accordingly. Time to result is very short for SARS-CoV-2-PCR negative pools (approx. 3 h) and still acceptable for individual SARS-CoV-2-PCR results for the participants of positive pools (approx. 7 h).

Discussion: Our pool testing method shows good sensitivity for time efficient SARS-CoV-2-PCR screening. The disadvantage of our pool testing method is the necessity to collect secondary samples, which in consequence may be of different sampling quality, as seen in cases where in spite of positive pool tests no positive participant could be identified, probably due to low viral load in early or late infection – SARS-CoV-2-PCR can be (weakly) positive for several weeks to months after infection. The widely used alternative method by Dorfman et al. [2] is based on pooling individual samples in the lab. No secondary samples are required, but sensitivity is reduced markedly due to the dilution effect [3]. Also, sample preparation takes longer because of the additional pooling step, and instrumentation complexity and lab consumable use are higher, e.g. for some PCR clean filter pipette tips in short supply up to today.

P-09-09

SARS-CoV-2 nucleocapsid protein mutations disrupt N gene amplification in frequently employed multiplex RT-PCR assays

Dominique Hilti[1]; Faina Wehrli[1]; Anna Roditscheff[2]; Martin Risch[3]; Lorenz Risch[3]; Adrian Egli[4]; Thomas Bodmer[2]; Nadia Wohlwend[1]

[1]Dr. Risch, Medizinische Mikrobiologie, Buchs SG, Switzerland; [2]Dr. Risch, Medizinische Mikrobiologie, Bern, Switzerland; [3]Dr. Risch, Allgemeine Innere Medizin, Buchs SG, Switzerland; [4]Universitätsspital Basel, Klinische Bakteriologie und Mykologie, Basel, Switzerland

Aims: Absence of SARS-CoV-2 PCR target signals may indicate the presence of new variants. We analysed the increase of N gene dropouts in the TaqPath COVID-19 CE-IVD RT-PCR Kit from Thermo Fisher Scientific (TaqPath) covering the viral ORF1ab, N and S genes. Whole genome sequencing was used to identify potential issues with the N gene PCR efficacy.

Materials and Methods: We analysed nasopharyngeal swabs and saliva samples from symptomatic patients as well as asymptomatic carriers from mass testing programs by RT-PCR with the TaqPath Kit. Complete N gene target failures (NGTF) were defined as missing N gene amplification in the presence of intact amplification of ORF1ab and S genes. Ultimately, whole genome sequencing was performed on matching samples with CT-values < 30 using a GridIon Nanopore sequencer (Oxford Nanopore Technologies - ONT, Oxford, UK).

Results: From 2nd of August 2021 to 1st of May 2022, we observed a total of 218'815 SARS-CoV-2 positive samples, with 168'101 samples matching the defined criteria. Out of these, 194 specimen with NGTF were identified (0.12 %). The samples originated from all regions of Switzerland and were mostly clonally unrelated. In large part, samples with NGTF were collected from October to December 2021 correlating with the infection wave attributable to the Delta variant (B.1.617.2) and its sub-lineages. During this time, a proportional increase of NGTF among all positive samples with a peak frequency of 1 % was observed. Frequency of NGTF swiftly fell once the Omicron lineages BA.1 and BA.2 became prevalent.

Sequencing revealed the nucleotide substitution G28922T (A217S) in 148 cases (88.6 %). 10 samples (6 %) carried the deletion 28913 – 28918 (del214/215), 8 samples (4.8 %) the deletion 28913 – 28915 (del214) and 1 sample (0.6 %) the deletion 28892 – 28930 (del207 – 219). Samples with intact N gene amplification lacked the specified mutations. Lineages included the Delta variant parental lineage B.1.617.2 (n=4), sub-lineages thereof (AY.4 (n=99), AY.4.3 (n=4), AY.33 (n=2), AY.36 (n=9), AY.39 (n=1), AY.43 (n=7), AY.43.3 (n=2), AY.46.6 (n=1), AY.98.1 (n=4), AY.122 (n=1) and AY.125 (n=11)) as well as the Omicron lineage BA.2 (n=1). The lineage of 24 specimen could not be determined.

Conclusion: The substitution A217S, as well as the deletions G214-, G214/215- and del207-219 in SARS-CoV-2 appear to be associated with the NGTF in the TaqPath Kit. These mutations were identified in several sub-lineages of the Delta variant as well as the Omicron BA.2 lineage and were apparently not linked to a particular variant. Importantly, a selection advantage associated with the NGTF could not be identified. The N gene is a common target in RT-PCRs e.g., Cepheid's Xpert Xpress SARS CoV 2 or the VIASURE SARS-CoV-2 (N1 + N2) Real Time PCR Detection Kit for BD MAX. Continuous monitoring and timely re-assessment using whole genome sequencing can thus improve the development of diagnostic tests.

P-09-10 / FV-08

Liquid chromatography tandem mass spectrometry for quantitative analysis of 11 oxygenated androgens in human serum.

Robert Zeidler; Ronald Biemann; Uta Ceglarek; Juergen Kratzsch; Berend Isermann; Alexander Gaudl

Universität Leipzig, Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Leipzig, Germany

Aims: 11-Oxygenated androgens (11-OAs) are discussed as potential biomarkers for adrenal androgen excess such as congenital adrenal hyperplasia (CAH) and polycystic ovary syndrome (PCOS). However, quantification of 11 OAs by LC-MS/MS still relies on extensive sample preparation including liquid-liquid extraction, derivatization and partial long runtimes, which is unsuitable for high-throughput analysis under routine laboratory settings. Aim of this study was to validate a method to quantify 11-OAs using a clinical routine LC-MS/MS method for steroid hormone profiling with minimal sample preparation.

Methods: A DIN ISO 15189 accredited LC-MS/MS method for quantitation of 7 serum steroids in daily routine use was extended by 11-ketoandrostendione (11-KA), 11-ketotestosterone (11-KT), 11β-hydroxytestosterone (11-OHT), 11β-hydroxytestosterone (11-OHT) and validated. In brief, online solid phase extraction was combined with reverse phase liquid chromatography using methanol/H2O/0.2 mM NH4F as mobile phase. Detection was conducted using a Sciex QTrap 6500plus in positive ionization and multiple reaction mode. Calibrators and controls were produced inhouse by spiking certified Chromsystems® calibration and control material for steroid hormone analysis covering expected endogenous concentration ranges. Method validation included thorough examination of reproducibility, recovery, linearity, sensitivity and specificity according to FDA guidelines. Possible effects of freeze/thaw cycles were addressed. For clinical verification, 13 CAH patients and healthy controls were compared.

Results: Chromatographic separation was achieved within a total run time of 6.6 min to simultaneously quantify 4 11-OAs as well as 7 other steroid hormones from the original method. Lower limits of quantification were well below endogenous ranges (63-320 pmol/l), recoveries ranged from 85% to 117% with CVs ≤ 15%. The concentration of the analytes were comparable to published values of healthy individuals and showed high stability across five freeze/thaw cycles (CV ≤ 3.1%). Clinical verification revealed, as expected, an increased concentration of 11-OAs and decreased ratios of androstendione or testosterone to 11-OAs in CAH patients.

Conclusion: We present a robust high-throughput method with high sensitivity for simultaneous quantification of four 11-OAs and seven routine steroid hormones using minimal sample preparation. By multiplex-design, ratios of clinically relevant steroids to 11 OAs are instantly accessible, allowing the use of 11-OAs for diagnosis and therapy monitoring in androgen excess-related disorders. In current studies, the method is used for determination of reference intervals (from birth to 80 years) as well as to investigate the relationship between 11-OAs with obesity, metabolic syndrome, and puberty.

P-09-11

MULTIMODAL SPATIALLY RESOLVED INVESTIGATION OF LIPIDS SIGNATURES IN NEEDLE BIOPSIES OF LIVER NEOPLASMS WITHIN THE MANNHEIM MOLECULAR INTERVENTION ENVIRONMENT (M2OLIE)

Miriam Rittel[1,2], Stefan Schmidt[1]; Emrullah Birgin[3]; Carina Ramallo Guevara[1]; Alexander Marx[4]; Axel Wellmann[5]; Cleo Weis[4]; Nuh Rahbari[3]; Carsten Hopf[1,2,6]

[1]Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences; [2]Institute of Medical Technology, Heidelberg University; [3]Department of Surgery, Medical Faculty Mannheim, Mannheim School of Medicine, Heidelberg University, Mannheim; [4]Institute of Pathology, University Medical Centre Mannheim; [5]Institute of Pathology Celle; [6]Medical Faculties Heidelberg and Mannheim, Heidelberg University

Introduction and Aims: Mass spectrometry imaging (MSI) is an indispensable tool for label-free spatially-resolved investigations of biological processes and of the molecular composition of various samples in fundamental research. However, the translation of MSI-based techniques into clinical routine for in-depth medical diagnosis, e.g. molecular pathology, remains a challenging task. Requirements in sample preparation for molecular analysis and time restrictions, as well as feasibility in a clinical context are often on opposing sides of the spectrum. Especially, targeting cancer and cancer sub-types in a fast and reliable way by MSI with diagnostic value for further treatment decisions is of particular interest. Here, we present a multimodal approach aligned with clinical routine practice for the assessment of molecular composition of different tumor tissue samples by fusing modern analytical tools and sophisticated clinical routine.

Methods: Within the framework of the M2OLIE research campus, we combined various spatially resolved techniques like infrared spectroscopy, immunohistochemistry and hematoxylin and eosin stainings with MSI-based molecular analysis to investigate the lipid and metabolite composition of primary hepatocellular carcinoma and metastases residing in the liver. Fresh-frozen liver tissue section were measured by means of high-precision untargeted matrix-assisted laser desorption/ionization (MALDI)-MSI in order to reveal the molecular fingerprint of cancer types for biomarker discovery. This includes resected tissue samples as well as biopsies. To this end, we have developed a device for biopsy embedding and MSI sampling, as well as a workflow for data fusion of modern molecular analysis with sophisticated clinical routine. By mapping this sample-specific information onto the MSI data a higher precision in analysis of neoplastic lesions can be accomplished.

Results: We analyzed liver cancer tissue sections with a multimodal approach. To this end, we combined the spatial information from infrared spectroscopy and histopathological evaluation to map tissue morphology-specific features onto the MSI data for in-depth molecular analysis, especially for tumorous regions. By precise co-registration this guided approach enabled transfer of information between adjacent tissue sections and of data from different modalities. Preliminary data revealed a differentiation of the tissue morphology and thus differences in the lipid and metabolite composition of specific histological features.

Discussion: These results promote a MSI-based routine that is feasible in clinical practice. Fusion of molecular information and clinical routine assessment for cancer classification could potentially result in benefits for treatment decisions based on higher level sample analysis.

P-09-12 / FV-09

Estimation of Continuous Reference Intervals using Real-World Data and refineR

Tatjana Ammer[1]; André Schützenmeister[2]; Hans-Ulrich Prokosch[3]; Manfred Rauh[4]; Christopher Rank[2]; Jakob Zierk[5]

[1]Friedrich-Alexander-Universität Erlangen-Nürnberg, Chair of Medical Informatics, Erlangen; Roche Diagnostics GmbH, R&D Biostatistics and Data Science, Penzberg, Germany; [2]Roche Diagnostics GmbH, R&D Biostatistics and Data Science, Penzberg, Germany; [3]Friedrich-Alexander-Universität Erlangen-Nürnberg, Chair of Medical Informatics, Erlangen, Germany; [4]Universitätsklinikum Erlangen, Department of Pediatrics and Adolescent Medicine, Erlangen, Germany; [5]Universitätsklinikum Erlangen, Department of Pediatrics and Adolescent Medicine; Universitätsklinikum Erlangen, Center of Medical Information and Communication Technology, Erlangen, Germany

Introduction: Most biomarkers and their corresponding reference intervals depend on covariates, such as sex, age, or ethnicity. While the derivation of reference intervals for categorical covariates like sex is straightforward, it is substantially more challenging to correctly represent the influence of continuous covariates. Most pronounced during physiological development in children, but extending beyond adulthood, many analytes show clinically relevant age-dependent dynamics. One current approach for the estimation of age-specific reference intervals is a discretization of the continuous covariate. However, this approach leads to unnatural, discontinuous transitions between reference intervals estimated for the artificial age groups. Therefore, continuous reference intervals are needed to adequately assess test results of these analytes. Here, we propose a novel method leveraging routine measurements and a recently published algorithm, refineR, to establish smooth, continuous reference intervals and percentile charts for biomarkers of interest.

Methods: We developed a fully automated pipeline for the generation of continuous reference intervals utilizing solely an indirect method (refineR) and real-world data. First, the input data is divided into fine-grained subgroups, ensuring sufficient amount of data points per group. Second, we apply the refineR algorithm to each group and use the estimated model to determine reference intervals and percentiles for each age bin. These percentiles are then automatically smoothed using a median and bilateral filter. Subsequently, the refineR algorithm is applied in an iterative way alternating between model estimation and smoothing of the percentile curves. The smoothed percentiles from iteration i then serve as regularization for model estimation in iteration i+1 to finally generate smooth, continuous reference intervals. The presented pipeline was applied to three important biomarkers with extensive pediatric dynamics (hemoglobin, alkaline phosphatase, and creatinine) using data obtained during patient care.

Results: The calculated percentile charts for hemoglobin, alkaline phosphatase and creatinine from birth to 18 years of age are in accordance with previously established reference intervals, demonstrating that the presented approach correctly models age-dependency and generates valid continuous reference intervals.

Conclusions: The provided pipeline enables the fully automated generation of high-precision percentile charts using real-world data, while requiring no additional tool, except the refineR algorithm. Providing precise percentile charts allows for accurately capturing the pronounced age-dependent dynamics that occur in many biomarkers, facilitating the interpretation of test results and ultimately improving patient care.

P-10-01

Das Modul Gesundheitsdaten als Basis für den Bachelor Biomedizinische Labordiagnostik an der ZHAW

Lisa Rigassi

Züricher Hochschule für Angewandte Wissenschaften, Departement Gesundheit, Studiengang Biomedizinische Labordiagnostik, Winterthur , Switzerland

Zielsetzung: Im Herbst 2022 startet ein schweizweit einzigartiger Bachelor Biomedizinische Labordiagnostik [1]. Die interdepartmentelle Ausrichtung zwischen "akademischen Gesundheitsberuf mit naturwissenschaftlichem Hintergrund" soll sich in den Modulen widerspiegeln. Das Modul Gesundheitsdaten im 1. Semester soll als Basismodul gestaltet werden, welches die zentralen Aspekte labordiagnostisch relevanter Daten in Gesundheitsversorgung und Forschung abbildet und eine Verbindung zu wesentlichen Bezugswissenschaften schafft.

Fragestellung: Welche Wissensstruktur unterstützt die Zielerreichung des Moduls? Was sind exemplarische Vorschläge zu Inhalten und didaktischen Methoden?

Material und Methoden: Die Inhalte des Moduls wurden anhand eines explorativen Brainstormings erarbeitet. Basierend auf den Ergebnissen wurde die Wissensstruktur entwickelt. Als Bezugsgrössen wurden das Modell der CanMEDS-Rollen [2] der labordiagnostische Prozess und ein Modell des Forschungsprozesses genutzt. Diese Wissensstruktur soll mit den involvierten Akteuren evaluiert werden. Als didaktische Methoden wurden u.a. Fallstudien und Workshops vorgesehen. Die Erreichung der Ziele soll durch kritische Validierung der Studierenden und Lehrenden geprüft werden.

Ergebnisse: Zur Strukturierung des Moduls Gesundheitsdaten wurden Leitfragen bezüglich Datenerhebung, Bereitstellung und Auswertung von Daten in der Labormedizin ermittelt und den Phasen des labordiagnostischen Analyseprozesses zugeteilt. Präanalytisch sind Fragen bezüglich Planung nötig: wer soll welche Daten aus welchem Grund bei wem erheben? In der Analytik ist die Erhebung zentral: z.B. mit welcher Methode und zu welchem Zweck? Postanalytische Fragestellungen befassen sich mit der Datenauswertung: was bedeuten die Daten? Wem sind sie zu melden? In einem zweiten Schritt wurden die Leitfragen den Rollen des CanMEDS-Modells zugeordnet und exemplarische Vorschläge zu konkreten Modulinhalte abgeleitet. Dazu gehören z.B. zum Manager das Managen von Daten in Laborinformationssystemen und wissenschaftliche Datenbanken, zum Communicator die interprofessionelle Kommunikation mit anderen Professionsangehörigen, Wissenschaftler:innen und Laien sowie zum Collaborator die Zusammenarbeit im Team, zwischen den verschiedenen Akteuren und zwischen Institutionen. Als relevante Bezugswissenschaften wurden Ethik, Recht, Informatik und Kommunikationswissenschaften abgeleitet.

Diskussion und Schlussfolgerung: Die Wissensstruktur kann wie folgt beschrieben werden: auf Basis der 7 CanMEDS-Rollen sind Daten in Präanalytik, Analytik und Postanalytik in der Gesundheitsversorgung sowie bei Planung, Erhebung und Auswertung in der Forschung zu managen. Den Rahmen bilden Recht und Ethik sowie Kommunikation und Informatik. Die vorliegende Modulstruktur ist ein erster Entwurf und sollte sowohl intra- als auch interprofessionell mit den Gesundheitsberufen, Naturwissenschaftler und Praxis diskutiert werden.

P-10-02

Clinical feasibility of a novel ultrasensitive multiplex NfL, Tau, UCHL-1 and GFAP assay for the evaluation of neuroaxonal and glial damage in serum samples of patients with multiple sclerosis and anti-IgLON5 disease

Justina Dargvainiene[1]; Thomas Grüter[2]; Ilya Ayzenberg[2]; Franziska E. Möllers[1]; Klaus-Peter Wandinger[1]; Klarissa Hanja Stürner[3]; Frank Leypoldt[1]; Ralf Junker[1]

[1]UKSH, Campus Kiel, Institut für klinische Chemie, Kiel, Germany; [2]St. Josef Hospital, Ruhr Universität Bochum, Klinik für Neurologie, Bochum, Germany; [3]UKSH, Campus Kiel, Klinik für Neurologie, Kiel, Germany

Introduction: Novel ultra-sensitive single molecule array (SiMoA) based detection of neurofilament light (NfL) and other neuroglial proteins is a promising tool for evaluation of neurodestruction. However, clinical applicability is hampered by considerable costs as well as absence of disease specific cut-off values.

Objective: We aimed to test the clinical validity of reduced cost protocol for SiMoA technology-based assay in two cohorts: 1) patients with relapsing-remitting multiple sclerosis (RRMS) and 2) patients with anti-IgLON5 disease. These inflammatory/neurodegenerative disorders were chosen because of their heterogeneous natures and urgent need for standardized biomarkers of disease activity/prognosis.

Methods: Serum samples were measured with Neurology 4-plex kit: NfL, Tau, ubiquitin C-terminal hydrolase L1 (UCHL-1) and glial fibrillary acidic protein (GFAP) - on fully automated HD-X-Analyzer (Quanterix) using standard and reduced cost protocols (measurements in duplicates vs. singlicates). In the RRMS cohort (N=114), serum samples were obtained prospectively at an outpatient clinic (UKSH, Campus Kiel); active disease was defined by new relapses and/or changes on MRI scans observed over the prior six months. In the cohort of treatment naive patients with anti-IgLON5 disease (N=27), the serum samples were recruited retrospectively from German network for Research on autoimmune encephalitis (GENERATE). Healthy controls (N=70) were analyzed for comparison. Biomarker concentrations were compared between patients and controls, correlated with disease activity/prognosis and cut-off values estimated when possible.

Results: The intra- and inter-assay variance for NfL and GFAP remained under 10%. In the RRMS cohort, higher NfL values were detected in active patients (N=42) compared to patients in remission (N=72) (12.1 (8.8-26.6) vs. 7.1 (4.8-10.7) pg/ml, p< 0.001) and the controls (9.2 (6.9-12.0)pg/ml, p< 0.001). An age-adapted NfL cut-off value of 8.5pg/ml predicted disease activity with 68% specificity and 76% sensitivity in patients under the age of 60 years (AUCROC=76.9 ± 0.05%, p< 0.0001). In the anti-IgLON5 cohort, NfL and GFAP concentrations were significantly increased (71.1±103.9pg/ml and 126.7±73.3pg/ml, respectively) compared to the controls (N=70) (9.2 (6.9-12.0)pg/ml and GFAP 67.0 (53.1-95.2)pg/ml respectively, p< 0.001). Low pretreatment NfL value was also an independent predictor for better treatment outcome. Tau and UCHL-1 allowed no differentiation between patients and controls.

Conclusions: Reduced cost protocols are feasible on fully-automated platform and could facilitate clinical application of serum neuronal biomarkers. While NfL discriminates fairly well between RRMS and controls, sensitivity and specificity for active disease as standalone marker is insufficient. In patients with anti-IgLON5 disease, low NfL level at treatment initiation may serve as a potential biomarker for response to immunotherapy and prognosis.

P-10-03

Apoptosis inhibition during cold storage of platelets better maintains both platelet functionality and survival

Irene Marini[1]; Yoko Tamamushi[1]; Lisann Pelzl[1]; Stefanie Nowak-Harnau[2]; Tamam Bakchoul[3]

[1]Institut für klinische und experimentelle Transfusionsmedizin, University of Tübingen, Tübingen, Germany, Forschung, Tübingen, Germany; [2]Institut für klinische und experimentelle Transfusionsmedizin, University of Tübingen, Tübingen, Germany, Blutspende, Tübingen, Germany; [3]Institut für klinische und experimentelle Transfusionsmedizin, University of Tübingen, Tübingen, Germany, Forschung und Blutspende, Tübingen, Germany

Introduction: Apheresis-derived platelet concentrates (APCs) is an essential medical therapy use to treat bleeding. Nevertheless, the storage at room temperature increases the risk of bacterial-infection post transfusion. Recently, we reported that cold-stored APCs show better platelet functionality but decreased platelet half-life caused by apoptosis activation (Marini et al., Haematologica 2019). In this study, we investigated the impact of an apoptosis inhibitor on platelet functionality and half-life during cold storage.

Methods: APCs were collected from healthy donors and stored for 7 and 10 days at 4℃ in the presence or in the absence of an apoptosis inhibitor (G04). Platelet apoptosis was assessed measuring the phosphatidylserine (PS) exposure and the mitochondrial membrane potential (MMP) by flow cytometry. Platelet functionality was investigated analyzing the expression of CD62, CD63 and PAC1 upon TRAP stimulation, by flow cytometry. Platelet aggregation ability, after TRAP and ristocetin incubation, was tested using an aggregometer. Next, thrombin generation was measured using a thrombogram. While, the adhesion ability was determined performing an in vitro assay. Platelet survival was analyzed using a NSG mouse model.

Results: Upon incubation with the apoptosis inhibitor a significant reduction of PS externalization was detected after 10 days of storage (p=0.002). Accordingly, significant higher levels of MMP were observed after G04 incubation (p=0.034). Interestingly, the inhibitor did not affect the platelet functionality. In fact, CD62 expression was comparable to untreated cells (p=0.086). While, the responsiveness of CD63 and PAC1 was better conserved in platelets stored with the inhibitor (day 10, CD63 p=0.035 and PAC1, p=0.005). Furthermore, on day 10 no differences in the platelet aggregation ability were detected with or without inhibitor (TRAP, p=0.591 and Ristocetin p=0.998) as well as comparable thrombin formation (p=0.602). Interestingly, a significant increase of the number of adherent cells was detected after platelets were treated with G04 (day 7, p= 0.0465). More importantly, a higher percentage of circulating human cold-stored platelets was detected in the mouse bloodstream upon incubation with G04 after 7 days of cold storage compared to untreated cells (5h post injection, p=0.046).

Conclusion: Our results show that the cold-induced platelet apoptosis, responsible for the faster destruction of cold-stored platelets in vivo, can be efficiently prevented using an apoptosis inhibitor. Furthermore, the inhibitor did not impair platelet functions like activation, aggregation, thrombin formation and adhesion. More importantly, the inhibitor better maintains cell survival in vivo. Therefore, incubation of APCs with apoptosis inhibitor(s) during cold storage, might be a promising strategy to prolong the storage time without impairing platelet's functionality and improving cell half-life.

P-10-04

Nachweis und Verteilung des prognostisch relevanten TERT Promotermutationsstatus bei multiphasisch differenzierten Schilddrüsenkarzinomen

Sandra Ehrhardt; Udo Siebolts, Liesa Siemss

Universitätsklinikum Halle (Saale), Institut für Pathologie, Halle, Germany

Einleitung:
Das klinisch relevante Schilddrüsenkarzinom ist das häufigste Malignom des endokrinen Systems. Multiphasische Schilddrüsenkarzinome sind, im Vergleich zu homogenen Tumoren, eine seltene, im wissenschaftlichen Kontext recht unerforschte Tumorentität. Bei diesen Tumoren findet man neben differenzierten Karzinomanteilen gleichzeitig schlecht- bzw. entdifferenzierte Anteile, wodurch die Tumorheterogenität zu Stande kommt. Ein Gen, welches bei Schilddrüsenkarzinomen eine prognostische Relevanz hat, ist das TERT Gen, welches für das Enzym Telomerase Reverse Transkriptase codiert [1]. In verschiedenen Studien hat man herausgefunden, dass vor allem die zwei somatischen Mutationen im Promoterbereich des Gens c.-124C>T (C228T) und c.-146C>T (C250T) für die Tumorgenese und -progression von besonderer Bedeutung sind [2,3]. Man hat ebenfalls herausgefunden, dass TERT Mutationen häufiger in schlecht differenzierten Schilddrüsenkarzinomen zu finden sind, während ein Auftreten in differenzierten Karzinomen eher selten der Fall ist [4]. Über die TERT Mutationsfrequenz und Verteilung in homogenen Schilddrüsentumoren wurden in der Vergangenheit viele Publikationen verfasst, sehr viel weniger erforscht sind die multiphasisch differenzierten Schilddrüsenkarzinome. Die Bestimmung des TERT Promotermutationsstatus als prognostischer Biomarker soll helfen diese Tumoren in ihrem Progressionsverhalten besser einzuschätzen.

Zielsetzung:
(1) Wie ist die TERT Mutationsfrequenz und – verteilung in multiphasisch differenzierten Schilddrüsenkarzinomen?
(2) Gibt es hinsichtlich des Auftretens und der Verteilung der TERT Promotermutation Unterschiede zwischen den häufig auftretenden homogenen und den seltenen multiphasisch differenzierten Schilddrüsenkarzinomen?

Methodik:
Ein Tumorkollektiv aus 14 multiphasisch differenzierten Schilddrüsenkarzinomen wurde mittels eines eigens entwickelten Pyrosequenzierungsassays auf das Vorhandensein der beiden prognostisch relevanten Hotspot Mutationen C228T und C250T im Promoterbereich des TERT Gens untersucht, die einzelnen Karzinomanteile wurden dabei jeweils getrennt voneinander analysiert.

Ergebnisse:

Die Pyrosequenzierung des multiphasisch differenzierten Schilddrüsenkarzinomkollektivs ergab, dass 71,4% der untersuchten Tumorproben die prognostisch relevante TERT Promotermutation tragen, 28% zeigten einen negativen TERT Status. Im untersuchten Kollektiv wurden die meisten TERT Promotermutationen unerwartet in den PTC Anteilen (75%) detektiert, die wenigsten in den PDTC Arealen (35,7%).

Schlussfolgerung:

Heterogene Schilddrüsenkarzinome sind Tumoren mit einer ungünstigen Prognose, dies bestätigt der Nachweis der prognostisch relevanten TERT Promotermutation in der Mehrheit der untersuchten Karzinome. Da die meisten TERT Mutationen in den PTC Anteilen detektiert wurden, sollte bei Tumorheterogenität stets der PTC Anteil oder besser jeder der unterschiedlich diff.Karzinomanteile molekularpathologisch untersucht werden.

P-10-05

Design und Validierung patientenspezifischer droplet digital PCR (ddPCR) Assays zur Detektion und Quantifizierung zirkulierender Tumor-DNA

Laura Mirbach[1]; Tobias Boch[2]; Angelika Duda[1]; Romy Eichner[1]; Sonja Loges[2]; Verena Haselmann[1]

[1]Universitätsmedizin Mannheim, Institut für Klinische Chemie, Mannheim, Germany; [2]DKFZ-Hector Krebsinstitut an der UMM, Abteilung Personalisierte Onkologie, Medizinische Fakultät Mannheim der Universität Heidelberg, Personalisierte Medizinische Onkologie (A420) DKFZ, Mannheim, Germany

Zielsetzung: Das Liquid Profiling gewinnt als Alternative zur gewebsbasierten Diagnostik zunehmend an Bedeutung. Der klinische Nutzen im Rahmen der Companion Diagnostik zur therapeutischen Stratifizierung konnte bereits für verschiedene Tumorentitäten, wie z.B. das Nicht-kleinzellige Bronchialkarzinom (NSCLC), nachgewiesen werden. Ein weiterer Vorteil des Liquid Profilings liegt in seiner Eignung zum Monitoring von Patienten im Verlauf im Sinne eines personalisierten Tumormarkers. Hierfür müssen patientenspezifische Sequenzvariationen im Primärtumor identifiziert und individuelle Assays entwickelt werden. Für dieses Ziel eignet sich insbesondere die droplet digital PCR (ddPCR), da sie neben einer hohen analytischen Sensitivität als offenes System die Etablierung laborentwickelter individueller Assays erlaubt.

Methoden: Für die Etablierung individueller ddPCRs müssen zunächst relevante Sequenzvariationen im Primärtumorgewebe identifiziert und das Vorliegen in der Keimbahn ausgeschlossen werden. Hieran schließt sich das Primer-/Probe-Design basierend auf einer TaqMan-qPCR an. Hierbei müssen spezifische Punkte im Hinblick auf die ddPCR berücksichtigt werden. Nach experimenteller Ermittlung der optimalen Annealingtemperatur erfolgt die Validierung durch Bestimmung des limit of blank, der false-positive-rate, des limit of detection sowie des limit of quantification mit anschließender Überprüfung von Präzision und Richtigkeit.

Ergebnisse: Exemplarisch wird der Fall einer Patientin mit NSCLC vorgestellt. Für diese Patienten wurden zwei relevante Sequenzvariationen im Primärtumorgewebe identifiziert, ein Basenaustausch sowie eine Fusion. Für beide Veränderungen wurden entsprechende personalisierte ddPCR-Assays etabliert und zur Verlaufskontrolle genutzt. Die Ergebnisse zeigen, dass sich dieser Ansatz eignet, um das Therapieansprechen im Verlauf zu überwachen.

Diskussion Und Schlussfolgerung: Das Liquid Profiling kann neben der Companion Diagnostik auch als personalisierter Tumormarker verwendet werden. Hierfür können mittels ddPCR patientenspezifische Assays entwickelt und zur Verlaufskontrolle eingesetzt werden. Dies stellt einen wichtigen Schritt dar, um neben einer personalisierten Therapie auch eine Personalisierung der Diagnostik als Voraussetzung für das Erreichen einer „Precision Medicine" in der Onkologie zu erzielen.

Invited abstracts - Symposia

Qualität in der Labormedizin. Symposium der Sektion Akkreditierung

CEN Spezifikationen und ISO Standards für die Qualität der Präanalytik von Genomanalysen. Wo stehen wir 2022?

EA-01

Uwe Oemüller

Qiagen GmbH, MDx Development, Hilden, Germany

Molecular in vitro diagnostics and research have allowed great progress in medicine including diagnostics. Further progress is increasingly established by new biomarker tests analyzing specimens' biomolecule profiles such as nucleic acids, proteins, and metabolites. However, profiles of these molecules can change significantly during specimen collection, transport, storage, and processing including analyte isolation, caused by post collection cellular changes such as gene inductions, gene down regulations, biomolecules modifications or degradation. This can make the outcome from diagnostics or research unreliable or even impossible because the analytical test will not determine the situation in the patient body but an artificial specimen analyte profile generated during the pre-analytical workflow. Preanalytical variables are a major error source for wrong diagnostic test results. High quality specimens with preserved analyte profiles as they were in the patients bodies are therefore crucial for reliable diagnostics, biomedical research and biobanking. Specifying, developing and verifying pre-analytical workflow parameters for diagnostics tests has consequently become a requirement by new European legislation.

The EU SPIDIA Consortium (2008-2013) developed new pre-analytical technologies for preserving molecular profiles in human specimen and generated broad evidence that guidance to laboratories on pre-analytical workflows improves analytical test results. Based on these results, the CEN/TC 140 for "in-vitro diagnostic medical devices" had released first 9 European Technical Specifications for pre-analytical workflows addressing different blood, other body fluids and tissue based molecular applications. In 2018 and 2019 they progressed to International Standards at the ISO/TC 212 for "clinical laboratory testing and in vitro diagnostic test systems". The successor EU SPIDIA4P consortium project (2017-2021), supported by a large international network, has broadened to a final portfolio of 22 pre-analytical CEN and ISO Standards intending to improve in vitro diagnostics and biomedical research, has developed corresponding External Quality Assurance (EQA) and is driving international implementation. The new standards can serve to fulfill requirements in the new EU In Vitro Diagnostic Regulation 2017/746 (IVDR). The SPIDIA4P project has received several awards including the "CEN-CENELEC Standards+Innovation Project Award 2021" for its important contribution of standardization and innovation to molecular diagnostics.

Gemeinsame Sitzung DGKL und DGNS: Aktuelle Entwicklungen und zukünftige Perspektiven für das Neugeborenenscreening

15 Jahre qualitätsgesichertes Neugeborenenscreening in Deutschland

EA-02

Uta Nennstiel

Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL), Sachgebiet GE4 Gesundheitsberichterstattung, Epidemiologie, Sozialmedizin, Kindergesundheit, Screeningzentrum, München-Oberschleißheim, Germany

Das Neugeborenen-Screening auf angeborene Stoffwechsel- und Hormonstörungen ist seit der Einführung des Screenings auf Phenylketonurie (PKU) Ende der 1960er Jahre eine der erfolgreichsten Maßnahmen der Sekundärprävention im Kindesalter und wird für bereits seit. Im Jahr 2004 nahm der Gemeinsamen Bundesausschuss (G-BA) das sog. „Erweiterte

Neugeborenen-Screening" (ENS) auf zehn metabolische und zwei endokrine Zielkrankheiten in die Kinder-Richtlinie auf und ergänzte es 2018 um Tyrosinämie Typ I, 2019 um schwere kombinierte Immundefekte (SCID) sowie 2021 um Sichelzellkrankheit und Spinale Muskelatrophie (SMA). Mit gesonderten Regelungen wurde das Mukoviszidose-Screening 2016 eingeführt [1]. Daneben unterliegt das ENS dem Gendiagnostikgesetz [2]. Einige Zielkrankheiten können bereits früh zu lebensbedrohlichen Stoffwechselkrisen oder zu bleibenden Schäden führen. Dies setzt eine Diagnosestellung und den Therapiebeginn bereits in den ersten Lebenstagen bis Wochen voraus. Die Zielkrankheiten des NBS sind selten und treffen in ihrer Gesamtheit eines von etwa 1.000 Neugeborenen [3]. Dies bedeutet wiederum, dass 99,9% der nicht betroffenen „gesunden" Neugeborenen bei dieser populationsbezogenen Reihenuntersuchung mit untersucht werden müssen. Dabei muss neben einer hohen Sensitivität insbesondere eine hohe Spezifität erreicht werden, um nur wenige Familien gesunder Kinder durch einen falsch positiven Befund zu beunruhigen [4]. Das ENS ist somit in erster Linie eine Public-Health-Maßnahme, die nach definierten Kriterien in einem von der Elternaufklärung bis zur Nachsorge gut strukturierten und organisierten sowie qualitätsgesichertem Programm durchzuführen wäre [5–9].

Die Screeningdaten aller elf deutschen Screeninglabore werden an die Deutsche Gesellschaft für Neugeborenscreening (DGNS) übermittelt, dort analysiert und in einem jährlichen Screeningreport publiziert [10]. Für den Zeitraum von 2006 bis 2019 liegen für Deutschland Screeningdaten für 10.022.333 Neugeborene vor. Bei 7.995 Kindern wurde die Verdachtsdiagnose in der Konfirmationsdiagnostik bestätigt, bei 807 Kindern ist unklar, ob die Konfirmationsdiagnostik durchgeführt wurde (lost to follow-up). Eine oder mehrere Folgeuntersuchungen waren bei 307.588 Neugeborenen erforderlich und wurden bei 246.253 Kindern (80,059 %) durchgeführt.

Die Daten zeigen, dass das ENS insgesamt erfolgreich umgesetzt wird, allerdings fehlen wichtige Komponenten eines qualitätsgesicherten Programms. Optimierungsbedarf ergibt sich beim Loss to follow-up, da von ca. 20 % der Neugeborenen mit kontrollbedürftigem Screeningbefund nicht bekannt ist, ob dieser jemals abgeklärt wurde. Eine verbesserte Rückmeldung der Diagnostik der behandelnden Ärzte an die Screeninglabore, sowie eine Nachverfolgung kontrollbedürftiger Screeningbefunde durch flächendeckende Trackingstrukturen ist hier nötig. Die Einführung eines Registers und eine geregelte Evaluation könnten die Qualität des Screening-Programms weiter verbessern.

Gemeinsames Symposium der Sektion TDM & Klinisch Toxikologische Analytik und der Internationalen Assoziation für TDM & Klinische Toxikologie (IATDMCT)

Therapeutisches Drug Monitoring bei Behandlung mit Depotantipsychotika

EA-03

Christoph Hiemke

Universitätsmedizin Mainz, Klinik für Psychiatrie und Psychotherapie, Mainz, Germany

Die Anwendung von Depot-Antipsychotika (Long-Acting Injectable antipsychotics, LAI) ist in den letzten Jahren konstant gestiegen. Anders als bei den meisten oralen Antipsychotika, deren Anwendung oftmals hinsichtlich erwünschter Therapieeffektivität und Arzneimitteltherapiesicherheit durch den Einsatz vom therapeutischen Drug Monitoring (TDM) optimiert wird, ist die Bedeutung von TDM bei Depot-Antipsychotika nach wie vor unklar (Schretsanitis et al. 2021). Weit verbreitet ist bei den Behandlern die Meinung, dass TDM nicht sinnvoll sei, da bei der Depotgabe, anders als bei oraler Gabe, stabile Wirkspiegel eingestellt werden. Darüber hinaus wird angezweifelt, ob die Referenzbereiche von Antipsychotika, die für die Akutbehandlung etabliert sind, auch für die Behandlung mit Depot-Antipsychotika gültig sind. In diesem Beitrag soll dargestellt werden, dass die Anwendung von TDM zur Therapiesteuerung auch Depot-Antipsychotika nützlich sein kann, weil auch bei Depotgabe die Wirkstoffkonzentrationen bei gleicher Dosis interindividuell hoch variabel sind und wie bei oraler Einnahme Alter, Geschlecht, Begleitmedikation oder metabolischer Phänotyp bzw. Genotyp bedeutsam sind. Mit Blick auf die gut etablierten therapeutischen Referenzbereichen oral verabreichter Antipsychotika scheinen die erwünschten klinischen Wirkungen von Depot-Antipsychotika bereits in niedrigeren Wirkstoffkonzentrationsbereichen erzielt zu werden. Es ist zu diskutieren, ob für Depot-Antipsychotika andere therapeutische Referenzbereiche zu empfehlen sein könnten. Die bisherigen Erkenntnisse hierzu sind aber nicht ausreichend valide, um die Empfehlung auszusprechen,

von den für orale Applikationsformen empfohlenen Referenzbereichen abzuweichen. TDM kann vor allem bei der Umstellung eines Antipsychotikums von der oralen auf die Depotformulierung nützlich sein, weil es hierdurch besser gelingt, patientenindividuelle pharmakokinetische Eigenschaften und Einflussfaktoren bei der Einstellung und bei der Dauertherapie mit einem Depot-Antipsychotikum zu kontrollieren. Wichtig ist es dabei auch, vor der Umstellung auf Depot die individuell optimalen Wirkstoffkonzentrationen zu bestimme, indem mehrfach in Phasen guten Ansprechens eine Blutspiegelmessung vorgenommen wird. Weitere Indikationen für TDM bei Depotantipsychotika sind Symptomverschlechterung, unerwünschte Arzneimittelwirkungen oder Einnahme von Medikamenten mit Wechselwirkungspotential.

Gemeinsames Symposium der Sektion TDM & Klinisch Toxikologische Analytik und der Internationalen Assoziation für TDM & Klinische Toxikologie (IATDMCT)

Einsatz der LCMS in der 24/7 Routine-Analytik - wo stehen wir aktuell?

EA-04

Frank Streit

Institut für Klinische Chemie und Interdisziplinäres UMG-Labor, Universitätsmedizin Göttingen

Das therapeutische Drug Monitoring (TDM) spielt eine wichtige Rolle in der Patientenversorgung eines Klinikums der Maximalversorgung. Die Bestimmung der aktuellen Medikamenten-Konzentration im Blut ermöglicht eine rasche Anpassung der Dosierung, um optimale Wirkspiegel zu erreichen bzw. um Toxizität zu verhindern. Dazu ist eine zeitnahe Analytik und Rückführung der Ergebnisse notwendig. Viele Medikamentenspiegel werden massenspektroskopisch ermittelt, was, wegen der Komplexität der Methodik, zumeist keinen Einsatz in der 24/7 Routine-Analytik erlaubt.

Wir haben TDM in den letzten Jahren mit dem CLAM2030-LCMS8060NX (Fa. Shimadzu) weiterentwickelt, das nun fast den Anforderungen für ein Klinikum genügt, wie wir Sie von den klinisch-chemischen und immunchemischen Analyzern her kennen. Mittels CLAM2030-LCMS8060NX erfolgt eine automatisierte Probenvorbereitung und eine kontinuierliche Analytik im Random-Access-Mode durch aufeinander abgestimmte Methoden. Dies wird durch eine Kalibrations-Stabilität über Wochen, eine hohe Stabilität der Reagenzien auf dem Gerät sowie einer intuitiven Bedienungsoberfläche und automatisierten Datenauswertung ermöglicht. Die bidirektionale Kommunikation mit unserem LIS-System (OPUS-L) erfolgt mittels HL7-Schnittstelle. Somit ist der 24/7 Betrieb auch durch Personal ohne spezielle massenspekrometrische Kenntnisse möglich.

Die derzeit verfügbaren, IVDR-konformen, automatisierten LCMS-Lösungen für TDM stellen noch keine Option für den Einsatz in der 24/7-Analytik dar, da Sie für Einzelproben ungeeignet sind und eine Abarbeitung und Datenauswertung durch speziell geschultes Personal bedürfen. Um unseren Anforderungen nachzukommen, sind wir daher gewissermaßen gezwungen, eigene Probenprozessierungen zu etablieren und zu validieren.

Update on Reference Intervals and Decision Limits - Symposium der Sektion Entscheidungsgrenzen/ Richtwerte

RIbench: A Benchmark for Evaluating Indirect Methods for Reference Interval Estimation

EA-05

Tatjana Ammer

Universitätsklinikum Erlangen, Kind- und Jugendklinik, Erlangen, Germany

Background: Precise reference intervals are essential for the interpretation of laboratory test results in medicine. Indirect methods leverage routine measurements containing a mixed distribution of non-pathological and pathological values to estimate reference intervals, rather than conducting a study with apparently healthy subjects (i.e. 'direct' method). In recent years, several such indirect methods have been developed. However, no standardized tool for the evaluation and comparison of indirect methods exists so far that can reveal the strengths and weaknesses of the different methods and guide algorithm selection and application.

Methods: We provide RIbench, a benchmarking suite that enables quantitative evaluation and comparison of existing and novel indirect methods. The benchmark contains simulated test sets for ten biomarkers mimicking real-world data (routine measurements). The non-pathological distribution of the biomarkers represent four common distribution types observed in laboratory practice: normal, skewed, heavily skewed, skewed-and-shifted. To identify limitations of the indirect methods, we added pathological distributions with varying location, extent of overlap, and fraction to the non-pathological distribution. Further, the sample size was varied to quantify the performance impact of the data set size. Overall, the benchmark suite contains 576 simulated tests sets per biomarker, 5,760 test sets in total. To evaluate the performance, we compute benchmark scores derived from the absolute z-score deviations between the estimated and true reference limits. We showcase the application of RIbench by evaluating five indirect methods, the Hoffmann method, and four modern approaches: TML, kosmic, TMC, and refineR. The results are compared against each another and a nonparametric direct method (N=120).

Results: For all methods, the pathological fraction had a strong influence on the results. Further, for TML, kosmic, TMC, and refineR, the sample size also strongly affected the performance. With a minimum sample size of 5,000 and a pathological fraction of up to 20%, these indirect methods still achieved results comparable or superior to the direct method.

Conclusions: We present RIbench, an open-source R-package that enables a quantitative and systematic evaluation and comparison of existing and novel indirect methods. Covering a variety of tests sets with varying difficulty, RIbench can serve as a valuable tool to reveal strengths and weaknesses, and enhance indirect methods, ultimately improving the estimation of reference intervals.

Informatik in der Labormedizin

MIO Laborbefund vor der Veröffentlichung

EA-06

Martina Sender; Uta Ripperger

mio42 GmbH , Berlin, Germany

Die Kassenärztliche Bundesvereinigung (KBV) trifft die notwendigen Festlegungen für die Inhalte der elektronischen Patientenakte, um deren semantische und syntaktische Interoperabilität zu gewährleisten (§ 355 SGB V). Als Tochtergesellschaft und im Auftrag der KBV entwickelt und spezifiziert die mio42 die sogenannten Medizinischen Informationsobjekte (kurz MIOs) für die elektronischen Patientenakte (ePA).

MIOs dienen dazu, medizinische Daten nach einem festgelegten Format auf Basis internationaler Standards und Terminologien zu dokumentieren. Sie können als kleine digitale Informationsbausteine verstanden werden, die universell verwendbar und kombinierbar sind. Dadurch wird der Austausch und die Verarbeitung der Daten zwischen einzelnen Akteur:innen innerhalb des Gesundheitswesens, unabhängig vom genutzten Softwaresystem, ermöglicht.

Für das MIO Laborbefund wurden bewährte Konzepte aus aktuell genutzten Qualitätsstandards und Richtlinien sowie Vorarbeiten von Fachgremien berücksichtigt, um eine breite fachübergreifende als auch sektorenübergreifende Akzeptanz zu erwirken. Die öffentliche Kommentierungsphase bezüglich der Spezifikation hat zum Ziel, das externe Feedback durch die Mitglieder von Verbänden und Organisationen als Vertreter:innen zukünftiger Verwender:innen oder Hersteller:innen von MIOs einzuholen. Im Anschluss erfolgt die Benehmensherstellung zur offiziellen Festlegung.

Die semantische Interoperabilität für konkrete Laboruntersuchungen wird ganz wesentlich durch die LOINC®-Codierung geschaffen, weil ein einzelner LOINC®-Code umfänglich die Eigenschaften einer Laboruntersuchung beschreibt. Eine SNOMED CT® Codierung kann für qualitative Labor-Ergebnisse verwendet werden. Zur Veranschaulichung wird kurz ein Beispiel präsentiert. Die Perspektive für die syntaktische Interoperabilität beim digitalen Labordatenaustausch ist, ein einheitliches Format zu haben (HL7 FHIR®), damit das MIO von sämtlichen Software-Systemen unmittelbar gelesen und angezeigt werden kann.

Informatik in der Labormedizin

Software im Labor - ein IVD?

EA-07

Thomas Streichert

Universitätsklinikum Köln, Institut für Klinische Chemie, Köln, Germany

Für Laboratorien ergibt sich aus dem erweiterten Geltungsbereich der IVDR eine neue Anforderung: Die IVDR definiert nicht nur ein Reagenz, Reagenzprodukt, Kalibrator, Kontrollmaterial, Kit, Instrument, Apparat, Gerät sondern auch eine

Software oder ein Softwaresystem als Medizinprodukt, sofern es vom Hersteller zur in-vitro-Untersuchung von aus dem menschlichen Körper stammenden Proben, einschließlich Blut- und Gewebespenden, bestimmt ist. So kann eine Software durchaus ein IVD im Sinne der IVDR werden. Viele Laboratorien haben im Laufe der Zeit Software entwickelt, beginnend vom einfachen Excel-Tool, über Algorithmen bis hin zu komplexen Lösungen zur Abbildung von diagnostischen Pfaden sowie der Unterstützung oder sogar vollautomatisierten Befundung oft eingebettet in die jeweiligen LIS. Dabei werden Laboratorien ggfs. (Software-)Hersteller im Sinne der IVDR. Dieser Vortrag versucht die kritischen und unkritischen Aspekte zu Software im Lichte der IVDR zu beleuchten.

Aspects of Quality Management in the Serological Immune Diagnostics

Problems in standardizing autoimmune diagnostics and potential solutions using the example of ANA and SmD antibodies in SLE diagnostics

EA-08

Walter Fierz

Heiligenschwend, Switzerland

The problems in standardizing immunological tests are mainly based on the individuality of the immune system of the patient and the variability of antigen presentation in different tests. The individual way the immune system recognizes antigens partly depends on its previous encounters with similar antigens, a phenomenon that is called "original antigenic sin". On the antigen selection and presentation side in the test, variabilities might be based on the particular expression system used, the glycosylation status of the antigens, the 3D structure of the antigens, and the accessibility of hidden epitopes, as e.g. with anti-b2-GPI antibodies. A third variability might come from the different avidities of the detected antibodies, dependent on the detection system, that may correlate with different clinical phenomena as e.g. seen with anti-dsDNA antibodies. Furthermore, the characteristics of the relation between a biomarker and a clinical diagnosis, like sensitivity and specificity, might depend on the prevalence of the underlying cause responsible for the biomarker on one side and for the disease on the other side.

Due to these standardization difficulties results from different test systems and therefore their clinical relevance cannot be directly compared. One way to improve the situation is to calculate the likelihood ratio of a particular result of a particular test that gives the relation between the occurrence of the result in patients versus controls and with it its diagnostic relevance.

Hype or hope – Wieviel KI braucht die Labormedizin?

Digitalisierung und KI in der Labormedizin

EA-09

Jakob Adler

Medical Laboratory for Clinical Chemistry, Microbiology, Infectious Diseases and Genetics "Prof. Schenk/Dr. Ansorge & Colleagues", Magdeburg, Germany

Digitalisierung. Kaum ein Wort hört man heute in allen Bereich unseres Lebens häufiger. Wir leben in einer Zeit, die geprägt ist von der nächsten großen gesellschaftlichen Veränderung seit der Industriellen Revolution: der „Digitalen Transformation". Doch was bedeutet das konkret für die Laboratoriumsmedizin? Scheinbar fernab vom (zukünftigen) Alltag aus elektronischer Patientenakte, MIO Laborbefund und Telemedizin gibt es eine enorm vielfältig Entwicklung im Bereich Digital Health. Welche Rolle spielen Big Tech Unternehmen wie Alphabet/Google, Amazon, Apple oder Intel? Gelten die auf uns einprasselnden Buzzwords wie Künstliche Intelligenz, Metaverse, Web 3.0, Industrie 4.0, Brave New Work, usw. auch für unsere Profession? Was gibt es bereits an routinetauglichen Anwendungen der künstlichen Intelligenz in der Labormedizin? Welche Gefahren können von solche Algorithmen ausgehen? Dieser Vortrag will einen niedrigschwelligen Einstieg in die Thematik Digitalisierung und Künstliche Intelligenz bieten und dabei den aktuellen Stand der Entwicklungen weltweit auch fernab des klassischen Gesundheitswesens beleuchten.

Hype or hope – Wieviel KI braucht die Labormedizin?

Explainable Artificial Intelligence - Anwendungsbeispiele für labormedizinische Daten

EA-10

Alexander Tolios

Medizinische Universität Wien, Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin, Wien, Austria

Machine learning-Algorithmen erlauben es in den Lebenswissenschaften, komplexe Muster in Daten zu finden, welche anschließend für klinische Vorhersagen genutzt werden können.

Aber der Preis für die Verwendung von solchen komplexen Methoden ist die eingeschränkte Interpretierbarkeit der algorithmischen Entscheidungsfindung.

Insbesondere im medizinischen Setting ist es jedoch wichtig, zu verstehen, warum ein Algorithmus zu einem bestimmten Ergebnis kommt, da ja die behandelnden Ärzte in der Lage sein müssen, die Entscheidung zu verstehen und ggf. zu hinterfragen.

Durch die Anwendung von "eXplainable Artificial Intelligence" (XAI)-Methoden besteht die Möglichkeit, schwierig zu interpretierbaren "black box"-Algorithmen (wie neuronale Netzwerke oder random forests) unter die Motorhaube zu schauen.

Im Rahmen dieses Vortrags sollen die gängigsten Methoden vorgestellt und deren Vor- und Nachteile gegenübergestellt werden.

Hype or hope – Wieviel KI braucht die Labormedizin?

Business Intelligence im Labor

EA-11

Martin Christmann

St. Bernward Krankenhaus GmbH, Zentrum für Labordiagnostik, Hildesheim, Germany

Business Intelligence ist ein Begriff aus dem Bereich der Wirtschaftsinformatik. Er beschreibt einen Prozess der unter Zuhilfenahme digitaler Algorithmen große Datenmengen, auch aus mehreren Quellsystemen, erfassen, konsolidieren und verständlich darstellen soll.

Die größte Herausforderung in diesem Prozess ist es die Integrität und Konsistenz der Daten sicherzustellen, auf deren Basis die weitere Prozessierung und letztendlich auch die Ableitung von Maßnahmen beruht.

Im Bereich der Laboratoriumsmedizin gibt es vielfältige Anwendungsfälle für diesen Analysenprozess und zuletzt auch einen wachsenden Bedarf an leicht bedienbaren Werkzeugen. Neben kommerziellen Anwendungen gibt es viele individuelle Wege diesen Analysenprozess zu verwirklichen, um sich einen schnellen und verlässlichen Überblick über die Kennzahlen des eigenen Labors zu verschaffen.

Anhand von Anwendungsbeispielen aus dem Krankenhauslabor werden hier die Möglichkeiten und Vorteile, aber auch die Grenzen und Hürden dieser Methodik, sowie der unterschiedlichen Herangehensweisen vorgestellt.

Update Endokrinologie

Auto-Antikörper-Diagnostik in der Diabetologie - Aktueller Stand der Analytik und klinische Anwendung in Deutschland

EA-12

Erwin Schleicher[1]; Markus Thaler[2]

[1]Universitätsklinikum der Eberhard-Krals-Universität Tübingen, Medizinsche Klinik IV, Tübingen, Germany; [2]Klinikum rechts der Isar der TU München, Institut für Klinische Chemie und Pathobiochemie, München, Germany

Autoantibodies against islet cells (IAAB), i.e. autoantibodies against glutamate decarboxylase, insulinoma antigen 2, zinc transporter -8, and insulin, develop early in type 1 diabetes mellitus (DM) when metabolic changes and clinical symptoms are still absent. Presence of IAAB therefore characterizes the first stage of the disease while stage 2 and 3 show dysglycemia and chronic hyperglycemia and overt clinical diabetes, respectively. Being positive years before clinical onset of the disease, IAAB are determined in individuals at high risk for type 1 DM. IAAB are positive in up to 85% of the newly diagnosed patients with type 1 DM indicating that every 6th patient with type 1 DM fails to show IAAB at the time of diagnosis. Novel studies indicate that screening for IAAB in early childhood may predict the disease years before manifestation. In adults, IAAB may serve to classify the DM as type 1 or type 2 / maturity-onset diabetes of the young (MODY) as well as patients as "autoimmune insulin deficient" or "severe insulin deficient" – when clinical and metabolic characteristics are ambiguous. A multitude of methods are employed to determine IAAB in the clinical laboratory. Methods allowing formation of the immune-complexes partially or totally in liquid phase are to be preferred. This is due to the fact that assay formats with solid-phase immobilized antigens seem not to reach sufficient sensitivities and specificities. As with other autoantibodies, comparability of different IAAB assay results is poor. Laboratories are therefore strongly encouraged to establish their own cut-off values in order to enable proper clinical interpretation of the test results. Ring trials for all IAAB mentioned above are currently available in Germany and contribute to analytical quality. Beyond that, the "Islet Autoantibody Standardization Program" as internationally coordinated endeavor aims to improve quality and comparability of IAAB assays.

Aus-, Fort- bzw. Weiterbildungsqualität bei nicht-ärztlichen Laborspezialisten - Symposium der Sektion Repetitorium

Digitale Kompetenz - "Hot-Skills" für die Führungskräfte der digitalen Zukunft

EA-13

Jakob Adler

Medical Laboratory for Clinical Chemistry, Microbiology, Infectious Diseases and Genetics , Clinical Chemistry, Magdeburg, Germany

Wir leben in der Zeit der „Digitalen Transformation". Wir merken immer wieder, dass viele unserer Arbeitsmittel und -abläufe den sich ständig ändernden Gegebenheiten scheinbar nicht mehr gewachsen sind. Doch was tun? Welche Fähigkeiten braucht der labormedizinische Nachwuchs, um in einer Welt aus Digital Health, Künstlicher Intelligenz und Metaverse den Aufgaben und Anforderungen an die eigene Profession gerecht zu werden? Was sind die „Hot Skills" der Zukunft? Wie wird man zum „Digital Talent"?

Im 21. Jahrhundert brauchen wir neben Lesen, Schreiben und Rechnen eine neue Grundfertigkeit: Programmieren. In einer immer digitaler werdenden Welt braucht es ein umfassendes technisches und informatisches Verständnis, um mit Trends wie Blockchain, Metaverse und Künstlicher Intelligenz sowie Notwendigkeiten wie der Cyber-Security umgehen zu können. Es braucht ein „algorithmisches Denken", um die Welt um uns herum zu verstehen und sie aktiv mitzugestalten. Wenn wir als Ärzt:innen nicht aktiv am digitalen Gesundheitswesen der Zukunft bauen, werden es andere tun. Big Tech Unternehmen wie Google, Amazon oder Apple stehen bereit und investieren Milliarden in die Entwicklung von Digital Health Anwendungen.

Die Sektion Junges Labor hat für sich erkannt, dass es eine neue Kompetenz erwerben muss: Digitale Kompetenz. Dieser Vortrag möchte die aktuellen Gegebenheiten beleuchten, die zur Gründung der Arbeitsgruppe Digitale Kompetenz geführt haben und einen Einblick in die Arbeitsweise und Inhalte der AG geben.

Zwischen Regulation und adhoc Analytik

Neue Anforderungen an das Diagnostiklabor durch SARS-CoV-2

EA-14

Burkhard Brandt

UKSH, Institut für Klinische Chemie, Kiel, Germany

Mit der Pandemie des SARS-CoV-2 Erregers kam eine bisher nicht dagewesene Flut von Aufträgen für eine PCR in die klinisch-chemischen Labore. Neben ihrer großen Anzahl kamen Probenmaterialein ins Labor, die bislang in den Routineabläufen, nur einen geringen Arbeitsaufwand darstellten, deren Entnahmequalität jedoch das Analysenergebnis wesentlich beeinflusst. Die dritte neue Herausforderung stellte die Notwendigkeit zur Steigerung der Bearbeitungskapazität in kürzester Zeit dar, da die Pandemie sich schnell in der Bevölkerung ausbreitete. Die Industrie reagierte schnell mit Assay- und Geräteentwicklungen, so dass 3 – 4 Monate nach den ersten Fällen halbautomatische und wenig später vollautomatische Systeme zur Verfügung standen. Dafür mussten Räume vorbereitet werden, die die dafür erforderlichen Medien (Strom, Wasser, Klimatisierung, Belüftung) enthielten, insbesondere dem Infektions- und Kontaminationsschutz genügten. Die Labore mussten die Assays ohne etabliertes Referenzmaterial validieren, den Probentransfer und die -vorbereitung unter dem besonderen Schutz der Mitarbeiter bewältigen und die Datensicherheit über ihr LIS für Einschleusung, Identifikation, Material und Befund einschl. Kommentierung und Übermittlung, auch an die Überwachungsstellen (GSA, RKI), gewährleisten. Darüber hinaus wurden auch PCR-Schnelltests bereitgestellt, die in die bestehenden Eilfallstrukturen zu integrieren waren. Die Versorgung mit Entnahmesystemen war im ganzen ersten Jahr der Pandemie knapp. Ein häufiger Herstellerwechsel erforderte Auswertungsanpassungen. Inzwischen ist die Versorgung stabil und mit der Einführung von inaktivierenden Medien im primären Probenröhrchen könnten Infektionsschutzmaßnahmen reduziert werden. Die entscheidende Einflussgröße bleibt trotz aller Validationen im Labor und im Gegensatz zur standardisierten venösen Blutentnahme die Entnahmequalität in Nase und Rachen. Klinisch erforderliche Entnahmen als BAL oder Bronchialabstrich/Sputum erfordern eine spezifische Probenaufbereitung. Nach wie vor schwierig, insb. in der Befundkommunikation, bleibt der Messwert der PCR, der Ct-Wert (Cycle Threshold). Er stellt anders als in der Klinischen Chemie verbreitet nicht direkt eine Stoffmenge dar. Die Wertelage bleibt darüber hinaus vom Assayhersteller und der individuellen Auswertung des qualitativen PCR-Assays abhängig. Mithilfe der inzwischen etablierten Referenzmaterialien von Referenzmaterialherstellern und des RKI konnte für die PCR-Methoden eine

Vergleichbarkeit hergestellt werden und ein Orientierungswert für den Ct eingeführt werden, der eine grobe Abschätzung der Kontagiosität der Patienten ermöglicht. Das RKI propagiert darüber die Entisolierung von Infizierten zu steuern. Damit ist für das Labor ein Grad der Referenzierung erreicht, der das Arbeiten in der SARS-CoV-2 Diagnostik erleichtert. Es ist jedoch nicht abzusehen, wann oder ob überhaupt die SARS-CoV-2 Pandemie ein Ende hat, deshalb werden hohe Anforderungen an das Labor bestehen bleiben.

Autorenverzeichnis

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Abdullah, Mohammed	P-02-04
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