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DGKL: 01. Analytische Qualität, Labormanagement

P-01-01

Comparative methodological verification of a novel clinical chemistry analyzer for routine laboratory diagnostics

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Introduction:

Confirming relevant performance characteristics (i.e. verification) of laboratory systems for their implementation in routine diagnostics is crucial for quality assurance in clinical laboratory. Thereby, a diagnostic laboratory method or analyzer is proven being suitable for fulfilling tasks with far-reaching decisions regarding patients' health as well as economic processes in clinical laboratory. Therefore, a variety of aspects must be taken into account during the practical implementation and laboratory system verification as well as transfer into clinical routine diagnostics.

Aims and Methods:

The main goal of the study was to re-evaluate the analytical performance of the cobas pure integrated solutions system under routine laboratory conditions. Measurements were performed comparing the cobas® 6000 routine clinical chemistry analyzer with the novel cobas pure integrated solutions system. The intra-day imprecision for the cobas pure integrated solutions system was determined by measuring quality control (QC) samples.

Results:

In total, 145 patient samples for comparative measurements on both clinical chemistry systems and six QC samples for intra-day imprecision analyzes on cobas pure integrated solutions system were included in the study. Six selected analytes each representing one of the six different test methods such as potentiometry (sodium, Na; N=24+1), turbidimetry (C-reactive protein, CrP; N=25+1), heterogeneous immunoassay (thyroid-stimulating hormone, TSH; N=23+1), IFCC standardized kinetic method (alanine aminotransferase, ALAT; N=23+1), colorimetry (albumin, ALB; N=25+1) as well as IFCC standardized colorimetry (alcaline phosphatase, AP; N=25+1) were assessed. The cobas pure integrated solutions system showed an average negative bias of 4.50 mmol/L for Na, 3.84 mg/L for CrP, 0.08 mU/L for TSH, 3.89 mU/L for ALAT, 6.55 U/L for AP and an average positive bias of 0.28 g/L for ALB. The corresponding coefficients of correlation (R2) ranged from 0.999 (Na, CrP, TSH, AP and ALB) to 1.000 (ALAT) suggesting an excellent statistical correlation between the two laboratory systems tested. Repeated measurements of QC samples on the cobas pure integrated solutions system exhibited an intra-day imprecision of 0.47 % for Na (mean = 145.79 mmol/L), 0.45 % for CrP (mean = 41.28 mg/L), 1.14 % for TSH (mean = 1.78 mU/L), 0.44 % for ALAT (mean = 130.70 U/L), 0.43 % for ALB (mean = 34.52 g/L) and 0.29 % for AP (mean = 247.25 U/L) complying with acceptance criteria.

Conclusion:

The results obtained by comparative analyses as well as imprecision data suggest suitability of the cobas pure integrated solutions system for routine clinical laboratory diagnostics.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-02

Validation of the S100b protein as a laboratory-developed test based on the matrix effect.

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Introduction:

The new European In Vitro Diagnostic (IVD) Regulation 2017/746 (IVDR) restricts the use of laboratory-developed tests (LDT) after 26th May 2022. The analysis of different matrices not validated by the manufacturer was also categorized as LDT. To reduce the turnaround time (TAT) of S100B protein in traumatic brain injury (TBI) in emergency settings, we would like to validate the S100B protein in plasma, which the manufacturer has only validated in serum.

Methods:

We enrolled in the study 51 patients with TBI and 85 patients without TBI. S100B concentrations were determined in 136 matching pairs of serum and lithium heparin blood samples. The analytical performances of the assay are evaluated according to its precision, accuracy, limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ), Cut off value, linearity, interference, and method comparison following the guidelines of the Clinical Laboratory Standards Institute. The concordance of the test results was assessed by linear regression, Passing Pablok regression, and Bland-Altman analysis. CT scans were performed as clinically indicated.

Results:

Coefficients of variation for within-run and between-day imprecision were 3.24%, 2.08 %, and 1.47 %, respectively for low-level (0.024 ng/ml), medium-level (0.170 ng/ml) and high-level (1.132 ng/ml) samples. The LoB was 0.011 ng/ml, LoD was 0.014 ng/ml, and the LoQ was 0.033 ng/ml. A linear relationship was demonstrated between 0.033ng/mL and 31.03 ng/mL (r2 =0.999). Method comparison shows that the assay correlates well with the reference method (r2 = 0.959), mean bias was -0.5% (95% CI -3.7% – 2.7%). With a sensitivity of 94.12% (84.08% - 97.98%) and a specificity of 69.41% (58.95% - 78.19%), the cut-off value was 0.105 µg/l. The assay is unaffected by icterus (bilirubin < 40 mg/dL), lipemia (Intralipid< 1500 mg/dL), and light hemolysis (Hb < 70 mg/dL).

Conclusion:

Plasma-based S100B tests showed acceptable performance and were comparable to serum-based tests. When quantifying the S100B protein in heparinized plasma, the hemolytic index should be considered.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-03

Reference Limit Estimator (RLE) – new functions and perspectives

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Introduction

The software tool “Reference Limit Estimator” (RLE), which has been developed since 2013, was further developed within the working group on reference limits. The tool is intended to allow simple and broad application for estimating reference limits

using indirect methods, both by scientific working groups and so-called routine laboratories that want to check their reference limits regularly.

Methods

The RLE uses the statistical software R for the analysis and Microsoft Excel as a user interface and for data checking and processing, such as cleaning the data for “first values” or “last values” or the targeted exclusion/inclusion of certain patient groups (e.g. pregnant women).

Results

In the latest version 50, the “Reference Limit Estimator” is adapted to the 64Bit environment and allows for a wide application spectrum of current R and Microsoft Office versions under Windows. Another significant advance is providing support for evaluation of the measurement stability over a period and the detection of drift effects.

The R algorithm uses the improved “Truncated Maximum Likelihood” model (TML) but is now open for the parallel integration of other models. Comparison of the estimated reference limits through the RLE with established reference limits, as well as calculation of confidence intervals of reference limits can be carried out. The RLE can now process very large data sets in a “Classic Mode”, which are outside the Excel limits.

In the meantime, the software has been evaluated for a large number of measurands with routine data.

Conclusions

The “Reference Limit Estimator” (RLE) provides users an up-to-date and easy-to-use tool. In addition to the established TML model, other models can now be integrated into the existing environment. The next goals are to 1) enable the user to establish continuous reference limits and 2) provide alternative user interfaces (e.g. Shiny) because of Excel limitations.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-04

Use of serum indices for the monitoring of sample quality

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Introduction

Serum indices (hemolysis (H), icteric (I) and lipemia (L)) are semi-quantitative indicators used in monitoring sample quality in clinical laboratories. The indices determination is mostly done by photometric measurement of automatic analyzers, usually by systems of clinical chemistry or coagulation. Automated systems for immunochemical analysis do not usually permit their recording.

The intended purpose is to identify interfering factors that significantly falsify a single analysis result depending on the method, and subsequently inform the clinician about the diminished significance of the value.

There are large differences in the definition of the index categories among manufacturers, resulting in discrepancies in observations, such as hemolysis rates. The aim of this study was to assess the influence of two distinct index classification schemes on result commentary, sample rejection, and the proportion of reported interferences.

Methods

A two-year dataset of HIL measurements was collected and analyzed based on submitters, class distribution of stages, and selected analytes. The study aimed to investigate the impact of two distinct serum index class schemes on result annotation and the subsequent analytical implications.

Results

The results indicate that different serum index schemes can yield varying observations, such as an apparent increase in low-grade hemolysis by either 11.7% or 40% more sample rejections for specific analytes. As per protocol CLSI EP7-A2, an interference factor test should involve at least two analyte concentrations and two concentrations of interfering factors, without necessarily requiring a dilution series. This leads to significant variability in bias reporting in assay data sheets, inaccurate transfer of interference assessment to the analytical result, and potentially premature sample rejection. The cross-manufacturer use of serum indices for quality assurance of analytical methods appears critical from this point of view. Therefore, the expanded application of HIL results in preanalytics for analytical methods of other assay manufacturers or platforms requires critical examination and raises the question of whether a semi-quantitative reporting of the serum indices is sufficient.

Discussion

The results of this study emphasize the necessity of a harmonized definition of serum indices as well as sufficient quality control to justify the quite permissible rejection of analytical results. The use of overly broad indices classes may lead to premature commentary and a higher proportion of reported analytical interference and unjustified rejection of samples as well as limitations in the cross-platform usability of serum-indices.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-05

Evaluation of the Fuji Dri-Chem NX700i Clinical Chemistry Analyzer including interference studies

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Introduction

The FUJI DRI-CHEM NX700i holds 28 colorimetric and 3 electrolyte test assays and analyzes up to 190 tests/hour (colorimetry + electrolytes). A STAT testing position is available. The colorimetric method slide is a multilayered slide composed of dry chemical ingredients needed for the reaction quantifying enzymes and substrates by colorimetric methods. The potentiometric method slide contains ion selective film electrodes. Calibration is done with QC cards except for CRP working with a calibrator. Each test needs 10µL of sample except for CRP (5µL) and ISE (50µL for all 3 tests).

Methods

In this study the Fuji Dri-Chem NX700i was evaluated using serum samples. The parameters investigated were albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), total bilirubin (TBIL), aspartate aminotransferase (AST/GOT), Alanine Aminotransferase (ALT/GPT), γ-glutamyltransferase (GGT), C-reactive protein (CRP), lipase (LIP), triglycerides (TG) and ammonia (NH3). Interferences from hemolytic, lipemic, icteric or highly elevated total protein samples were excluded according to the manufacturers method specific declarations and studied separately. Method comparison was done by measuring 50 samples for each analyte at the same time on the NX700i and the Siemens Atellica CH analyzer. For each parameter inter- (controls, n=10) and intra-assay (patient samples, n=10) coefficients of variation (CV) were calculated for low, medium and high concentrations. The manufacturer lists known interfering substances for every analyte. The measurement of CRP involves Glucose as part of the reactions and is listed as interfering substance above the concentration of 400 mg/dl (22.2 mmol/L). This was tested by measuring CRP at glucose concentrations from 150 mg/dl to 860 mg/dl. The effect of very low and high protein concentrations and haemolytic, icteric and lipemic samples were tested.

Results

Inter-assay CVs (n=10) were 1.1 to 7.7% (Low), 0.7 to 3.9% (Medium) and 0.8 to 5.5% (High). Intra-assay CVs (n=10) were < 0.01 to 9.5% (Low), < 0.01 to 3.7% (Medium) and 0.7 to 3.3% (High). Method comparison (Passing-Bablok Regression) of routine samples from hospital patients revealed good agreement of the two methods with correlation coefficients of r = 0.98 and higher except for LIP (0.83), NH3 (0.87) and Alb (0.93). In this study there were false low CRP values above Glucose values of 417 mg/dl.

Conclusion

The FUJI DRI-CHEM NX700i shows good intra- and inter-assay precision with low CVs and excellent correlation with the Siemens Atellica CH analyzer.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-06

Einfluss suboptimaler Präanalytik auf die labormedizinische Ergebnisqualität

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Zielsetzung: In bevölkerungsbezogenen Studien mit Bioproben sind die präanalytischen Möglichkeiten oft eingeschränkt und können die Genauigkeit von Labormessungen beeinflussen. Häufig bleibt unklar, welcher Anteil der beobachteten Unterschiede auf Defizite in der Präanalytik zurückzuführen ist. Der Einfluss suboptimaler Präanalytik auf die labormedizinische Ergebnisqualität wurde in einer Studie an ausgewählten Analyten systematisch untersucht.

Methoden: Von geschulten Untersuchungsteams wurden an 454 Personen aus der Bevölkerung (Alter 16-74 Jahre) durch venöse Blutentnahme je 2 EDTA-Vollblutröhrchen und 4 Serumröhrchen abgenommen und ins Epidemiologische Zentrallabor (Epi-Lab) des RKI gebracht. Ein EDTA-Röhrchen wurde nach der Abnahme gekühlt, das andere ungekühlt gelagert und transportiert. Zwei Serumröhrchen wurde nach 30-45 Minuten Standzeit bei Raumtemperatur 12 min lang mit 2500 x g zentrifugiert. Ein Röhrchen wurde anschließend bei 4 °C gekühlt, das andere bei Raumtemperatur gelagert. Auch von den nicht zentrifugierten Serumröhrchen wurde eines gekühlt und eines ungekühlt gelagert. Die Proben wurden einmal wöchentlich ins Epi-Lab gebracht. Hier wurden die nicht zentrifugierten Serumproben zentrifugiert und alle Proben bis zur Analyse gekühlt. Aus dem EDTA-Vollblut wurde HbA1c bestimmt (Tosoh HLC-723G8), aus den Serumröhrchen Gesamt-Cholesterol, HDL-Cholesterol, 25-OH-Vitamin D und Anti-HBs-IgG (Abbott Alinity). Die Messwerte der präanalytischen Varianten wurden vergleichend statistisch ausgewertet (Stata/SE 17.0), die Messwerte mit optimaler Präanalytik den Teilnehmenden mitgeteilt. Die Teilnahme erfolgte nach schriftlicher informierter Einwilligung, positive Datenschutz- und Ethikvoten lagen vor.

Ergebnisse: In die vergleichenden Auswertungen konnten 115 EDTA- und 116 Serumprobenpaare einbezogen werden. Die Zeitspanne zwischen Blutabnahme und Eintreffen der Proben im Epi-Lab betrug im Mittel 4,5 Tage (Spannbreite 1 - 7 Tage). Zwischen gekühlten und ungekühlten Proben fiel die Abweichung der Messwerte am stärksten aus beim HDL-Cholesterol (Median der Differenz 9,7 %), am geringsten beim HbA1c (Median der Differenz 0,9 %). Zwischen zentrifugierten und nicht zentrifugierten gekühlten Proben fiel die Abweichung der Messwerte am stärksten aus beim 25-OH-Vitamin D (Median der Differenz 3,5 %), am geringsten beim HDL-Cholesterol (Median der Differenz 1,8 %). Die Gesamtrangfolge zunehmender Abweichungen aufgrund fehlender Kühlung oder Zentrifugation lautet: HbA1c (Kühlung), Gesamt-Cholesterol (Kühlung), HDL-Cholesterol (Zentrifugation), Anti-HBs-IgG (Zentrifugation), Anti-HBs-IgG (Kühlung), Gesamt-Cholesterol (Zentrifugation), 25-OH-Vitamin D (Zentrifugation), 25-OH-Vitamin D (Kühlung), HDL-Cholesterol (Kühlung).

Diskussion und Schlussfolgerung: Für epidemiologische Studien unter erschwerten Feldbedingungen können bei bestimmten Fragestellungen Kompromisse in der Präanalytik akzeptabel sein, insbesondere bei der Kühlkette.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-07

Quantification of Interleukin 6 (IL-6) concentrations in the presence of soluble IL-6 receptor (sIL-6R) and soluble glycoprotein 130 (sgp130)

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Introduction: Interleukin 6 (IL-6) is a critical cytokine involved in numerous physiological and pathological processes. Effects of IL-6 on target cells are mediated via classic-signaling (involving membrane-bound IL-6-receptor, IL-6R) or trans-signaling, where IL-6 forms a complex with soluble IL-6R (sIL-6R) and this complex then binds membrane-bound gp130. While only few cells express IL-6R and hence are responsive for classic IL-6 signaling, gp130 is expressed on all cells. Soluble gp130 (sgp130) is found in the circulation and was shown to bind circulating IL-6/sIL-6R-complexes hence neutralizing IL6-trans signaling at low IL-6 concentrations. Whether inter-individual concentration-differences of sIL-6R and sgp130 influence analytical quantification of IL-6 remains to be elucidated.

Methods: We aimed to investigate the impact of different concentrations of sIL-6R and sgp130 on the quantification of IL-6 by using a commercial immunoassay (Roche Diagnostics GmbH) in the presence of varying concentrations of sIL-6R and sgp130.

Results: At low IL-6 concentrations (below 1 ng/ml) the presence of sIL-6R impaired the detection of IL-6. At IL-6 concentrations of 1 ng/ml or higher the interference caused by sIL-6R was no longer observed. Interestingly, the addition of sgp130 markedly reduced the detection of IL-6, regardless of initial IL-6 concentrations.

Conclusions: The presence of sIL-6R impairs the accurate quantification of IL-6 at low concentrations. As IL-6 concentrations increase the interference caused by sIL-6R diminishes. The addition of sgp130 significantly reduces measured concentrations of IL-6. Hence, plasma concentrations of sIL-6R and sgp130 need to be taken into consideration when measuring IL-6 levels. Further research is warranted to improve IL-6 measurements in the presence of sIL-6R and sgp130 and hence allow more accurate interpretation of IL-6 levels with regards to associated pathologies.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-08

Comparison of urine analytes measured with two different technologies (Vergleich einiger Urinanalyte gemessen mit zwei unterschiedlichen Technologien)

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Zielsetztung

Aufgrund des Konzepts der Rückführbarkeit auf Standards oder Referenzmethoden höherer Ordnung sollte im Vergleich von Technologien, die denselben Analyten mit unterschiedlichen Methoden messen, ähnliche Ergebnisse liefern. Derzeit liegen nur wenige Daten zum Vergleich der Ortho Vitros 7600-Plattform mit einem anderen häufig verwendeten System, nämlich Roche Cobas 6000, vor. Daher haben wir die beiden Analysesysteme verglichen, indem wir 6 häufig geordnete Analyten gemessen haben.

Methoden

Wir haben die Methoden zur Bestimmung von Creatinin, Harnsäure, Harnstoff, Natrium, Kalium und Phosphat auf Roche Cobas und Ortho Vitros evaluiert. Beide Analysegeräte wurden gemäß den Anweisungen des Herstellers betrieben. Wir haben die Präzision in der Serie und von Tag zu Tag auf dem Vitros-System mit im Handel erhältlichen Kontrollmaterialien (Ortho Performancce Verifier) überprüft. Zusätzlich haben wir Methodenvergleiche mit mehr als 100 Patientenproben durchgeführt, die den linearen Messbereich der Geräte abdeckten. Die Analytik erfolgte mit frischem Probenmaterial (Urin) auf beiden Instrumenten innerhalb eines Zeitunterschieds von 2 Stunden.

Ergebnisse

Unsere Ergebnisse zeigen, dass die Variationskoeffizienten in der Serie je nach Analyt und gemessener Konzentrationen zwischen 0.43 % und 1.91 % lagen. Zur Ermittlung der statistischen Daten beim Methodenvergleiche verwendeten wir die Passing-Bablok-Methode. Die Korrelationskoeffizienten (r) lagen zwischen 0,994 und 0,999. Die Steigungen der verschiedenen Parameter lagen zwischen 0,89 und 1,09. Die vom Hersteller vorgeschlagenen linearen Bereiche wurden getestet und bestätigt. Bei der Vitros-Technologie haben wir keine Verschleppungseffekte festgestellt, da Einweg-Pipettenspitzen und trägergebundene Reagenzien (Slides) verwendet werden.

Diskussion und Schlussfolgerung

Insgesamt sind die beiden unterschiedlichen Technologien zur Bestimmung von den hier untersuchten Analyten vergleichbar. Die Kalibrierung in Bezug zur einer Referenzmethode oder einem Kalibrator höherer Ordnung (sogenannte Rückführbarkeit) gewährleisten einen zuverlässigen Vergleich.

DGKL: 01. Analytische Qualität, Labormanagement

P-01-09

Nachhaltige Laborprozesse, smart konstruiert und umgesetzt

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Zielsetzung

In der Kürze der zur Verfügung stehenden Zeit und dem erforderlichen Fokus auf eine gezielte und personalisierte Diagnostik für die Patient:innen, ist es dem Labor in der Interaktion zwischen Einsender, der eigenen Analytik, Konsillaboren und institutionellen Empfängern (KBV, etc.) nicht immer möglich, das komplette diagnostische Potenzial auszuschöpfen. Ein Großteil der HR muss in manuelle Prozesse investiert und wesentliche verfügbare Informationen werden nicht in Anspruch genommen.

Wir wollen aufzeigen, wie die konsequente Nutzung der Digitalisierung und der vorhandenen Standards die Laborprozesse deutlich vereinfachen, konsequenter und genauer machen kann.

Dabei ist das Ziel das reibungslose Zusammenspiel der folgenden Komponenten :

- Arzt- und Krankenhausinformationssysteme
- Order- und Laborsysteme,
- Automation,
- Analytik,
- Validation und
- der Verzicht auf proprietäre Formate und Mechanismen.

Gemeinsam mit mehreren Marktteilnehmern wollen wir diesen papierlosen und medienbruchfreien Ablauf belastbar aufzeigen.

Abgedeckt werden sollten zudem die folgenden 4 Bereiche:

- Anforderung: Unterstützung des Einsenders in der Auswahl korrekter Parameter in einer intuitiven Oberfläche
- Validation: Unterstützung des Laborarztes oder der Laborärztin in der Befundung
- Befundauskunft: Hinweise und Möglichkeiten einer gezielten Reflextestung
- Bidirektionaler Austausch von Patienten- und Falldaten

Dabei soll auch auf das Change-Management im Labor und beim Einsender eingegangen werden.

Ergebnisse

Am Beispiel verschiedener Bereiche wie Endokrinologie, Gerinnung und Infektiologie wird aufgezeigt, wie einerseits

- Medizinische Hinweise
- Leitlinien
- Formelle Grundlagen (EBM-Regeln)

bei Anforderung und Bearbeitung berücksichtigt werden und damit Nachfragen, Falschanforderungen verhindert oder vermieden werden können und andererseits der Laborarzt bei der Validation der Ergebnisse ohne dabei Verluste durch Medienbrüche oder proprietäre Formatezu riskieren.

Diskussion und Schlussfolgerung

Für eine nachhaltige Implementierung wurde auf internationale Standards und Schnittstellen wie FHIR, Snomed und LOINC gesetzt. Dabei unterstützen eine moderne Software-Architektur, aber auch ein partnerschaftlicher Umgang / Mindset unter allen Projektbeteiligen. Im Vortrag sollen auch die Medizinprodukt Anforderungen kritisch beleuchtet und diskutiert werden.

DGKL: 02. Autoimmunerkrankung und Allergie

P-02-01

The emergence of myeloid-derived suppressor cells in a murine model of pulmonary fibrosis

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Background: The immune system plays a major role in pulmonary fibrosis. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of innate immunity with remarkable immune suppressive functions. We hypothesize that the strong anti-inflammatory activity of MDSCs may dampen pulmonary fibrosis by inhibiting local inflammation and its transition to lung fibrosis. Aim: Here, we aim to investigate the emergence of both polymorphonuclear (PMN)- and monocytic (M)-MDSCs and their pattern of generation, activity and recruitment in pulmonary fibrosis. Method: Murine model was performed by administration of bleomycin in C57BL/6 mice. Immunological, histopathological and clinical changes of mice respiratory tract were investigated at days 3, 7, 14, and 21 after bleomycin challenge. Results: Strikingly, we found entirely different pattern of PMN- and M-MDSCs generation and recruitment after bleomycin challenge. The number of PMN-MDSCs was increased on day 3 reaching a maximum on day 21 after bleomycin challenge. However, while the number of M-MDSCs were higher on day 3, their numbers decreased over time reaching to the minimum on day 21. The suppressive activity of PMN-MDSCs was mainly pronounced on day 3, but M-MDSCs retained their suppressive activity from day 3 to day 21. The changes in the number and activity of MDSCs were also correlated with lung histopathological and respiratory mechanics changes. Conclusion: We showed that the number and activity of PMN- and M-MDSCs are different from each in acute inflammatory and fibrotic stages. These findings suggest that MDSCs are involved in modulating the immunological, histopathological and functional changes in pulmonary fibrosis.

Keywords: Myeloid-derived suppressor cells, Animal model, Pulmonary fibrosis

DGKL: 02. Autoimmunerkrankung und Allergie

P-02-02

The role of Arginase 1 therapy on myeloid-derived suppressor cells in a murine model of chronic asthma and influenza virus-induced asthma exacerbation

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Background: Asthma exacerbation leads to the worsening of respiratory symptoms, often induced by respiratory viral infections. Myeloid derived suppressor cells (MDSC) play a crucial role in inflammatory diseases such as asthma by suppressing the T-cell response. Methods: Here, we aim to investigate the efficacy of Arginase 1 (Arg-1), one of the key products of MDSCs, on airway inflammation and responsiveness in a chronic murine model of house dust mite (HDM) induced asthma and Influenza virus (IAV) H1N1 A, /Puerto Rico/8/1934 induced asthma-exacerbation. The mice were treated intraperitoneally with Arg-1, three times per week for five weeks. Lung-function, immunological, biochemical and histopathological analysis were performed three days after last HDM or IAV challenge. Results: We demonstrated that the number and suppressive activity of MDSCs on T cells are altered between experimental and therapy groups. In vivo lung function studies showed a distinct improvement of lung function parameters in Arg-1 treated exacerbated asthmatic mice. Histopathological and immunological analyses correlated with these findings. Conclusion: Our results provide evidence that MDSCs and their main product Arg-1 play a crucial role in the pathogenesis of chronic asthma and IAV induced asthma exacerbation. Arg-1 treatment impacts immunological, functional, and histopathological features in the lung, improving airway inflammation and responsiveness in asthmatic and exacerbated asthmatic mice.

DGKL: 02. Autoimmunerkrankung und Allergie

P-02-03

The Role of Myeloid-Derived Suppressor Cells in a Murine Model of Influenza Virus-Induced Asthma Exacerbation

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Rationale: Myeloid-derived suppressor cells (MDSCs) are a phenotypically heterogenous group of cells that potently suppress the immune response. There is a growing body of evidence supporting the important role of MDSCs in a variety of lung diseases, such as asthma. However, the role of MDSCs in asthma exacerbation has not been investigated. Here, we studied the role of MDSCs in a murine model of influenza virus-induced asthma exacerbation.

Methods: BALB/c mice were exposed to house dust mite (HDM) three times a week for a total of five weeks to induce a chronic asthmatic phenotype. Asthma exacerbation was then induced by additional exposure to the A/Hamburg/5/2009 hemagglutinin 1 neuraminidase 1 (H1N1) influenza virus. Number and suppressive activity of polymorphonuclear (PMN-) and monocytic (M-) MDSCs were assessed on different time points.

Results: Analysis of the bronchoalveolar lavage fluid (BALF) revealed an upregulation of Th2 cytokines (IL4, IL-5 and IL-13) in asthmatic mice and an increase of IFN-γ and TNF-α levels in exacerbated asthmatic mice. Lung inflammatory features were found to be significantly increased in asthmatic mice, while lung function was significantly impaired in exacerbated

asthmatic mice. Both the number and suppressive activity of MDSCs were significantly increased in asthmatic mice. The number of lung M-MDSCs was significantly increased on day 1 in exacerbated asthmatic mice compared to non-exacerbated mice. Furthermore, we observed a trend of increased number as well as suppressive activity of lung MDSCs on day 1 and day 6 in exacerbated compared to non-exacerbated asthmatic mice.

Conclusion: These findings further establish the role of MDSCs in the pathogenesis of asthma. Here, we provide some of the first evidence supporting a potential important role of MDSCs in asthma exacerbation. Additional experiments such as depletion, adoptive transfer and single cell analysis of MDSCs are ongoing to further define the role of MDSCs in asthma exacerbation.

DGKL: 07. Herzkreislauferkrankungen

P-02-04

Quantitation of plasma arginine and dimethylarginines by LC/MS/MS: Potential cardiovascular biomarkers for antiphospholipid syndrome

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Introduction: Endothelial dysfunction (ED) is a maladapted endothelial phenotype characterized by reduced nitric oxide bioavailability, increased oxidative stress, elevated expression of proinflammatory and prothrombotic factors and reduced endothelium-derived vasodilation. Autoimmune antibodies could cause ED and development of cardiovascular complications in patients with antiphospholipid syndrome (APS), rheumatoid arthritis (RA) or lupus erythematosus (LE). The mechanism whereby autoantibodies increase vascular permeability is not completely characterized. Antiphospholipid antibodies are commonly binding the surface of resting endothelial cells and this in turn leads to endothelial activation that triggers a pro-oxidative state. It was hypothesized that asymmetric dimethylarginine (ADMA) and its metabolites acts as a major causative factor in the induction of ED via a pathway of increased endogenous nitric oxide synthase. We aimed to evaluate the utility of plasma ADMA levels as potential cardiovascular biomarkers in the development of autoimmune-associated CVD.

Methods: The development and validation of a liquid chromatography-tandem mass spectrometric (LC/MS/MS) method for quantification of plasma levels of ADMA and other related molecules in ADMA pathway was performed. A LCMS8060NX coupled to Nexera (TM)X2 UHPLC (both Shimadzu Corporation) was used. The plasma samples were obtained from 50 patients and divided into 5 equal groups: I –APS patients with lupus anticoagulant (LAC), II –APS-patients with anticardiolipin and anti-beta2-glycoprotein 1 antibodies, III –APS- and RA- or LE-patients, IV –RA- or LE-patients and V –healthy volunteers. Sample preparation was performed by ultrafiltration using a 30 kDa Filter (Vivaspin 500 Sartorius Stedim Lab Ltd. UK).

Results: Arginine and ADMA plasma levels were increased in APS patients in relation to RA and LE patients. Our results also revealed that the plasma ratio of arginine and ADMA, an indicator of physiological arginine methylation status, was significantly elevated in patients with APS and RA/LE.

Conclusion: This finding might help to identify endothelial dysfunction in the management of autoimmune diseases.

DGKL: 07. Herzkreislauferkrankungen

P-02-05

Dysregulation of telomeric genes in Hutchinson Gilford Progeria

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Introduction

Hutchinson Gilford Progeria (HGP) is a model disease for premature aging and premature atherosclerosis. Due to a mutation in lamin A, the cell nuclear membrane is pathologically altered. As human telomeres are attached to the nuclear matrix, we systematically investigated the gene expression of potential TPE-OLD (telomere position effect over long distances) candidate genes, that is, genes whose expression may be regulated via telomeric looping structures. In preliminary work, it was shown that at least one TPE-OLD gene, PPP2R2C, is dysregulated in HGP (1).

Methods

Genes whose expression was increased or decreased in HGP in gene array studies were compared to genes whose expression was increased or decreased in stress-related aging. Gene arrays were performed using Affymetrix gene expression chip HG U133 Plus 2. Non-replicative stress was induced by UV-B radiation (6 x 88 J / m2 over three days). TPE-OLD genes were identified using a recently developed method (https://tpe-old.uni-rostock.de/). Gene enrichment analysis was performed with g:Profiler (2). To investigate whether telomere lengthening influences the outcome, fibroblasts were immortalized with the catalytic subunit of human telomerase and cells were passaged up to at least 2-fold telomere extension.

Results

Genes overexpressed in HGP relative to healthy control cells were enriched in TPE-OLD genes (p=0.0179). A similar result was obtained for hTERT immortalized HGP (p=0.0023), even though the gene expression changes were in most cases lower in magnitude. By contrast, differentially expressed genes in cells treated with UV-B were not enriched in TPE-OLD genes. Differentially expressed TPE-OLD genes in HGP often feature biological processes such as development, differentiation and cell cycle, including transcription-factor-mediated processes.

Conclusions

The data suggest that telomere dysfunction contributes to gene expression changes in HGP, even if the mean telomere length is not notably changed. The dysregulation of genes that regulate development and growth, in particular, is important both for understanding the pathology and for new therapeutic approaches.

DGKL: 07. Herzkreislauferkrankungen

P-02-06

Biomarker für die Langzeitprognose von Patienten mit stabiler koronarer Herzkrankheit

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Zielsetzung

Risikofaktoren der koronaren Herzerkrankung sind heute gut untersucht. Für Biomarker, die als Folge einer koronaren Herzerkrankung in das Blut gelangen, besteht dagegen noch weiterer Untersuchungsbedarf. Unsere Studie hatte die Prüfung zum Ziel, ob in der Klinik etablierte Biomarker wie Troponin, NTproBNP, Copeptin, Interleukin-6 und CRP geeignet sind, um

a) zwischen koronargesunden und koronarkranken Patienten zu unterscheiden und

b) eine Aussage zu dem Langzeit-Mortalitätsrisiko (10 Jahre) von Patienten mit stabiler KHK zu machen.

Methode

Wir haben in der Patientenkohorte der Leipziger LIFE-HEART Study Biomarker mit potentieller diagnostischer Wertigkeit für die KHK untersucht. Hierzu analysierten wir das Blut von 3072 Patienten (davon 36% Frauen) mit Verdacht auf KHK und einer mittleren Nachbeobachtungszeit von 10 Jahren. Alle Patienten erhielten zum Zeitpunkt des Studieneinschlusses eine medizinisch indizierte Koronarangiographie. Zu den untersuchten Biomarkern gehörten hs-cTNT, NT-proBNP, Copeptin, hsCRP und Interleukin-6.

Die Patienten wurden nach dem Schweregrad der KHK stratifiziert: CAD 0 (keine Koronarsklerose), CAD1 (nicht-obstruktiv, d.h., Stenose < 50%) und CAD 2 (≥ eine Stenose ≥ 50%). Zwei Gruppenvergleiche (GC) der Biomarker in Gruppen mit unterschiedlichem Schweregrad der koronaren Herzerkrankung wurden analysiert: GC1: CAD0 + 1 vs. CAD2 und GC2: CAD0 vs. CAD1 + 2.

Die Schwergrad der Stenosierung CAD0, CAD1 und CAD2 wurden bei 1271, 631, bzw. 1170 Patienten festgestellt.

Ergebnisse

Unter Berücksichtigung der klassischen Risikofaktoren, unterschieden sich hs-cTnT, NT-proBNP und IL-6 signifikant in beiden GC. Diese drei Biomarker erlaubten in der univariaten Analyse Patienten mit schwerer KHK von Patienten ohne KHK zu unterscheiden. hsCRP ermöglichte zusätzlich in GC2 die Unterscheidung von Patienten ohne KHK von Patienten mit leichten und schweren Koronarstenosen.

Nach der multivariaten Analyse mit Einbeziehung aller Biomarker und Einflussfaktoren blieben hs-cTnT, NT-proBNP und IL-6 in GC1 signifikant. In GC2 erreichten hs-cTnT ($p < 0,001$) und Copeptin ($p = 0,014$) Signifikanz.

Die 10-Jahresüberlebensrate in den Gruppen CAD0, CAD1 und CAD2 betrug 88,3%, 77,3% und 72,4%. Durch die Einbeziehung von hs-cTnT, NT-proBNP, Copeptin und IL-6 wurde die Risikovorhersage verbessert ($p < 0,001$).

Schlussfolgerung

Die untersuchten Biomarker hs-cTNT, NT-proBNP, Copeptin, hsCRP) und Interleukin-6 können zu einer präzisen Identifizierung des Schwergrades der KHK und des Mortalitätsrisikos bei KHK-Patienten beitragen. Die schnelle Zugänglichkeit der Biomarker Diagnostik in der Klinischen Routine-Analytik erlaubt jetzt eine Translation dieser Befunde in die Klinik für eine primäre und sekundäre KHK-Prävention. Unsere kürzlich publizierte Studie (Netto J et al, Nutrition 2022) zählt zu den weltweit größten Langzeitstudien über 10 Jahre von Patienten mit stabiler KHK, wie dies kürzlich in einer Metaanalyse gewürdigt wurde.

DGKL: 07. Herzkreislauferkrankungen

P-02-07

C-terminal peptides of α-1-antitrypsin as putative biomarker in PiMM and PiZZ COPD patients

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Alpha-1-antitrypsin (AAT) is an acute phase glycoprotein and broad spectrum anti-protease that is pivotal to keep balance between protease/antiprotease, especially in the lungs. Therefore, inherited severe PiZZ (Gly342Lys) deficiency of AAT (AATD) represents a genetic risk factor for early onset COPD with emphysema. In fact, COPD patients with normal PiMM variant of AAT have 80 to 220 mg/dl of plasma AAT protein whereas PiZZ patients have about 57 mg/dl of AAT. The dominant theory for AATD-related COPD is an imbalance in protease/anti-protease ratio. Therefore, augmentation therapy (AATat) with human plasma purified pharmaceutical preparations of AAT is used to retard disease progression.

In inflammatory environments, AAT ca be subjected to (un)specific proteolytic cleavage, resulting in generation of carboxyl(C)-terminal peptides. Peptides and other post-translationally modified forms of AAT have received little attention in clinical research areas, although these form might express unique biological activities and be useful as biomarkers. Recently, we developed a novel UPLC-MS/MS multiplex approach to quantify AAT peptides and found significant upregulation in acute inflammatory diseases, like ARDS. Plasma levels of AAT peptides have not been investigated in chronic inflammatory conditions, like COPD. Therefore, we conducted a single-center study to learn whether peptides of AAT are present in plasma of COPD patients, and if yes, whether these putative peptides are specific for genotype related COPD.

We enrolled 111 clinically stable and well-matched COPD patients, including 67 PiMM and 44 PiZZ patients, of which 21 received standard AATat. As expected, plasma AAT levels were lower in PiZZ than PiMM patients (mean±SD, 28±9 vs. 152±26 mg/dl, $p < 0.001$), but rose after augmentation therapy. According to our results, C-terminal peptides of AAT, particularly C36 and C42 peptide, are detectable in PiMM (0.075±0.047 and 0.086±0.035 µM) but not in PiZZ COPD patients (below LLOQ). In line, C36 and C42 were measurable in plasma of PiZZ patients receiving AATat while concentrations achieved ~50% of PiMM patients. In the complete patient cohort, we found a strong positive correlation between AAT and C36 or C42 plasma concentrations suggesting that proteolytic cleavage of AAT is a source of peptides.

We show that C-terminal peptides of AAT differ in PiMM and PiZZ COPD patients, which allows us to suggest that these peptides could be used to discriminate between AAT variants in COPD and might reflect efficiency of AATat in PiZZ patients. Furthermore, peptides might be valid as novel biomarkers for prediction and/or characterization of exacerbations in COPD patients.

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DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-03-01

BioRef: A national infrastructure for generating precise reference intervals for diagnostic medicine

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Introduction/Aim: Reference intervals for patient test results are in standard use across many medical disciplines, allowing physicians to identify potentially pathological test results with relative ease. The process of inferring cohort-specific reference intervals is, however, often ignored due to the high costs and cumbersome effort associated with such a task. Determining reference intervals based on data collected during daily clinical routine using fully automated computational resources may help to lower the associated costs and personalize the reference intervals to the respective cohort population and enhance patient care.

Methods: With the BioRef project, we have developed a multi-center computational framework, where specialized web applications estimate and assess patient group-specific reference intervals based on clinical routine data from four Swiss Hospitals. We have established a common legal governance and interoperability framework for our clinical partners to share their data either to a central database via a national and secure data sharing network or providing their data in a

decentralized way via “TI4Health”, a secure and encrypted data-accessing system, allowing each data provider to abide to the restrictions laid out by their ethics waivers.

Results: The deployed web applications, which allow intuitive and interactive data stratification by patient factors (such as age, administrative sex and personal medical history) and laboratory analysis features (such as device, analyzer and test kit identifier) are accessible for registered physicians and researchers. As we are evaluating our deployed framework, we are currently establishing the onboarding of future national and international partners, refining the statistical analysis for multi-cohort patient queries and adjusting the web-interfaces to build clinically viable diagnostic tools.

Conclusion: We present that establishing an opportunity for clinical physicians and researchers to define precise reference intervals in a convenient and reproducible way on-the-fly is a vital part of practicing precision medicine today.

DGKL: 03. Biobanking

P-03-02

Analyzing the protein content in human milk for the effects of different pasteurization methods

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Introduction

In the setup of human milk banking, donated human milk (HM) is frequently pasteurized to reduce potential pathogens and to ensure the safety of premature infants. However, it is known that heat treatment can affect the protein composition of HM. In this work, alterations of selected whey proteins were investigated after the application of three different pasteurization approaches.

Methods

Breast milk samples (n = 15) were Holder-pasteurized (62.5 ± 0.5 °C for 30 min) using either water bath (WB-HoP) or dry temperature (DT-HoP) treatment or were subjected to High Temperature Short Time Treatment (HTST; 62 °C for 5 sec). To enable protein analysis, samples were pretreated by filtration and centrifugation. In the resulting whey, secretory immunoglobulin A (sIgA) and lactoferrin (LF) concentrations were determined by commercially available enzyme-linked immunosorbent assays before and after heat treatment. Alkaline phosphatase activity (ALP) was measured via enzyme activity assay (BioVision, Milpitas, CA, USA).

Results

Both HoP methods resulted in almost a complete decrease of ALP activity (WB-HoP = 0.3 ± 0.4 %, DT-HoP = 0.5 ± 0.4 %), whereas after HTST 52.8 ± 12.2 % was retained (all $p < 0.001$). The sIgA retention was significantly higher after WB-HoP (73.2 ± 13.5%) and after HTST (80.4 ± 22.7 %) than after DT-HoP (57.0 ± 14.4%, all $p < 0.01$). In terms of retention of LF, the two HoP methods did not differ significantly (WB-HoP = 47.0 ± 40.0 % vs. DT-HoP = 25.0 ± 9.7 %). Compared to both HoP methods, HTST showed significantly higher retentions of LF (69.9 ± 41.8 %, all $p < 0.01$).

Conclusion

Holder pasteurization by dry tempering (DT-HoP) seems to have a stronger impact on the quality of human milk than the other two approaches (WB-HoP, HTST). In terms of the protein retention, HTST seems to be a good alternative to the current gold standard HoP.

DGKL: 03. Biobanking

P-03-03

Impact of centrifugation and storage conditions on non-fluorescent and fluorescent Nanoparticle Tracking Analysis in plasma blood samples

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BACKGROUND-AIM

Stability of small extracellular particles (SEP, 45-1000nm) under different pre-analytical conditions is a prerequisite for (Fluorescent) Nanoparticle Tracking Analysis (NTA) in blood samples acquired by health integrated biobanking (HIBB). In this study the impact of different pre-analytical conditions typical for HIBB on (Fluorescence) Nanoparticle Tracking Analysis (NTA) are investigated.

METHODS

Blood was drawn from healthy volunteers in collection tubes with three different anticoagulants (n=10 for each anticoagulant) and centrifuged with 2000g or 4000g. NTA measurement was performed either directly after centrifugation, after storage at room temperature for four hours (2000g) and after freezing at -80C and thawing (2000g). For fluorescent NTA, samples were pre-incubated with the lipophilic fluorescent dye CellMask™ Green.

ZetaView device (Particle Metrix) was used for NTA quantification of small extracellular particles.

RESULTS

Albeit different volunteers donated blood the different anticoagulation tubes, the mean concentration of small extracellular particles was similar in all three tubes after direct measurement indicating a minor influence of the anticoagulant used.

Only in the citrate tube, a (non-significant) drop in SEP concentration after increased centrifugation speed was observed in the non-fluorescent NTA measurement. Although not always significant, there was a clear tendency of loss in SEP concentration after four hours of storage in all the three different collection tube types. There was no loss in SEP concentration after a freeze-thaw cycle.

There was no significant difference in SEP concentration in the fluorescent NTA after increased centrifugation speed, prolonged storage or a freeze-thaw cycle. This was true independent from the tube anticoagulant used.

CONCLUSIONS

The results are to inform researches planning large-scale NTA measurements in samples acquired by HIBB.

In general, NTA in fluorescence mode seems to be less affected by different pre-analytical conditions compared to non-fluorescence NTA and therefore more suitable for HIBB.

DGKL: 03. Biobanking

P-03-04

A Two-Step Procedure for Timely and Precise Identification of Hospitalized Diabetic Patients

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Background and Introduction

Automated sample collection from diabetic hospitalized patients for biobanking can be challenging due to incomplete patient data at the time of sample selection. This study evaluates a two-step procedure based on machine learning algorithms and natural language processing for timely and precise identification of diabetic patients in the context of healthcare-integrated biobanking.

Method

In the first step of the procedure, logistic regression (LR), and conditional inference forest (CIF) models were trained for the early identification of diabetic individuals on a training dataset (n=550) containing laboratory values from the first 72 hours of hospital stay. Models were then evaluated in a test dataset (n=235) together with a simple rule based laboratory cut-off classifier (LCC). In the second step, laboratory parameters blood glucose and HbA1c, ICD-10 codes or information from discharge summaries extracted by a natural language processing software (NLP-DS) were evaluated for the removal of false positive samples to achieve optimal overall specificity and precission of sample selection.

Results

In the test dataset, evaluation metrics (recall/precision/F1-score) were 71%/86%/0.78 for CIF, 77%/70%/0.74 for LR, and 73%/68%/0.70 for LCC. NLP-DS was the best performing second (review) step (93%/100%/0.97). Combining first-step models with NLP-DS resulted in overall metrics 66%/100%/0.80 for CIF&NLP-DS, 72%/100%/0.84 for LCC&NLP-DS, and 66%/100%/0.80 for LR&NLP-DS. The total case/sample removal rate after the review step was 13% (CIF&NLP-DS), 33% (LR&NLP-DS) and 35% (LCC&NLP-DS).

Conclusion

A machine learning–based two-step procedure seems to be an efficient method for timely identification of diabetic patients and highly precise sample selection enabling targeted automated sample collection for healthcare-integrated biobanking.

DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-03-05

In silico characterization of high-fluorescent cells from cerebrospinal fluid: a supportive diagnostic tool for clinical decision

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Introduction: Automated cytological analysis of cerebrospinal fluid (CSF) represents a convenient platform for accurate and fast quantification of total nucleated cells. These are subsequently classified by a laboratory analyzer as mononuclear (MN: lymphocytes, monocytes), polymorphonuclear (PM: e.g. granulocytes) and high-fluorescent (HF: e.g. plasma cells, erythro-/macrophages, and tumor/malignant cells). CSF HF cells detected on an automated hematology analyzer are currently considered as a research laboratory parameter. Nevertheless, studies have already suggested a diagnostic association with defined clinical patterns. Hence, there is a clinical need for sustained in silico characterization of CSF HF cells combining multiparametric laboratory results with clinical data in well-established patient cohorts.

Aims and Methods: The main goal of the study was to improve the certainty of clinical diagnoses of neurologically associated diseases including HF cells as novel supportive diagnostic criterion. Thereby, we aimed to analyze the diagnostic capability of HF cells characterizing HF cell positive versus HF cell negative cases assigned to various neurologic diagnoses groups such as inflammation, hemorrhage, neoplasia and others.

Results: The results obtained by comparative cytological analysis of manual and automated cell differentiation correlated well for each CSF cell subtype MN, PMN and HF (R2 = 0.85, 0.87 and 0.61, respectively). Diagnosis based correlation values for manual and automated cell differentiation ranged between R2 = 0.66 and 0.95 for MN, R2 = 0.71 and 0.95 for PMN, and R2 = 0.05 and 0.89 for HF showing an insufficient correlation for central nervous system (CNS) associated inflammation (R2 = 0.05), hemorrhage (R2 = 0.22) and other diagnoses (R2 = 0.11) for the HF cell subtype. The correlation coefficient for HF cells in CNS associated neoplasia group was 0.89 indicating diagnostic relevance of HF cells for patients with malignancy. Finally, considering HF cell positive versus HF negative cases underlying a multiparametric analysis with focus on each individual diagnosis group, we assume a supportive predicting and/or diagnostic capability for CSF HF in defined clinical settings.

Conclusion: CSF HF cells detected on a laboratory hematology analyzer seem to have a prognostic and/or diagnostic value for particular diagnoses groups. However, additional laboratory CSF parameters to be included in the clinical decision might further sharpen the multiparametric analysis approach being essential for a reliable diagnostic prediction.

DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-03-06

A general purpose heatmap function in the R environment

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Introduction: Visualisation of high dimensional data remains one of the main challenges in communicating results. Especially in the context of transcriptional data, but also in applications like multiplexed ELISAs, the visual presentation becomes more difficult to manage. For this problem, the heatmap has been established as one of the methods of choice. The ability to display more than 100 variables as well as additional annotational information without creating clutter sets it apart from other methods like bar charts or radar plots. While there are software solutions, they remain poorly optimised and ill fit for customisation. In this project, a software solution has been generated to create heatmaps with a focus on ease of use, customisability, and reproducibility.

Methods: The R programming language is used with a focus on the tidyverse family. The visualisation is created with the ggplot2 package.

Results: Given a table with the data do be visualised, the package will create a heatmap with a default blue-red colour gradient. By default, the first column is expected to be the identifiers for the variables so be plotted on the x-axis, while the rest of non-numeric columns are defaulted to be annotation columns. Rows and columns can be clustered with a variety of distance measures. The returned object can be further customised using the ggplot2 framework; such customisations include different colour gradients, distance measures for clustering, normalisation method and colours for as well as number of annotations.

Conclusion:The software presented here has the goal to drive research through providing an easy to use and highly customisable framework with which to create heatmaps and help in visualisation of high dimensional data. It will support the understandability of high dimensional data and support the interpretation as well as hypothesis generation for any kind of research projects.

DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-03-07

Continuous reference intervals determined with the Shiny application AdRI

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Introduction: Reference intervals are an important part of the interpretation of medical laboratory results. Especially in children and adolescents, their limits sometimes can change very rapidly with age [1]. We suggest continuous methods to better represent the age-dependent progression of reference intervals. A user-friendly Shiny application called AdRI (Age-dependent Reference Intervals), available at https://github.com/SandraKla/AdRI, has been developed for this purpose.

Methods: Generalized additive models for location, scale, and shape parameters (GAMLSS) were developed by Rigby and Stasinopoulos and implemented in the gamlss R package, which provides a variety of features and capabilities for univariate statistical regression modelling and statistical learning [2]. The purpose of the Shiny application AdRI is illustrated using 40 biochemical markers from the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) [3].

Results: Depending on the additive term used, we obtain different smoothed percentile curves of laboratory values. For alkaline phosphatase (ALP), the GAMLSS with P-splines for females and with Decision Trees for males has the lowest Generalized Akaike Information Criterion (GAIC). All models recognize that the range for ALP of the models is wider after birth and then narrows to follow a relatively constant course over a long period of childhood. With the onset of puberty, the range widens again for both sexes and decreases sharply towards adulthood.

Conclusion: We demonstrate the superiority of continuously modeled reference intervals compared to fixed age groups and provide the Shiny application AdRI to make the technique easily accessible to clinicians and other experts. The influence of pathological values, hyperparameters and the distribution of data over age should be the subject of further investigation, given that the database of laboratory values in newborns and children is generally small.

DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-03-08

A machine learning-derived, blood count based algorithm improves prediction of sepsis

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Introduction: Delay in diagnosing sepsis results in potentially preventable deaths. Mainly due to their complexity or limited applicability, machine learning models to predict sepsis have not yet become part of clinical routines. For this reason, we created a machine learning model that only requires complete blood count (CBC) diagnostics.

Methods: Non-intensive care unit (non-ICU) data from a German tertiary care centre were collected from January 2014 to December 2021. Patient age, sex, and CBC parameters (haemoglobin, platelets, mean corpuscular volume, white and red blood cells) were utilised to train a boosted random forest, which predicts sepsis with ICU admission. Two external validations were conducted using data from the Greifswald University Hospital and the Medical Information Mart for Intensive Care IV database (MIMIC-IV). Using a subset of laboratory data including also procalcitonin, an analogous model was trained with procalcitonin as an additional feature.

Results: After exclusion, 1,381,358 laboratory requests (2016 from sepsis cases) were available. The derived CBC model shows an area under the receiver operating characteristic (AUROC) of 0.872 [CI: 0.857–0.887] for predicting sepsis. External validations show AUROCs of 0.805 [CI: 0.787–0.824] for the Greifswald University Hospital and 0.845 [CI: 0.837–0.852] for MIMIC-IV. The model including procalcitonin revealed a significantly higher performance [AUROC: 0.857; CI: 0.836–0.877] than procalcitonin alone [AUROC: 0.790; CI: 0.759–0.821; $p < 0.001$]. Thus, the CBC model can facilitate early sepsis prediction in non-ICU patients with high robustness in external validations.

Conclusion: Our results demonstrate that a machine learning-derived algorithm based on routine CBC results improves diagnosis of sepsis, allowing earlier detection of patients at risk. Its implementation in clinical decision support systems has strong potential to provide an essential time advantage and increase patient safety.

DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-03-09

Comparison of electronic acute kidney injury alerts for identification of patients at risk of adverse outcomes

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Introduction:

Creatinine based algorithms for electronic alert systems (e-alerts) to detect in-hospital acute kidney injury (AKI) are discussed to be an effective a way for identification of patients at risk of adverse outcomes. A variety of algorithms has been used in studies. Acute kidney disease (AKD) has been described as prolonged kidney damage after acute kidney injury. The association between e-alerts and AKD at hospital discharge has not been studied yet for different algorithms.

Methods:

Creatinine records from a tertiary university hospital were analyzed retrospectively to directly compare five different algorithms for e-alerts. Endpoints were in-hospital mortality or dialysis and AKD at hospital discharge.

Results:

From 6580 hospitalized patients, 2395 (36.4%) had one or more e-alerts. The algorithm that most closely reflects current KDIGO 2012 definition for AKI generated more often (30.9%) and in sum earlier e-alerts as compared to each other algorithm. In addition, the KDIGO-mimicking e-alert had the highest odds ratio for mortality and dialysis during hospital stay before (OR 12.0 CI [9.6-15.0]) and after (OR 6.2 [4.8-8.1]) adjustment for co-variables. After stratification for increase of creatinine (stadium I-III) and combination with another e-alert the modified version of the KDIGO-mimicking e-alert had the highest odds ratio (41.1[25.8-65.7], 57.3[32.8-65.7], 163.0[79.2-335.6] for stadium I,II and III respectively) for AKD at hospital discharge compared to the other e-alerts after adjustment for co-variables.

Conclusion:

All e-alerts can detect patients at increased risk of adverse outcomes during hospital stay (mortality and dialysis) and at discharge from hospital (AKD). A simple modification of the best performing alert (mimicking KDIGO definition for AKI) can further improve risk prediction for AKD at discharge.

DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-03-10

Classifictaion of Mass Spectrometry Profiles of Organic Acids Using Statistical Models

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Background and Introduction

The use of statistical models in conjunction with spectral data like infrared spectroscopy or mass spectrometry data has, in medical and scientific contexts, enabled automated classification with high accuracy, precision and recall.

We theorize that machine learning models can be used to analyze mass spectrometry data of organic acids and distinguish between spectra indicating physiological and pathological states.

Methods

79 organic acid mass spectrometry profiles derived from quality control assessment data were used to train and test a random forest model.

Labels for physiological and pathological spectra as well as different disease entities were assigned to the data based on the correct diagnosis according to the respective quality control assessment documents as well as the interpretation of the spectra by a physician.

The data was preprocessed and spectra with large variation of the average area of non disease related sample peaks after normalization to the internal reference standard were excluded.

The data set was balanced by random undersampling and split into training and test data with a ratio of 3 to 1.

A random forest model was designed using the Python Scikit-learn library. Hyperparameters were optimized. The model was trained to classify into physiological and pathological spectra using the training data set and model performance was evaluated using the test data set with accuracy, F1 score, precision and recall as performance metrics and five fold cross validation.

Results

Using quality assesment data an accuracy of 92,8% was reached for the distinction between physiological and pathological spectra using a random forest model. The F1 score reached was 0.92 with a precision of 93% and recall of 87 %.

Conclusion

Machine learning models can be used to analyze mass spectrometry data of organic acids and distinguish between spectra indicating physiological and pathological states with high accuracy and precision.

To improve and validate our model we plan on creating larger datasets using data available from routine measurements of organic acid mass spectrometry profiles.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-01

Associations of lipoprotein subclasses with bone turnover and bone strength in the general population

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Introduction: Dyslipidemia was previously not only reported to be a cornerstone of atherosclerosis but also to be related to low bone mineral density, fractures and the occurrence of osteoporosis [1, 2]. Indeed, circulating low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations seem to be involved in bone remodeling [3, 4]. While LDL-C and HDL-C concentrations are regularly measured in patient care a more detailed picture on an individual's lipoprotein status can be obtained by analyzing lipoprotein subclasses [5]. The aim of the present study was to examine whether lipoprotein subclasses are associated with bone turnover and strength.

Methods: Lipoprotein subclasses were determined by nuclear magnetic resonance (NMR) spectroscopy in samples from 794 men, 335 premenopausal and 387 postmenopausal women who participated in the Study of Health in Pomerania (SHIP-TREND-0). The associations between the lipoprotein subclasses (exposure) and the serum concentrations of two bone turnover markers (intact amino-terminal propeptide of type I procollagen (PINP) indicating bone formation, and C-terminal telopeptides of type I collagen (CTX) indicating bone resorption) and the ultrasound-based heel stiffness index were analyzed using sex-specific linear regression models. Whenever significant interactions with menopausal status were observed, analyses were performed separately for pre-and postmenopausal women. All models were adjusted for age, waist circumference, physical activity, diabetes mellitus, high-sensitivity C-reactive protein concentration and measurement device and a correction for multiple testing was applied.

Results: In general, in men and women, an increasing content of triglycerides, phospholipids and cholesterol, as well as an increasing number of apolipoproteins (Apo-B, Apo-A1 and Apo-A2) were related to a decrease in bone turnover. Most, but not all, associations were seen for bone formation and bone resorption processes simultaneously. Yet, in men, several associations were restricted to bone resorption while in women several associations were restricted to bone formation. Finally, in men, in intermediate density lipoprotein particles (IDL) an increasing cholesterol content and an increasing number of Apo-B, as well as an increasing triglyceride content in LDL and HDL particles was related to a lower ultrasound-based bone stiffness. In women, associations between the lipoprotein subclasses and the ultrasound-based bone stiffness index were absent.

Conclusion: The present analyses revealed several interesting associations that need to be further evaluated. Most notably the impact of IDL particles on bone turnover and strength in men, which would have been unnoted using classical serum lipid markers like LDL-C and HDL-C, seems of interest. Additionally, in premenopausal women, the effect of triglycerides on bone formation deserves further investigation.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-02

Childhood sexual abuse is associated with higher total ghrelin serum levels in adulthood: Results from a large, population-based study

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Background: Ghrelin is an orexigenic peptide hormone synthesized in times of stress and hunger and alterations of the ghrelin system following acute stressors could be repeatedly shown in humans. However, little data exists on long-term effects of trauma on the ghrelin system. We aimed to investigate the influence of childhood trauma on total ghrelin serum levels in a large, population-based study.

Methods: Total serum ghrelin was measured in 1666 participants of a population-based cross-sectional study ('LIFE study'). The Childhood Trauma Screener (CTS) was used for the assessment of childhood trauma in the final sample (N=1086; mean age: 57.10 ± 16.23 years; 632 males, 454 females). Multiple linear regression analyses and generalized linear models were chosen to examine the association between childhood trauma and total serum ghrelin concentrations.

Results: Childhood sexual abuse went along with significantly higher ghrelin serum levels in the total sample ($\beta = 0.114$, $t = 3.958$; $p = 0.00008$) and in women ($\beta = 0.142$, $t = 3.115$; $p = 0.002$), but not in men ($\beta = 0.055$; $t = 1.388$; $p = 0.166$). Women with severe emotional neglect in the childhood had higher ghrelin levels than those without (odds ratio = 1.204; $p = 0.018$). For the CTS Sum Score and other CTS sub-scale scores, no significant association with ghrelin serum levels was found.

Conclusion: Our study is the first to show associations between childhood sexual trauma and total ghrelin levels in adults in a large, community-based sample. Our results should initiate further research of the role of ghrelin in human stress response in prospective study designs.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-03

Photometric urinary iodine assays - (pre-)analytical pitfalls and potentials

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Introduction

Iodine is one of the most important trace elements and has a major influence on the development of our brains in childhood and on the supply of thyroid hormones throughout our lives. In epidemiological studies urinary iodine is used to determine iodine supply within populations, since more than 90 % of the daily iodine intake is excreted in urine. ICP-MS is considered as the reference method for iodine quantification, however this method is not widely available. Therefore, the WHO recommends the Sandell-Kolthoff method with a photometric detection. For this method the sample preparation is critical to ensure optimal accuracy and precision.

Material and Methods

In this work, we compared different modifications of established sample preparation methods. The methods were derived from an assay developed at the Robert Koch Institute (RKI), variating the amount of sample and addition of sodium hydroxide and ammonium persulfate in compliance with the WHO recommendations. The measurements were performed in triplicates on a Gallery analyzer (Thermo Scientific), which is UV-VIS based and using the Sandell-Kolthoff method. The results were compared to the RKI (measured on a Cobas Mira analyzer, Roche) and ICP-MS as a reference assay. In addition to the WHO recommendations, the kinetic of the reaction was monitored and the reaction constant was determined for each sample instead of the end-point of the reaction. To ensure the quality of all measurements one pooled urine sample, a quality control (Recipe, ClinChek Trace elements) and reference material (NIST 3668) with two concentration levels were analyzed.

Results and Discussion

Overall, the quality assessment samples demonstrated good agreement with the target values. For 2 of 3 modifications both imprecision (CV) and bias were less than 9%. For ICP-MS, the NIST samples showed a positive bias of approximately 15%. Compared to ICP-MS all sample preparation methods showed acceptable values for the Passing-Bablok regression with intercepts between -2.11 – 6.83, slopes between 0.84 – 0.91, and Pearson correlations of 0.96 – 0.98. However, during the establishment of each method it was observed that the ratio of sample volume to amount of ammonium persulfate, the amount of sodium hydroxide and the quantification strategy had a major impact on the final results.

Conclusion

For urinary iodine methods the sample preparation strategy should be selected carefully. The different sample preparation methods had an impact on the results and each had its distinct advantages and disadvantages.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-04

HbA1c Bestimmung mittels Routine-HPLC – Einfluss von Hb-Varianten

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Zielsetzung: Die High Pressure Liquid Chromatography (HPLC) stellt den Goldstandard in der HbA1c-Diagnostik dar und ist eine sehr präzise Methode, mit der die ab Oktober 2023 geforderte relative Abweichung von 3% der RiliBÄK (1) erreicht wird. Mit der Methode werden auch einige Hb-Varianten detektiert. Allerdings ist der Einfluss verschiedener Hb-Varianten auf die HbA1c-Messung nicht immer klar (2).

Methoden: Von 8277 HbA1c-Routineproben wurden 34 abnormale Routine HbA1c-Chromatogramme mit Varianten-Flag in der HPLC (Tosoh Bioscience G11, Variant Modus, Laufdauer 1 Minute) selektioniert und mittels immunologischer Messung (Tina-quant Gen. 3, Roche Cobas) gegengemessen. Diese Proben wurden in einem längeren HPLC-Lauf (Biorad, Variant II, β-Thal-Methode, Laufdauer 6 Minuten) auf Hb-Varianten untersucht.

Ergebnisse: Bei häufigen heterozygoten Varianten (HbS, HbE, HbC) und unter Berücksichtigung der Hb-Variante Riccarton war eine gute Übereinstimmung der beiden Methoden von 82,4% (28/34) zu finden. In 17,6% (6/34) Fällen war die HPLC Methode nicht plausibel (Ergebnis nicht valide, diskrepant zu den Vorwerten oder nicht mit Blutzucker-Werten des Patienten vereinbar). Hierbei handelte es sich am ehesten um folgende Varianten: Hb Le Lamentin, homozytoges HbE und weitere nicht sicher zu charakterisierende Varianten. In zwei dieser Proben (5,9%) lag ein hoher HbF-Wert >30% zugrunde, der ebenfalls zu nicht validen HbA1c-Werten führte.

Diskussion und Schlussfolgerung: Durch den breiten Einsatz der HPLC für die Bestimmung von HbA1c fallen immer mehr anormale Hb-Muster auf. In diesen Fällen stellt sich die Frage, inwieweit eine Interferenz mit der HbA1c-Methode vorliegt. Diese Stichproben-Untersuchung bestätigt, dass in der Mehrheit der Fälle häufige heterozygote Hb-Varianten eine valide HbA1c-Messung ermöglichen (3). Sobald homozygote Formen oder seltenere Varianten vorliegen, ist die Bestimmung von HbA1c herausfordernder (4). Bei Patienten mit hohen HbF-Werten sollte keine HbA1c-Messung erfolgen, sondern auf Alternativen zurückgegriffen werden.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-05

Erniedrigter Omega-3 Index und erhöhter ω6/ω3 – Quotient bei Schwangeren mit Gestationsdiabetes mellitus

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Zielsetzung

Gestationsdiabetes mellitus (GDM) ist definiert als eine Glukosetoleranzstörung, die erstmals in der Schwangerschaft mit einem 75-g-oralen Glukosetoleranztest (OGTT) aus venösem Plasma diagnostiziert wird. Die Pathophysiologie des GDM ist ähnlich der des Typ-2-DM und wird auf eine zunehmende Insulinresistenz mit abfallender β-Zell-Kompensation zurückgeführt. In mehreren Studien zeigten sich Unterschiede im Fettsäureprofil zwischen Schwangeren mit und ohne GDM. Die Rolle der Omega-3 Fettsäuren in der Pathogenese des Typ-2-DM wird durch verschiedene Studien unterschiedlich beurteilt, eine kürzlich durchgeführte Meta-Analyse (1) kam jedoch zum Ergebnis, dass der Omega-3 Index negativ mit Typ-2-DM korreliert. Gleiches wird auch für GDM postuliert. Der Omega-3 Index (gemessen in Erythrozyten) hat sich als Langzeitparameter für die Versorgung mit den ω3-Fettsäuren Eicosapentaen- (EPA) und Docosahexaensäure (DHA) in den letzten 8 bis 12 Wochen etabliert und wird als prozentualer Anteil von EPA plus DHA an den Gesamtfettsäuren angegeben.

Methoden

Es erfolgte die gaschromatische-massenspektrometrische Analyse (2) von 35 Fettsäuren in Erythrozyten von Schwangeren mit (n=102) und ohne GDM (Kontrollen, n=102). Die extrahierten Fettsäuren (FS) wurden zu Methylester (FAME) derivatisiert und anschließend mittels Gaschromatographie-Tandem-Massenspektrometrie (GC/MS/MS) mit chemischer Ionisation (NH3) gemessen. Die Quantifizierung erfolgte mit der FS C17:1 als interner Standard. Intra- und inter-assay Variationskoeffizienten waren für alle FS < 10%.

Ergebnisse

Der Omega-3 Index liegt mit 2,70 ± 0,14 % bei Schwangeren mit GDM signifikant niedriger als bei den Kontrollen mit 3,27 ± 0,16 % ($p = 0,018$), während der ω6/ω3 – Quotient mit 6,80 ± 0,37 signifikant höher im Vergleich zu den Kontrollen mit 4,96 ± 0,14 liegt ($p < 0,001$). Der Quotient der Delta-6-Desaturasen ω6 (18:3/2) ist bei Schwangeren mit GDM mit 0,38 ± 0,02 % signifikant kleiner ($p = 0,025$) als bei den Kontrollen mit 2,10 ± 0,35 %. Während für EPA kein Unterschied zwischen Schwangeren mit und ohne GDM festgestellt werden konnte (0,18 ± 0,02 % vs 0,19 ± 0,01 %) ist die DHA bei Schwangeren mit GDM mit 2,52 ± 0,13 % im Vergleich zu Kontrollen mit 3,08 ± 0,15 % signifikant erniedrigt ($p = 0,014$).

Diskussion und Schlussfolgerung

Es bestehen signifikante Unterschiede in den Fettsäurekonzentrationen in den Erythrozyten von Schwangeren mit und ohne GDM. Diese liegen insbesondere in niedrigeren Omega-3 Fettsäurekonzentrationen im Vergleich zu Omega-6 Fettsäuren bei Schwangeren mit GDM. Eine mögliche Ursache könnte eine erniedrigte Delta-6 Desaturasenaktivität bei Schwangeren mit GDM sein, ähnlich wie bei Typ-2-DM. Die Frage nach dem geeigneten Material, Serum vs Erythrozyten, für die Feststellung von Fettsäurenveränderungen, bleibt ebenso wie die Frage nach Gesamtfettsäuren vs Fettsäuren bestimmter Lipidklassen weiterhin ungeklärt.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-06

Associations between Circulating Chemerin and Adiponectin Concentrations with Lipoprotein Subclasses in a Population-Based Study

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Introduction: Various cross-sectional studies have observed associations of the adipokines chemerin and adiponectin with the clinically most established lipid parameters total cholesterol, HDL-cholesterol, LDL-cholesterol, and total triglycerides. However, the lipids examined in these studies are heterogeneous with respect to size, density, and chemical composition and further separation of lipoproteins in subclasses could help to elucidate the complex interaction between adipokines and lipid metabolism. Therefore, this study aimed to analyze the associations of adiponectin and chemerin with lipoprotein subclasses in a population-based study.

Methods: Lipoprotein subclasses were determined by nuclear magnetic resonance (NMR) spectroscopy in samples from 3286 participants of the Study of Health in Pomerania (SHIP-TREND-0). The associations between adiponectin/chemerin (exposure) and lipoprotein subclasses (outcome) were analyzed using linear regression models, adjusting for necessary confounders and multiple testing.

Results: The analyses revealed a broad range of significant associations between adiponectin/chemerin and lipoprotein subclasses. When comparing the results between adiponectin and chemerin, it became obvious that the associations are usually in opposite directions (exceptions: content of cholesterol, Apo-A1, Apo-A2 in HDL-4). In general, high adiponectin concentrations were associated with a favorable lipid profile, whereas high chemerin concentrations were linked to an unfavorable lipid profile. Interestingly, the direction of the associations between adipokines and cholesterol, triglycerides, and Apo-B content across LDL-subfractions changed with increasing density and corresponding decreasing size of the LDL-particles. In different subpopulations, the detected regression results were quite stable. For chemerin, the effects seem to be primarily driven by disease (diabetes, hypertension, cardiovascular diseases, cancer, respiratory diseases, thyroid diseases, renal diseases) and obesity, whereas for adiponectin, effects were largely independent of the health status.

Conclusion: Compared to the clinically most established lipid parameters, the present analysis of lipid subclasses provides a more accurate picture of the relationships between adipokines and lipid metabolism. Overall, we observed that a high chemerin concentration was associated with an unfavorable and atherogenic lipoprotein subclass profile, whereas for high adiponectin concentration the opposite was observed.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-07

Protein Tyrosine Phosphatases as Molecular Targets of Inflammation-induced Insulin Resistance and Muscle Atrophy

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Introduction

Up to 50% of critically ill patients develop atrophy of skeletal muscles contributing to intensive care unit (ICU)-acquired weakness (ICUAW). This manifests in reduced muscle strength, prolonged hospitalization and rehabilitation. Systemic inflammation, sepsis, and insulin resistance are risk factors for ICUAW. However, no specific treatment options are available and underlying factors are largely unclear. Notably, in skeletal muscle, inflammatory and metabolic pathways (e.g. insulin signaling) are closely connected. Insulin signaling is regulated by reversible protein phosphorylation by protein tyrosine phosphatases (PTPs). Yet, the importance of PTPs in inflammation-induced skeletal muscle pathologies is uncertain.

Methods

C57Bl6/N mice were exposed to polymicrobial sepsis by caecal ligation and puncture (CLP) surgery. Sham-operated mice served as controls. Gene expression analysis was performed by RNAseq and qPCR in M. tibialis anterior. Furthermore, qPCR was done on RNA isolated from M. vastus lateralis of ICUAW patients 5 and 15 days after ICU-admission. Patients who underwent elective orthopedic surgery served as controls. In vitro, differentiated C2C12 muscle cells were treated with proinflammatory cytokines (i.e. TNF, IL-1β, IL-6) and vehicle, respectively, for 2, 16 and 24h. Gene and protein expression analyses were performed by standard procedures. Pharmacological inhibition of PTPs was conducted to investigate their impact on insulin signaling.

Results

CLP animals showed an increased expression in Ptpn1/PTP1B and Ptpn2/TC-PTP, which are involved in insulin signaling. Further, an increase in insulin receptor/Insr and a time-dependent reduction in Irs1 expression was seen, indicative for inflammatory insulin resistance. Ptpn1/PTP1B, Ptpn2/TC-PTP and Insr were also found upregulated in ICUAW patients, comparable to findings in septic animals. In vitro, TNF and IL-6 caused an upregulation of Ptpn2 but not Ptpn1. By contrast, Irs1 was downregulated after cytokine stimulation, as seen in muscle tissue by RNAseq. Importantly, the broad-spectrum PTP inhibitor vanadate resulted in enhanced phosphorylation of the insulin receptor even in the absence of insulin, highlighting the relevance of PTPs for insulin signaling in C2C12 muscle cells. Furthermore, PTP1B inhibition resulted in higher phosphorylation of the insulin receptor and the downstream effector AKT, suggesting PTPs as novel targets in septic muscle atrophy and ICUAW.

Conclusion

Our data implicate a relevant role of PTP1B and TC-PTP in peripheral insulin resistance during inflammatory muscle atrophy. The inhibition of PTPs lead to a higher phosphorylation of insulin signaling components indicative for an improved insulin sensitivity. Thus, targeting PTPs could alleviate the negative impact of insulin resistance on muscle atrophy during systemic inflammation.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-08

Gene expression changes in adipose tissue in individuals with metabolic syndrome following lifestyle-induced weight loss

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Aims: Lifestyle-induced weight loss (LIWL) is regarded as efficient therapy to reverse or ameliorate metabolic syndrome (MetS). Here we investigate the effect of lifestyle- induced weight loss gene expression in adipose tissue before and after 6 month LIWL.

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). Before and following LIWL, 43 participants underwent fat tissue biopsy. In subjects with documented weight loss of at least 5% after 6 months, differentially expressed genes (DEGs) were analyzed in paired tissue samples by microarray analysis. DEGs were correlated with parameters defining the metabolic syndrome and obtained results were compared with gene expression data of two other weight loss cohorts.

Results: We identified 642 DEGs. Pathway enrichment analysis revealed upregulation of genes related to cholesterol metabolism and reverse cholesterol transport in adipocytes. Spearman correlation with the parameters characteristic for the definition of the metabolic syndrome (BMI, HDL cholesterol, triglycerides (TGs), blood pressure and fasting plasma glucose (FPG)) revealed that 6 genes positively and 6 negatively correlated with BMI reduction in LIWL. 5 DEGs were correlated with TG changes and one DEG (retinol dehydrogenase 5; RDH5) showed an inverse correlation with HDL cholesterol in LIWL. Fasting plasma glucose, RRsystolic and RRdiastolic showed no correlation with the top 20 DEGs in LIWL. We compared our results with gene expression data of obese children and obese individuals following surgical weight loss. Six DEGs were identified in all three cohorts. The genes glycerol-3-phosphate dehydrogenase 1 like (GPD1L), gasdermin B (GSDMB) and molybdenum cofactor synthesis 1 (MOCS1) were upregulated after weight loss, whereas CD248, NAD(P)H quinone dehydrogenase 1 (NQO1) and tenomodulin (TNMD) were downregulated.

Discussion: We identified genes that are differentially expressed following LIWL in subcutaneous adipose tissue. Further studies are needed to investigate the specific role of these genes for metabolic syndrome.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-09

Deciphering postprandial hypoglycemia: dominant influence of insulin sensitivity in patients without diabetes

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Introduction

Postprandial hypoglycemia describes a condition with abnormally low blood glucose within a few hours after carbohydrate-rich meals. Underlying mechanisms remain subject of investigation, with current hypotheses including an unusually rapid increase in blood glucose, excessive insulin release, or prolonged presence of elevated insulin levels. We now analyzed these possibilities in a large sample of oral glucose tolerance tests.

Methods

1457 patients without diabetes underwent prolonged (180 min) 75 g oral glucose tolerance tests as part of clinical work up at the endocrinology division at Ulm University Hospital between 2012 and 2023. Blood samples were taken before and 15 min after glucose ingestion and thereafter every 30 min. Glucose was enzymatically measured from fluoride/citrate plasma or fluoride plasma (before 2016), insulin and C-peptide were measured by immunoassays. Groups were compared by unpaired, two-tailed t-tests and correlations were tested by multivariate linear regression analyses in R. Reported are median and IQR.

Results

Of analyzed 1457 patients (median: 43 (IQR: 22) years, 927 women), 499 had blood glucose below 70 mg/dl and 103 even developed blood glucose below 54 mg/dl (afterwards termed hypoglycemia group) at 180 min post oral glucose load. Such low glucose levels were not yet present at 120 min, where only 6 patients had blood glucose below 54 mg/dl.

The groups with and without postprandial hypoglycemia were comparable in sex and age. However, the hypoglycemia group had a slightly lower HbA1c (5.4 vs 5.5%; p=0.03).

Fasting glucose was lower in the hypoglycemia group (92 (12) vs 100 (14) mg/dl; $p < 0.0001$). Peak glycemia was reached in both groups at 30 min and the increment in plasma glucose was comparable between groups (p=0.5).

Insulin secretion was assessed as Stumvoll's first and second phase as well as AUC C-peptid0-30/Glucose0-30 and was lower in the hypoglycemia group in unadjusted models (all $p < 0.0001$). After the appropriate adjustment for insulin sensitivity, AUC C-peptid0-30/Glucose0-30 and Stumvoll's second phase were comparable between groups (both $p > 0.3$). However, Stumvoll's first phase of insulin secretion was slightly lower in the hypoglycemia group (p=0.02).

Insulin sensitivity, as assessed both at fasting (HOMA-IR) and during the oGTT (modified Stumvoll-Index) was higher in the hypoglycemia group (both $p < 0.0001$).

Conclusion

Our data indicate that postprandial hypoglycemia affects a significant number of patients in a university hospital's endocrinology setting. Rise in blood glucose and excess insulin secretion do not substantially contribute to this phenomenon. However, those with postprandial hypoglycemia exhibited notably higher insulin sensitivity. This likely also contributes to better every day glucose control, as reflected by lower HbA1c. Our data underscore that in cases of suspected postprandial hypoglycemia, a prolonged oGTT is necessary to ascertain the diagnosis.

DGKL: 05. Endokrinologie und Stoffwechsel

P-04-10

CD248 induces sterile inflammation in diabetic kidney disease

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Aims: The dysfunction of pericytes plays a major role in the pathogenesis of diabetic kidney disease (DKD), which is the leading cause of end-stage renal disease. We and others have identified the pericyte-specific transmembrane receptor CD248, which plays a specific role in DKD progression. However, the underlying molecular mechanisms are incompletely understood.

Methods and Results: Our results show that increased expression of the transmembrane receptor CD248 in glucose-stressed pericytes in vitro and during DKD in vivo is associated with the assembly of a protein complex that mediates the unfolded protein response (UPRosome) comprising HSP90 and IRE1α. CD248 causally promotes inflammation via inhibition of IRE1α-mediated XBP1 splicing. Knockdown or knockout of CD248 reverses, while overexpression of CD248 induces maladaptive UPR signaling and inflammation, indicating a causal interaction. Both UPR proteins IRE1α and PERK regulate UPR by controlling proapoptotic and proinflammatory genes such as Death receptor 5 (DR5). Our data revealed that DR5--associated proinflammatory signaling is reduced in high glucose-exposed pericytes upon knockdown of CD248. This effect is abolished when the endoribonuclease domain of IRE1α is inhibited, indicating that high glucose-induced CD248 enhances DR5 activation in pericytes via inhibition of IRE1α.

Conclusion: We identified CD248 as a regulator of DR5-associated proinflammatory signaling in renal pericytes under diabetic conditions. This research is expected to provide new mechanistic insights and identify the transmembrane receptor CD248 as a potential new druggable target to prevent proinflammatory signaling in DKD.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-01

Altered phosphoproteome in resting Glanzmann thrombasthenia platelets

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Introduction

For primary hemostasis, the GPIIb/IIIa complex is essential to induce platelet aggregation after vascular injury. Patients who have a defective GPIIb/IIIa complex (both qualitatively and quantitatively) develop a thrombocytopathy (Glanzmann thrombasthenia, GT). In previous studies, GT patients have been shown to exhibit altered spreading in the process of platelet shape change. Based on this, this study aimed to further investigate the signaling pathways downstream of the GPIIb/IIIa complex. Because platelets lack a nucleus, signaling pathways are often regulated via posttranslational modifications, such as phosphorylation. Based on this, the phosphoproteome of resting platelets should be studied to analyze possible changes in signal transduction. The phosphoproteome analysis should provide an overview of which signal effector proteins are already modified in resting cells in order to identify possible targets for further analyses.

Methods

For the study, healthy platelets, GT platelets and also in vitro GT platelets (healthy platelets incubated with a GPIIb/IIIa inhibitor) were examined. For this purpose, first highly purified platelets (HPP) were prepared, the proteins were digested (in-solution), the peptides were purified and the phosphopeptides were enriched using titanium dioxide beads. This was followed by both global proteome and phosphoproteome analysis by LC-MS/MS.

Results

The results of the global proteome showed that the expression of the GPIIb/IIIa complex is significantly reduced in GT platelets. Also decreased in GT platelets is the amount of the three fibrinogen subunits (α, β und γ). Phosphoproteomic analysis revealed that the proteome of GT patient platelets is significantly altered compared to the control platelets and in vitro GT platelets. A total of 157 on/off phosphosites were identified, with 147 of the off phosphosites assigned to the GT patient collective.

Conclusion

Proteomic analysis showed that the GPIIb/IIIa complex is expressed intracellularly in GT platelets but in significantly reduced amounts. In addition, the amount of fibrinogen is also reduced, indicating a defect in the intracellular uptake of fibrinogen from plasma. Furthermore, the phosphoproteome of resting GT platelets was studied for the first time. Significant differences were found between control platelets, in vitro GT platelets, and GT patient platelets. This shows that already in resting platelets phosphorylations are downregulated in GT patients and therefore different signaling pathways are affected.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-02

EDTA-associated pseudothrombocytopenia: definition and real-world occurrence

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Objective: To better characterize the occurrence and extent of anticoagulant-associated pseudothrombocytopenia (PTCP) in the daily routine of a high-throughput clinical laboratory in order to draw conclusions on a more precise definition of this phenomenon.

Methods: Concomitant platelet counts in both EDTA and citrate whole blood (WB), performed in our laboratory over a period of four years and 9 months, were analyzed, calculating the correlation, as well as the absolute difference in the platelet counts obtained from both materials, cross-referencing these measures with automated flags for platelet aggregates and the results of the visual examination for platelet aggregates of peripheral blood smears.

Results: Platelet counts in both materials were strongly correlated ($\rho = 0.86$; $p < 0.0001$) but are on average significantly higher in EDTA WB than in citrate WB (median difference: 11±14.8/nl, $p < 0.0001$). This is in spite of numerous instances of EDTA-associated PTCP recorded in our data, where the opposite is the case. The automated flag for possible platelet aggregates was shown to be very unspecific, while a machine-learning algorithm suggested the difference in platelet counts between EDTA and citrate WB as a much better predictor of platelet aggregates.

Conclusions: EDTA-associated PTCP is a regular occurrence in the hematology laboratory. Differences in platelet counts between EDTA and citrate WB appear to be a far better predictor of PTCP than automated flags. A clear and useful definition of PTCP is still missing, however, and cannot be derived from our data either, indicating the need for further research.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-03

A workflow for the periodical review of reference intervals in the clinical hematology laboratory

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Introduction: Reference intervals (RI) are essential for the correct interpretation of laboratory tests and thereby contributing to an optimal patient care. RIs are not only affected by factors such as age, gender, and other demographic factors, but as well may vary between different assays and analyser platforms. Therefore, it is essential to periodically review RIs in the clinical laboratory, given that both laboratory methods and the patient population may change over time.

Methods: RefineR, a comprehensive R package, was used to estimate RIs from real-world laboratory data using an indirect approach. Different RIs, including previous RIs and potential RIs from literature, were compared to the indirect method. Anonymized data from all routine hematology patients in 2022 were included in this study. 14 routine hematology parameters (complete blood count with differential plus reticulocytes: WBC, RBC, Hb, Hct, MCV, MCH, MCHC, PLT, neutrophils, eosinophils, basophils, lymphocytes, monocytes, and reticulocytes) were evaluated.

Results:

Acceptance criteria for RIs were developed based on the reference change values (RCV) concept, whereas the examined RIs were considered acceptable if the estimated limits were within the range of the estimated limits plus/minus the tolerable RCV deviation. The “multiple of the tolerable deviation” was calculated and highlighted colour-coded as a heatmap. For some parameters, such as RBC, Hb, Hct, and leucocyte differential parameters, the indirect RI estimation did not provide reasonable intervals, probably due to the high proportion of pathological results in the clinical data. Therefore, a manual pre-selection is necessary to exclude parameters in which the estimated RIs are not plausible. However, most parameters could be evaluated in a reliable and easy way, thereby facilitating the periodic review process.

Conclusion: This study provides a workflow for the periodic review of RIs. Supplementary to the manual review the RCV deviations and the heatmap representation may aid clinical laboratories ensuring accuracy of their RIs and facilitating the review process.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-04

Von Willebrand Factor significantly modulates FVIII-containing immune complexes and suppresses FVIII-specific secondary immune response in murine haemophilia A.

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Introduction:

Haemophilia A is an X-linked bleeding disorder due to dysfunctional or absent coagulation factor VIII (FVIII). FVIII replacement therapy can cause the development of high-affinity IgG anti-FVIII antibodies (inhibitors) that neutralize FVIII, resulting in therapy failure in up to 40% of the patients (1). Previously, we could show that inhibitors and FVIII form immune complexes (FVIII-ICs) that bind effector molecules (C1q and Fc gamma receptors; FcgR), potentially forcing subsequent immune reactions (2). Data indicate that von Willebrand Factor (VWF) as the FVIII chaperone mediates immuno-protective effects in initial inhibitor formation, but the impact on potential harmful FVIII-ICs is unknown. Therefore, we investigated the impact of VWF on FVIII-ICs formation and murine secondary FVIII-specific immune response.

Methods:

FVIII-ICs were generated using recombinant human FVIII and 7 monoclonal antibodies against different FVIII epitopes or serum from FVIII-immunized haemophilia A mice (HA mice). FVIII-ICs characteristics were investigated in the presence/absence of plasma-derived VWF (Biotest AG) in terms of effector molecule binding (C1q, FcgR; in–house ELISA) or size (analytical ultracentrifugation with fluorescence detection, ProteomeLab XL-I, Beckman Coulter). FVIII-specific recall response with/without VWF was investigated in vitro by FVIII-restimulation of splenocytes from FVIII-immunized haemophilia A mice (B6;129S4-F8tm2Kaz; in-house ELISPOT) or in vivo using single FVIII challenge of primed splenocytes after adoptive transfer into naïve HA mice.

Results/Conclusion:

FVIII and inhibitors form immune complexes of heterogeneous size that bind to C1q and FcgRs, in contrast to monomeric IgG. In the presence of VWF, effector molecule binding was suppressed in a dose-dependent manner, up to complete prevention. VWF increased the amount of free IgG but did not entirely prevent FVIII-ICs formation. Of note, the size of FVIII-ICs increased significantly by VWF incorporation into the complexes, which may shield the Fc-fragments of IgG.

VWF abolished the differentiation of antibody-secreting cells from memory B-cells in vitro, which was translated in a significantly reduced FVIII recall response in vivo. Thus, VWF modifies FVIII-ICs function and inhibits FVIII recall response, suggesting therapeutic potential in terms of inhibitor eradication.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-05

Validierung von Referenzwerten für regulatorische T-Lymphozyten

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Zielsetzung:

Regulatorische T-Lymphozyten (Treg) sind eine Untergruppe der T-Helfer-Lymphozyten (TH, CD4+), die eine wichtige Rolle bei der Aufrechterhaltung der Immuntoleranz und der Verhinderung von Autoimmunität spielen. Die Bestimmung der Treg erfolgt mittels durchflusszytometrischer Analyse. Derzeit gibt es keine allgemein anerkannten Referenzwerte für Treg. Darüber hinaus wird die Validierung von laborinternen Referenzwerten für Treg aufgrund der Verwendung unterschiedlicher Geräte und Antikörper zwischen Durchflusszytometrie-Laboratorien empfohlen.

In diesem Beitrag berichten wir über Ergebnisse der Validierung eines Referenzintervalls für Treg basierend auf zwei unterschiedlichen Methoden: (1) die direkte Treg-Messung einer Gesundkohorte und (2) die indirekte Berechnung des Referenzintervalls aus der Routinediagnostik des Durchflusszytometrie-Labors des Instituts für Klinische Chemie am Universitätsklinikum Schleswig-Holstein (UKSH).

Methoden:

Zur Berechnung der Referenzwerte wurden die Treg bei 130 erwachsenen Patienten (Alter >18 Jahre) mit normwertigen TBNK-Zell-Immunstatus (CD3, CD4, CD8, CD19, CD16/CD56, Multitest 6-color TBNK reagent, Trucount tubes, BD) durchflusszytometrisch bestimmt. Die Treg wurden als CD45+CD4+CD25high+CD127low/- Zellen mit den Antikörpern CD45-PE-Cy7 (Klon HI30, BD), CD4-FITC (Klon SK3, BD), CD25-APC (Klon CD25-3G10, Invitrogen), CD127-PE (Klon HIL-7R-M21, BD) identifiziert. Die Messungen wurden am CantoII (BD) durchgeführt und mittels FACS Diva Software (BD) ausgewertet. Das Referenzintervall wurde als 2,5 – 97,5 % definiert. Zur Überprüfung der Referenzwerte wurde parallel das Referenzintervall aus den Routinelabordaten (N=813) mit der Truncated Minimum Chi-Quadrat Methode (TMC) berechnet.

Ergebnisse:

Der Treg-Anteil erreichte in der untersuchten Gesundkohorte (Durchschnittsalter 46,47+/-14,28, Min-Max: 19-79) im Mittel 7,78+/-2,07% der TH. Basierend auf den Messungen dieser erwachsenen Gesundkohorte betrug die 10. - 90. Perzentile 5,1 - 10,6% und ergab ein 95% Referenzintervall für Treg von 3,57 - 12,03% der TH. Das Referenzintervall mit TMC berechnet, betrug 5,17 - 12,7% der TH.

Diskussion und Schlussfolgerungen:

Das Treg Referenzintervall, welches nach den nach wie vor geltenden Empfehlungen der IFCC als Goldstandard, methodisch aus einer Gesundkohorte bestimmt wurde, zeigte eine sehr gute Übereinstimmung mit dem aus den Labordaten der Routinediagnostik indirekt berechnetem Referenzintervall und konnte somit validiert werden. Des Weiteren sind die ermittelten Ergebnisse konform mit einer multizentrischen Studie, in welcher eine 10. – 90. Perzentile der Treg von 5 – 10% der TH anhand von 330 Gesunden in 6 Zentren ermittelt wurde. Das für Erwachsene direkt ermittelte 95% Referenzintervall von 3,57 - 12,03% der TH wird somit im UKSH etabliert und kann aufgrund von fehlenden veröffentlichten Referenzintervallen für Treg zudem als Anhaltspunkt für andere Labore dienen.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-06

Investigation of shear-stress mediated formation of platelet-leukocyte-aggregates in a Flow-Chamber-Model

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Introduction

The main function of platelets is within hemostasis, but they also play a major role in inflammatory processes. For example, together with leukocytes they can form so-called platelet-leukocyte aggregates (PLA), which can be distinguished in sub-groups like platelet-neutrophile aggregates (PNA) or platelet-monocyte aggregates (PMA). The occurrence of such aggregates is predominantly described in connection with inflammatory or thrombotic situations. Therefore, they are also considered as potential biomarkers for some diseases such as chronic obstructive pulmonary disease, consumptive coagulopathy, or sepsis.

In patients with implanted extracorporeal membrane oxygenation (ECMO) or left ventricular assist device (LVAD), who also showed increased PLA levels, the occurrence of these PLA has been associated with inflammatory processes. We investigated whether the shear stress exerted on cells by ECMO or LVAD can also lead to PLA formation using a flow chamber model.

Methods

D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) anticoagulated blood was collected from healthy donors and for part of the experiments a platelet inhibitor was added to the blood 15 min before shear stress exposure. Whole blood was pumped at a defined rate through a PDMS-glass-based flow chamber (height 100 µm, width 1000 µm, length 5 cm, with or without a 75% stenosis), causing a defined shear stress on the cells. We chose a low shear rate of 500s-1 and a high shear rate of 5000 s-1. In the stenosis area, the shear rates were 2000 s-1 and 20000 s-1. After shear stress exposure, PLA, PNA and PMA were determined by flow cytometry.

The study received a positive vote from the ethics committee (AZ: 2022-1015).

Results

At shear rates of 500 s-1 and 5000 s-1 in the flow chamber without stenosis the measurements showed a significant increase in PNA and PMA relative to the respective control. An increased appearance of PLA was observed only at 5000 s-1. Expression of CD62P on platelets was not increased by shear rates.

In the flow chamber with a 75% stenosis, it was shown that PLA, PNA, and PMA were significantly increased at 500 s-1 and 5000 s-1. Increased CD62P expression was only observed at 5000 s-1.

When comparing the samples with 5000 s-1 shear rate of the model without stenosis and the samples with 500 s-1 shear rate and the model with stenosis (2000 s-1 at the stenosis), it was shown that significantly more PLA, PNA, and PMA were formed in the samples from the stenotic flow chamber.

Conclusion

This study demonstrated that shear stress exposure can lead to increased formation of PLA, PNA, and PMA. Thereby, platelet activation does not seem to be necessary. However, acceleration of cells due to stenosis seems to have a higher influence on PLA formation than the degree of shear rate alone. Whether these results can also be confirmed in patients with ECMO or LVAD needs to be investigated in further studies.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-07

Antibody-activated Partial Thromboplastin Time assays (aaPTT): new tools in coagulation diagnostics

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Introduction:

The activated Partial Thromboplastin Time (aPTT) is one of the most commonly used diagnostic coagulation tests in clinical laboratories. Routine aPTT testing is generally used to screen for congenital or acquired bleeding disorders, to detect lupus anticoagulant or to monitor levels of anticoagulation in patients receiving heparin. We recently identified the FXII surface binding site and unexpectedly found that the C-terminal portion of the FXII proline-rich (PR)-domain was essential for surface-driven FXII activation and coagulation. We developed an antibody against a peptide in the center of the surface activation site (designated PR3). In contrast to currently used surface-based contact activators with poorly characterized FXII-activating activity, anti-PR3 antibodies precisely and stoichiometrically activated FXII fully in solution [1].

Materials/Methods:

FXII-activating antibodies were generated as described previously. To establish an antibody-activated PTT (aaPTT) assay we use our FXII-activating antibodies (αPR3) in (i) chromogenic, (ii) thrombin generation and (iii) clotting assays. We evaluate the responsiveness of the three different type of coagulation assays by analysing platelet poor plasma of healthy blood donors.

Results:

Validation of the method is performed with regards to sensitivity, accuracy and precision according to good clinical laboratory practice. An accuracy and precision will be estimated for within laboratory performance including within-run, between days, and inter-operator comparison.

Discussion/Conclusion:

In conclusion, we describe an antibody mediated coagulation assay that allows for making better, more standardized and more sensitive coagulation assays to screen for clotting factor deficiency, lupus anticoagulant or monitoring heparin.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-08

Detection of CAR T-cells by flow cytometry – three cases

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Introduction: CAR (chimeric antigen receptor) T-cell therapy is a relatively new method of treating therapy refractory or recurring B-cell neoplasia like B-cell lymphoma or acute lymphoblastic leukemia of the B-cell linage. CAR T-cells are engineered T-cells that express an immunoglobuline single chain against a specific antigen. In case of CAR T-cells designed for use in B-cell neoplasia the antigen is often CD19 and the single chain antibody expressed against CD19 is FMC63. In

addition to FMC63 CAR T-cells contain a hinge domain, a transmembrane domain and an intracellular domain for signal transduction.

To engineer CAR T-cells the patient's own T-cells are isolated, genetically modified and then amplified. In the end, the T-cells (now CAR T-cells) are re-administered to the patient who has been lymphodepleted prior to that.

Different manufacturers developed CAR T-cells with different antigen specificity. Currently, in Germany there are four approved therapeutics for B-cell neoplasia: Kymriah©, Yescarta©, Tecartus© and Breyanzi©.

Methods: CAR T-cells of three patients were measured with flow cytometry with an antibody against FMC63. Analyses were performed on different time points after CAR T-cell administration, the first measurement was done approximately after one week. The relative amount of CAR T-cells was identified (CAR T-cells/lymphocytes) and the absolute count was calculated. As control for the specificity of the antibody we used a healthy control/ a patient not being treated with CAR T-cells.

Results and conclusion: Here we present data of three patients that were treated with CAR T-cells. Two patients were treated for diffuse large B-cell lymphoma (DLBCL) and the third is a patient with B- cell acute lymphoblastic leukemia (B-ALL). We show the change of number of CAR T-cells over time and also how different products (Kymriah© and Tecartus©) lead to different results.

DGKL: 06. Hämatologie und Hämostaseologie

P-05-09

EVALUATION OF CELL DEATH BEHAVIOR IN SEZARY SYNDROME PATIENTS BY A NOVEL INTEGRATED FLOW CYTOMETRIC PANEL

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Background:

The diagnosis of the cutaneous T cell lymphoma (CTCL) entity Sézary syndrome (SS) relies on the identification of blood Sézary cells (SC) by different marker proteins (CD7 loss, CD26 loss, and CD158k gain) by flow cytometry. Treatment of CTCL is challenging since its pathogenesis is characterized by resistance to apoptosis rather than hyperproliferation, but the exact mechanisms of cell death resistance are not yet fully understood.

Methods:

28 SS patients (CTCL stage IV) diagnosed according to the WHO – EORTC classification of CTCL and the criteria of the ISCL were included in this study. Peripheral blood mononuclear cells (PBMCs) were isolated, and stained for the SC markers CD7 and CD26 loss and CD158k gain. The cells were treated with dimethyl fumarate (DMF), bexarotene, and mitomycin c in vitro and cell death was measured and compared to untreated controls.

Results:

Spontaneous and therapeutically induced cell death were measured and correlated to cellular marker profiles of SS patient samples. In the marker-positive cells, spontaneous cell death sensitivity was reduced. Different treatments in vitro managed to induce cell death in the marker-positive cell population compared to untreated controls. Repeated analysis after three months of treatment did not show the previously identified cell death resistance of the marker-positive T cells anymore.

Conclusions:

We propose this novel integrated approach comprising the evaluation of the SC marker expression and analysis of cell death sensitivity to CTCL treatments that can enable a better therapy stratification.

DGKL: 06. Hämatologie und Hämostaseologie

P-06-01

Correlation analyses of a genetic with three functional clotting assays intended for laboratory testing of activated protein C resistance

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Introduction:

Activated protein C (APC) is an important regulator of coagulation by cleaving activated factors V (FV) and VIII (FVIII). When FV and/or FVIII responses to APC are reduced or absent the hypercoagulability disorder APC resistance (APC-R) is present and the risk of venous thrombosis is increased. APC-R can occur as an acquired (caused by e.g., advanced age, trauma, hematological malignancies, pregnancy, or oral contraceptive use) or hereditary condition, the latter being most commonly caused by the “FV Leiden” mutation. This single point mutation in the F5 gene leads to an amino acid substitution and thus inability of APC to bind and cleave activated FV, causing APC-R in an autosomal dominant-inherited manner. In clinical and laboratory practice, the detection of APC-R is essential for identifying individuals at risk of thrombotic events, which finally may guide recommendations for use of prophylactic anticoagulant therapy. Several functional clotting tests based on variants of activated partial thromboplastin time (aPTT) or Russel Viper Venom test (RVVT) can be used for APC-R testing, often being susceptible for incorrect interpretation because of interferences with prevalent anticoagulants. Therefore, genetic testing of FV Leiden for confirmation of abnormal functional clotting tests remains pivotal in the standard approach for laboratory APC-R diagnostics.

Aims and Methods:

The main goal of the study is to correlate the F5 genotype with the results of three different functional clotting APC-R assays considering clinical and radiological findings. Thereby, we aim at retrospective analyses of 5231 patient samples received for APC-R diagnostics in the period from 2013 to date.

Results:

We will systematically evaluate the results of genetic analysis of F5 and functional APC-R testing in our laboratory over the period of the last ten years. Comparative analyses of F5 genotyping of individuals (wild type, heterozygous, and homozygous FV Leiden background) as well as their correlation with three different clotting APC-R assays will enable us to identify incongruent APC-R cases. Moreover, considering clinical patient information and radiological findings we will be able to determine predictive values of functional APC-R assays applied.

Conclusion:

APC-R is a well-established risk factor for venous thrombosis. Its testing is clinically important as it facilitates identifying individuals at risk of thrombosis, guide clinical decision-making in terms of anticoagulant therapy, and provide valuable information for prevention and management of thrombosis driven events. However, both genetic and functional clotting assays show advantages as well as disadvantages that need to be considered when APC-R is tested.

DGKL: 06. Hämatologie und Hämostaseologie

P-06-02

Implementierung einer halbautomatischen Multimer-Analyse des von-Willebrand-Faktors

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Das von-Willebrand-Syndrom (vWS) ist eine häufige Blutungserkrankung und seine Diagnostik bedarf spezialisierter Methoden. Die Multimerenanalyse detektiert qualitative und strukturelle Veränderungen des vWF und dient damit der Klassifizierung des vWS im Rahmen einer Stufendiagnostik. Diese komplexe Multimerenanalyse erfolgt häufig manuell mittels Agarose-Gelelektrophorese. Seit wenigen Jahren ist ein für die in-vitro-Diagnostik geeignetes, kommerzielles Assaykit, Hydragel vWF multimers von Sebia, für die halbautomatische Analyse verfügbar. Bisher gibt es in Deutschland keine Referenzbereiche für diese Technik.

Wir zeigen die Validation dieses Assaysystems nach den Richtlinien des Clinical and Laboratory Standards Institute (EP5, EP28) und die Etablierung von validen Referenzbereichen sowohl für gesunde Probanden als auch für vWS-Patienten mit unterschiedlichen Subtypen. Unsere Daten werden dazu beitragen, die Diagnostik von vWS-Patienten durch standardisierte Tests und schnellere Befundung zu verbessern.

DGKL: 06. Hämatologie und Hämostaseologie

P-06-03

Heparin-Calibrated Chromogenic Anti-Xa Assay for the Measurement of Apixaban, Edoxaban and Rivaroxaban: A Retrospective Study.

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Introduction: To measure the effect of direct factor Xa inhibitors apixaban, edoxaban and rivaroxaban, inhibitor-specific chromogenic anti-Xa assays are recommended. However, in many laboratories these assays are not routinely available, for example in the Institute of Clinical Chemistry at the University Medical Centre Mannheim, where inhibitor-specific anti-Xa assays are only performed during routine working hours. The only alternatively and continuously available assay, is a heparin-calibrated chromogenic anti-Xa assay. Although an overall good correlation of factor Xa inhibition measured by inhibitor-specific and heparin-calibrated assays has been reported, most studies are based on a relatively small number of patient samples.

Here, we performed a retrospective analysis to determine the relationship between heparin-calibrated anti-Xa measurements and apixaban, edoxaban and rivaroxaban levels measured by inhibitor-specific anti-Xa assays.

Methods:

We examined samples from patients under therapy with apixaban, edoxaban or rivaroxaban, for which measurement of inhibitor-specific as well as heparin-calibrated anti-Xa assays were requested. Patient samples from January 2018 to April 2023 were included. Anti-Xa assay was performed using the Innovance Heparin Assay calibrated either with Innovance Heparin calibrator or with Technoview drug-specific calibrators.

Results:

A total of 1,158 specimens (592 apixaban, 521 rivaroxaban and 45 edoxaban) were analyzed. For all three direct factor Xa inhibitors, we found a linear correlation which was slightly higher for apixaban (R2 = 0,97) and rivaroxaban (R2 = 0,96) than edoxaban (R2 = 0,93).

Conclusion:

In summary, our retrospective analysis from a large sample collective confirms that a heparin-calibrated anti-Xa assay can be used to screen for clinically significant apixaban, edoxaban or rivaroxaban levels when inhibitor-specific chromogenic anti-Xa assays are not available.

DGKL: 06. Hämatologie und Hämostaseologie

P-06-04

The PLT-F – a fast and easy to measure parameter if the routine method is interfered - showed a good comparison with the FCM method

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An exact platelet value is always essential, especially if the platelet value is in critically low ranges and decides on the administration of a platelet concentrate. In general platelets are safely determined in routine with impedance technology.

In some cases of certain interferences (fragmentocytes, giant platelets) impedance technology cannot safely separate erythrocytes and platelets, as the volumes of both cell classes may overlap. The values (PLT-I) are therefore unreliable and require verification using alternative methods.

Analysis systems of the XN-series provide a fluorescence-optic counting of platelets (PLT-F) after expression of the cells with a dedicated marker, the counting volume is 5-fold higher compared to routine measurement. In case of e.g. giant platelets or fragmentocytes this may provide a correct value of the platelets comparable with the reference method recommended by the ICSH - the flow cytometric measurement of monoclonal antibodys. (CD61/CD41).

PLT-F can be initially selected or is automatically selected as a reflex measurement if the impedance PLT-histogram is abnormal or if the PLT value is below a customer-specific limit.

If the deviation of both methods impedance technology (PLT-I) versus fluorescence-optic counting (PLT-F) is too large, some laboratories use classical methods of platelet counting as confirmation, such as the platelet counting according to Fonio or the platelet chamber count (Neubauer chamber).

The aim of the study is to prove that the fluorescence-optic measurement of platelets PLT-F provides a reliable result in all cases and can replace an additional Fonio test, which is used in the laboratory until now.

This is a significant benefit to the workflow, as more and more samples must be distributed among fewer staff. Complete automation would significantly improve the workflow while providing significant diagnostic benefits, as the administration of a platelet concentrate in many cases depends on an exact result that cannot be guaranteed manually.

We investigated 63 samples with a high delta between PLT-I and PLT-F or an abnormal PLT histogram and additionally determined the platelet count by flow cytometric measurement with monoclonal antibodies (CD61/CD41). Most of them

thrombocytopenic samples. For 52 samples a fourth measurement was carried out, the determination of the platelet count according to Fonio.

The correlation coefficients of platelet counts measured by CD61/CD41 were highest with XN PLT-F (r=0.9938). Correlation of CD61/CD41 staining with Fonio method revealed values of r=0.8396.

The platelet value from the PLT-F channel was completely comparable with the reference method CD61/64 for almost all samples. The platelet counting according to Fonio has a poor correlation with the reference method and is influenced by subjective factors. As a result, the PLT-F method will be used in our laboratory as a confirmation test, if the routine measurement (impedance count) is interfered.

DGKL: 14. Onkologie

P-06-05

A novel serum extracellular vesicle protein signature to monitor glioblastoma tumor progression

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BACKGROUND

Detection of tumor progression in glioblastoma patients remains a major challenge. Extracellular vesicles (EVs) are potential biomarkers and can be detected in the blood of tumor patients. In this study, we evaluated the potential of serum-derived EVs from glio-blastoma patients to serve as a marker for tumor progression.

METHODS

EVs from serum of GB patients and healthy volunteers were separated by size ex-clusion chromatography and ultracentrifugation. EVs were characterized by multiple methods in accordance with MISEV2018. (EV Track ID: EV200097). Putative glioblastoma EV markers were defined by using a proximity-extension assay and bead-based flow cytometry. Tumor progression was defined according to modified RANO criteria.

RESULTS

EVs from the serum of glioblastoma patients (n = 67) showed an upregulation of CD29 ($p = 0.08$), CD44 ($p < 0.0001$), CD81 ($p < 0.0001$), CD146 ($p < 0.0001$), C1QA ($p = 0.003$), and histone H3 ($p < 0.0001$) as compared to serum EVs from healthy volunteers.

For two independent cohorts of glioblastoma patients, we noted significant upregulation of C1QA, CD44, and histone H3 upon tumor progression, but not in patients with stable disease. In a multivariable logistic regression analysis, a combination of CD29, CD44, CD81, C1QA, and histone H3 correlated with RANO-defined tumor progression with an AUC of 0.76.

CONCLUSION

Measurement of CD29, CD44, CD81, C1QA, and histone H3 in serum-derived EVs of glioblastoma patients, along with standard MRI assessment, could improve detection of true tumor progression and thus be a useful tool for clinical decision making.

DGKL: 14. Onkologie

P-06-06

Validierung eines Enzyme-linked Immunosorbent Assay zur Bestimmung der Melanoma Inhibitory Activity (MIA) Konzentration im Serum

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Zielsetzung:

Das Protein Melanoma Inhibitory Activity (MIA) wird als Serum Tumormarker des malignen Melanoms diskutiert. Das Ziel der aktuellen Studie war die Untersuchung präanalytischer Faktoren und die Bewertung der analytischen Leistungsmerkmale des Quantikine ELISA Human MIA Immunoassay (R&D Systems Inc., USA). Zudem sollten laborinterne Grenzwerte indirekt anhand eines großen Datensatzes statistisch berechnet werden, und ein Vergleich zur Wertelage eines zweiten human MIA ELISA (Roche Diagnostics GmbH, Deutschland) erfolgen.

Methoden:

Neben Untersuchungen zu Präzision und Richtigkeit des Assays wurden Serumproben (n=24) bei Raumtemperatur, 4-6 °C und -20 °C maximal 14 Tage gelagert oder dreimalig gefroren und aufgetaut. Zur Interferenztestung wurden Serumproben (n=4) mit Hämolysat, direkten Bilirubin Standard und künstlicher Lipidemulsion versetzt. MIA Grenzwerte für ≥ 18 Jahre (n=2147) wurden ohne klinische Informationen mittels Reference Limit Estimator der DGKL indirekt anhand des gesamten klinikinternen Datensatzes berechnet. Passing-Bablok-Regression und Bland-Altman-Diagramm dienten zum Vergleich des R&D und Roche MIA ELISA (n=19).

Ergebnisse:

Die Variationskoeffizienten lagen Intra- und Inter-Assay bei ≤ 6,6 % und ≤ 7,2 %. Die relativen Messabweichungen waren ≤ 12,6 %. Serumproben waren bei 4-6 °C und -20 °C bis Tag 14 stabil (keine Abweichung > 10 % des Ausgangswerts). Hingegen fielen die MIA Konzentrationen nach 2 Tagen bei Raumtemperatur < 90 % des Ausgangswerts ab. Ein dreimaliger Einfrier-Auftau-Zyklus hatte keinen signifikanten Einfluss auf die MIA Konzentration. Freies Hämoglobin (bis 4605 mg/l), Lipidemulsion (Triglyzeride bis 4298 mg/dl, Roche L-Index bis 884) und direkter Bilirubin Standard (bis 43,9 mg/dl) führten zu keiner Interferenz. Der laborinterne MIA Grenzwert (97,5. Perzentile) für alle Personen ≥ 18 Jahre lag bei ≤ 1154 pg/ml (90 % Konfidenzintervall: 1120-1418 pg/ml). Für männliche und weibliche Personen ≥ 18 Jahre waren die MIA Grenzwerte ≤ 1144 pg/ml (1100-1388 pg/ml) und ≤ 1406 pg/ml (1090-1450 pg/ml). Die MIA Konzentrationen männlicher und weiblicher Personen ≥18 Jahre unterschieden sich jedoch nicht signifikant. Für Personen ≥ 18 Jahre zeigte sich keine Altersabhängigkeit. R&D und Roche ELISA korrelierten signifikant positiv (r=0,938), jedoch lag die mittels Roche ELISA gemessene MIA Konzentration im Mittel 87 % über der MIA Konzentration des R&D ELISA. Die Passing-Bablok-Regression ergab: R&D MIA [pg/ml] = 262 pg/ml + 0,09*(Roche MIA [pg/ml]).

Diskussion und Schlussfolgerung:

Präzision und Richtigkeit des Assays sind für die Diagnostik geeignet. Relevante präanalytische Faktoren der Stabilität und Interferenzanfälligkeit konnten geklärt werden. MIA sollte bei Raumtemperatur innerhalb von 1 Tag gemessen werden. Für

Lagerung und Versand ist MIA bei 4-6 °C oder -20 °C mindestens 14 Tage stabil. R&D und Roche MIA ELISA haben eine unterschiedliche Wertelage, korrelieren jedoch gut.

DGKL: 14. Onkologie

P-06-07

Machine learning-based development of an LC-MS multi method for the quantification of a large number of oral antitumor drugs and subsets thereof

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Introduction

Personalized medicine is an ongoing trend in the treatment of many diseases. At the same time, growing evidence supports a broader use of therapeutic drug monitoring (TDM) to support personalized medicine, particularly in fields such as oncology, toxicity and efficacy are particularly critical issues. Nevertheless, the development of assays struggles to keep pace with the rapid introduction of new drugs. Therefore, there is an urgent need for innovative methods that accelerate assay development and allow seamless inclusion of newly approved drugs and adaptation to smaller subgroups whenever it is required by scientific or clinical circumstances.

Methods

To address this need, two machine learning approaches, i.e. a regression based approach and an artificial neural network (ANN), were applied and evaluated for an efficient and time-saving development of a liquid chromatography mass spectrometry (LC-MS) method for the simultaneous quantification of 73 oral antitumor drugs (OADs) and five active metabolites in human plasma. The workflow consisted of several steps including training, retention time prediction and comparison of the two machine-learning approaches. The superior approach was subsequently used to stipulate the optimal gradient, followed by individual prediction and measurement of the individual retention times of all 78 oncological agents.

Results

Both machine learning approaches predicted retention times highly accurately. (mean difference ± standard deviation (SD): 2.08% ± 9.44% ANN; 1.78% ± 1.93% regression-based approach), whereas the regression-based approach was superior to the ANN. Using the regression-based approach, an optimal gradient with a run time of 17.92 minutes and mean differences ± SD of 0.67% ± 5.16% were predicted and experimentally confirmed, followed by a full validation according to EMA and FDA guidelines. Furthermore, the possibility to use this approach for time-effective development of optimal methods for subsets of the 78 compounds was demonstrated by an exemplary modification to a subset of 14 uro-oncology agents. For this subset, a significant reduction in run time of 8.63 minutes could be demonstrated.

Conclusion

Using a regression-based approach, an LC-MS method for the simultaneous quantification of 73 OADs and five active metabolites was developed and validated with a run time of 17.29 min. We could demonstrate that the applicability of a machine-learning approach allows (1) time-effective method development of a large set of compounds, (2) to easily include newly approved compounds and (3) effectively customize a method to individual scientific or clinical situations, where only a subset of compounds might be of interest.

DGKL: 03. Biobanking

P-06-08

Intracellular signaling and non-canonical functions of Thrombomodulin

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Introduction:

Changes in endothelial proliferation, migration and barrier function are essential in various diseases like atherosclerosis and diabetes-associated vascular complications. Recent studies showed that thrombomodulin (TM), best known for activating protein C, is able to modulate these cellular functions independent of its role in clotting. However, the mechanisms of intracellular TM signaling remain poorly understood. Therefore, we aim to identify these mechanisms of cellular signaling by TM and its impact on endothelial function.

Methods:

We used an endothelial cell line (EA.hy926) and generated an EA.hy926TMnull cell line using CRISPR/Cas9. We conducted functional experiments on wildtype EA.hy926 cells and EA.hy926TMnull cells. Cells were challenged with aPC and thrombin to determine the role of these coagulation regulators. To assess proliferation, we used CFSE staining and measured the fluorescence with FACS. For migration, we conducted a scratch assay over a timeframe of twelve hours. TEER-assay (transtrans epithelial electrical resistance), Evans blue-bound albumin permeability assay, and VE-cadherin and F-actin immunofluorescence staining was done to study barrier function. In addition, we generated TM mutants lacking the cytoplasmic domain (TM-ΔC) and we exchanged the TM transmembrane domain (TM-ΔT) with that of the structurally similar protein CD248. In current studies, we are transfecting the EA.hy926TMnull cells with these plasmids to generate cells expressing these TM-mutants. To identify the intracellular interactome of TM, we generated a full length TM plasmid fused with TurboID. TurboID leads to biotinylation of proteins that are within a spherical radius of 10 nm of the TurboID tag. After cell lysis the biotinylated proteins are purified and analysed by mass-spectrometry.

Results and Conclusion:

We have optimized all the above-mentioned experiments and workflows in EA.hy926 wildtype cells. Thrombin treatment reduced barrier function in endothelial cells. EA.hy926TMnull cell line showed reduced proliferation and barrier function. Also, morphological differences in the Ea.hy926TMnull cells were observed. In ongoing experiments transfected cells, expressing the different TM-mutants, are analyzed. We expect to identify new functions of TM in maintaining the endothelial cell barrier.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-01

Evaluierung der Leistung eines Scores aus CRP, TRAIL und IP-10 bei der Diskriminierung zwischen bakterieller und viraler Infektion im Vergleich zu CRP und PCT im klinischen Routinesetting während der Corona-Pandemie

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Mit dem sog. BV-Score wurde 2015 eine neue Methode vorgestellt, die basierend auf der rechnerischen Integration der Konzentrationen von CRP, TRAIL und IP-10 als serologische Immunmarker eine schnelle und zuverlässige Diskriminierung zwischen bakteriellen und viralen Infektionen ermöglichen soll. Bisher durchgeführte Studien an Kohorten mit selektiven Ein- und Ausschlusskriterien zeigten eine hohe Leistungsfähigkeit des Scores mit Überlegenheit gegenüber etablierten einzelnen Standardparametern. Unsere Untersuchungen sollten prüfen, ob diese Resultate auch in einer Routinediagnostiksituation reproduzierbar sind.

Wir verglichen die Güte des Scores am stationären Patientenklientel unseres städtischen Klinikums während der Coronapandemie mit der von CRP und PCT. An 72 Patientenseren, deren Restmaterial am Tag der initialen Infektionsdiagnostik innerhalb von 8h bei -20°C eingefroren worden war, wurden die Messungen retrospektiv durchgeführt. Die Messung von CRP, TRAIL und IP-10 erfolgte mittels CLIA (Grenzwert für virale Infektion < 36, bakterielle Infektion > 64, uneindeutig 35-64) auf einem Liaison XL (Fa. Diasorin, Dietzenbach, DE). CRP wurde immunturbidimetrisch (Grenzwert für bakterielle Infektion ≥40 mg/l) und PCT (Grenzwert für bakterielle Infektion ≥0,5 µg/l) mittels Sandwich-ECLIA auf einem Cobas 8000 (Fa. Roche, Mannheim, DE) gemessen. Die statistische Datenauswertung erfolgte anonymisiert in der Software SPSS. In Korrelation mit der klinischen Abschlussdiagnose wurden Sensitivität und Spezifität sowie ROC-Kurven für den Score, den CRP- und den PCT-Wert erstellt.

In unserer Stichprobe erreichte CRP für die Detektion einer bakteriellen Infektion eine Sensitivität von 83,3% und eine Spezifität von 60%. PCT zeigte eine Sens. von 69% und eine Spez. von 100%. Der Score aus CRP, TRAIL und IP-10 erreichte bei alleiniger Wertung eindeutiger Ergebnisse eine Sens. von 90,2% und eine Spez. von 55,6%. Der Anteil der uneindeutigen Ergebnisse lag bei 7,5%. Die AUCs lagen für CRP bei 0,831, für das PCT bei 0,913 und für die Signatur bei 0,783.

Im Rahmen unserer Testungen war das PCT allen anderen Parametern überlegen. Schwächen der BV-Signatur bei rein viralen Infektionen entstehen am ehesten aufgrund von zwei Mechanismen: Einerseits verschieben alle Zustände, die mit einem erhöhten CRP einhergehen (schwere virale Infektionen, Operationen, Tumore) den Score in Richtung „bakteriell“, andererseits führt eine rasche Abnahme von IP-10 und TRAIL im Infektionserlauf zu einem überschießenden Einfluss des CRP. Bisherige Untersuchungen mit einem höheren Anteil an pädiatrischen Patienten in einer frühen Infektionsphase von vor allem Atemwegsinfektionen bildeten eine unkompliziertere analytische Umgebung ab. Im stationären Routinebetrieb kann ein Einsatz unter Einhaltung einer kurzen Zeitverzögerung bei bekanntem Symptombeginn mit genauer Kenntnis der Einflussfaktoren und korrekten Interpretation in Zusammenschau der gesamten Befundkonstellation hilfreich sein.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-02

Enhanced early T cell activation and protection from hyper-inflammation in smoke-exposed Cox4i2-/- influenza infected mice

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Respiratory virus infections are major precipitators of chronic obstructive pulmonary disease (COPD) exacerbations. Mitochondrial reactive oxygen species (mtROS) play an important role in cellular immune function but increased mtROS levels may have an adverse effect in COPD. Cox4i2 regulates ROS production at complex III of the mitochondrial electron transport chain and downregulation is protective against the development of emphysema.

We hypothesize that Cox4i2-/- mice are protected from smoke-induced hyper-inflammation but also exhibit impaired antiviral cellular immunity and therefore show higher virus load following infection. We further postulate that spleen cells

from wildtype (WT) smoke exposed (SE) and Cox4i2-/- mice are less responsive to ex vivo restimulation with virus antigenic stimuli and show a suppressed Interferon-γ (IFN-γ).

We analyzed lung histology in terms of inflammation and mucus production, as well as BAL Albumin leakage, and virus load via qPCR. We also analyzed IFN-γ production following ex vivo stimulation of spleen cell homogenates by ELISPOT and T cell activation markers from lung T cells by flow cytometry. C57BL/6 (WT) and Cox4i2-/- mice were exposed for two weeks to smoke (SE) or room air (RA), with or without H1N1 influenza A/Puerto Rico/8/1934 (PR8) infection. Mice were sacrificed 3 or 10 days post infection (DPI).

Unexpectedly, there was no difference in viral load at 3 DPI, but at 10 DPI WT SE mice showed a trend for higher virus load when compared to WT RA mice ($p=0.0541$) . Influenza infection increased perivascular and peribronchiolar inflammation in all groups after 3 DPI ($p < 0.0001$), with no changes in mucus production. At 10 DPI, peribronchiolar and perivascular inflammation was still increased after infection ($p < 0.0001$) with a significant decrease in Cox4i2-/- SE mice vs. WT SE mice ($p=0.0377$) and a trend for a decrease in Cox4i2-/- SE vs. Cox4i2-/- RA mice ($p=0.0548$). Infection led to increased albumin leakage in all groups but not Cox4i2-/- SE mice with a significant decrease in Cox4i2-/ SE vs. WT RA and Cox4i2-/- RA mice ($p=0.006$ & $p=0.004$). Restimulation with UV inactivated virus or virus-derived immunogenic peptides showed impaired IFN-γ production in Cox4i2-/- ($p=0.0125$ & $p=0.248$). CD69 on CD4+ T cells increased after infection ($p < 0.0001$). With significant increases in Cox4i2-/- SE mice vs. both WT groups at 3 DPI ($p=0.0208$ & $p=0.0097$). In CD8 T cells Cox4i2-/- SE mice showed similar upregulation as WT RA mice.

Taken together Cox4i2-/- mice did not show impaired T cell activation albeit decreased albumin leakage, perivascular inflammation and IFN-γ production at 10 DPI indicate a protection effect over hyper-inflammation, especially in Cox4i2-/- SE mice. Future steps include prolongation of smoke exposure of mice and translation in the human context including testing of COPD patients' peripheral blood mononuclear cells and human precision cut lung slices.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-03

Vitamin D in cellular immunity regulation in chronic hepatitis B patients.

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Introduction: To study the relationship of 25-hydroxyl vitamin D3 with peripheral blood T lymphocyte immune function in chronic hepatitis B patients.

Methods: The laboratory data for patients with chronic hepatitis B, chronic hepatitis C were analyzed. Serum 25-hydroxyl vitamin D, hepatitis B virus serological markers, and subsets of T lymphocytes were determined. These patients were divided into three groups based on serum 25-hydroxyl vitamin D level.

Results: In CHB patients the proportion of CD4+ T lymphocytes and the ratio of CD4+/CD8+ significantly decreased ($p < 0.05$) as the level of 25-hydroxyl vitamin D decreased, but the proportion of CD8+ increased ($p < 0.05$). Vitamin D showed a moderate negative influence on the CD8 cell count in CHF patients.

Conclusions: Vitamin D may take a role in the immunologic function adjustment and immune tolerance in the natural course of chronic HBV infection, and high levels of vitamin D may be able to achieve sustained virological response.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-04

Use of Myeloid Activation Markers with or without a lymphocyte subset test for the management of acute unclear fever

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Use of Myeloid Activation Markers with or without a lymphocyte subset test for the management of acute unclear fever

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Keywords: CD169, Siglec-1, CD64, HLA-DR, myeloid activation, acute infection, viral infection, bacterial infection.

Abstract

Fever and clinical signs of infection can be caused by a variety of pathogens and are often difficult to differentiate clinically. The early detection and differentiation of infection origin in emergency patients is a daily and ever-present challenge for clinicians. Established laboratory markers, e.g., CRP and PCT, are often not sufficient to determine a definitive diagnosis. So far there has been a lack of reliable, sensitive, and fast turnaround time point-of-care- diagnostics to precisely distinguish between viral and bacterial cause of infection.

In this multicenter study we aim to determine the expression of the three surface markers neutrophil CD64, monocyte CD169 and HLA-DR in a total of 160 patients presenting to the emergency department of university hospitals Giessen and Marburg (UKGM) with fever or clinical suspicion of infection. Therefore, a fast flow cytometric approach is used, and expression levels are compared to standard laboratory parameters, e.g., CRP and PCT as well as microbiology work up. Independently, lymphocyte subset analysis with adjunct activation markers CD25 and HLA-DR is performed for each sample. Preliminary results suggest a correlation between neutrophil CD64 levels and conventional infection parameters, such as CRP ($p=0.02$) and significant differences between marker expression levels among acutely infected patients vs. non- infected controls ($p=0.03$).

With the help of lymphocyte subset analysis and myeloid activation in combination or taken alone we aim to gather information not only on the expression levels of myeloid CD64, CD169 and HLA-DR in acute bacterial and/or viral infection, but further on adaptive immune cell activation and dynamics.

Objectives are further to contribute to biomarker research and thus improve patient care, point of care diagnostics and the reduction of antibiotic overuse.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-05

Comparison of three procalcitonin reagents in SARS COV 2 patients

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Comparison of three procalcitonin reagents in SARS COV 2 patients

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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 original Thermofisher Scientific / Brahms reagent for the Kryptor (Brahms) in plasma from COVID-19 patients with a clinical suspicion for bacterial superinfection.

Method: PCT reagents from Diazyme Laboratories for the ADVIA (Siemens), Thermofisher Scientific /Brahms for the Centaur (Siemens) and for the Kryptor (Brahms) have been compared using SARS COV2 positive patient samples

Results: We were able to determine significant differences between the three 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 ($p < 0.01$). 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.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-06

SARS-CoV-2 Vaccination Companion Diagnostics: Perspectives and Limitations of Post-Vaccination Serology in Wildtype B.1 and Omikron BA.5.1

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SARS-CoV-2 Vaccination Companion Diagnostics: Perspectives and Limitations of Post-Vaccination Serology in Wildtype B.1 and Omikron BA.5.1

Purpose: Vaccination against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is carried out according to fixed immunization-schedules instead of individualized (re-)immunization in accordance with personal immune responsiveness and adapted to the current variant of concern.

Methods: We developed diagnostic strategies based on antibodies against SARS-CoV-2 spike protein receptor-binding domain (S1-AB) and their neutralizing capacity in surrogate assays as well as in full virus neutralization tests in cell culture. Neutralization against wildtype B.1 and Omikron BA.5.1 six months after vaccination with unmodified mRNA vaccine was evaluated and led to proposals for individualized (re-)vaccination schedules. For this purpose, 124 subjects were monitored by serological testing before, during and six months after vaccination against SARS-CoV-2 with the mRNA-based vaccine Spikevax (Moderna, Cambridge, MA, USA).

Results: Vaccination responses varied substantially interindividually and with regard to the investigated variant of concern. Six months after the second vaccination, 92% of the sera exhibited sufficient neutralizing capacity against wildtype B.1, but only 20% showed similar neutralization of Omikron BA.5.1. At this time point, participants still positive for the full virus NT in B.1 strain could be discriminated by S1-AB levels ≥1000 U/mL as a diagnostic tool for gauging ex-vivo immune-responsiveness, while sera inhibiting BA5.1 could not be distinguished from non-inhibiting sera by serum levels of S1-AB.

Conclusion: Our studies support individualized (re-)vaccination schemes based on simple serological tests suitable for health care routine shaping vaccination-programs more time- and cost efficient as well as reducing the amount of side-effects. Nevertheless, it also shows that not only the vaccines against SARS-CoV-2 themselves but also the serological tests of immune responsiveness thereto have to be regularly adapted to the emergent new variants of concern. Moreover, the diagnostic guidelines should be revised to encompass ex-vivo correlates of immune protection. In this context, it will be interesting to determine the discriminative power of the 2nd-generation S1-AB-test currently under roll-out.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-07

Serum NfL als Biomarker bei erregerbedingten Meningitiden und Enzephalitiden

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Zielsetzung:

Bei verschiedenen Erkrankungen des zentralen Nervensystems werden neuronale Strukturproteine wie Neurofilament light (NfL) als Folge einer neuroaxonalen Schädigung freigesetzt. Dies geschieht unabhängig von der zugrundeliegenden Pathologie. Die Spezifität von NfL für Neurone, die von der Pathologie unabhängige Freisetzung sowie die Messbarkeit im Serum macht dieses Protein zu einem attraktiven Biomarker der neuroaxonalen Schädigung. Ein diagnostischer und prognostischer Mehrwert konnte für einige neurologische Erkrankungen wie z.B. Multiple Sklerose, Amyotrophe Lateralsklerose und verschiedene Demenzerkrankungen gezeigt werden. Bisher wenig untersucht sind NfL-Werte bei viralen und bakteriellen Meningitiden und Enzephalitiden. Jedoch spielt die neuronale Destruktion auch hier eine wichtige Rolle, sodass Serum NfL (sNfL) als Biomarker zur Abschätzung des Krankheitsverlaufs und der Prognose denkbar ist. Diese Studie untersucht die sNfL Konzentration bei Patienten mit Herpes-Enzephalitis (HSE), bakterieller und aseptischer Meningitis (BM und AM) und korreliert die Werte mit klinischen Parametern.

Methoden:

Von 90 Patienten (30 HSE, 27 BM, 33 AM) wurden definierte klinische Daten erfasst und von allen verfügbaren Serumproben (HSE N=64, BM N=55 and AM=40) sNfL mit dem NF-light Kit am single molecule array HD-X-Analyser (Quanterix) gemessen. In der HSE-Gruppe wurde außerdem im Liquor und Serum mittels sekundärer Immunhistochemie auf Rattenhirnschnitten und indirekter Immunfluoreszenz auf Primärkulturneuronen nach postinfektiösen neuronalen Oberflächenantikörpern gesucht. Anschließend erfolgten die statistische Auswertung und Korrelation der Messergebnisse mit klinischen Parametern.

Ergebnisse:

Patienten mit HSE und BM hatten signifikant höhere Serum NfL-Konzentrationen als Patienten mit AM. Dies galt sowohl für die akute (Tag 0-7) als auch für die subakute (Tag 8-21) Erkrankungsphase. Weiterhin korrelierten höhere sNfL Werte mit einem schlechteren Outcome (gemessen an der modified ranking scale). In der HSE-Gruppe konnte zusätzlich eine Tendenz zu höheren sNfL Werten bei Patienten, die im Krankheitsverlauf neuronale Oberflächenantikörper entwickelten, beobachtet werden.

Diskussion und Schlussfolgerung:

Unsere Ergebnisse zeigen, dass das sNfL als zusätzlicher Biomarker der neuroaxonalen Schädigung bei Patienten mit erregerbedingten Meningitiden und Enzephalitiden herangezogen werden kann. Auch Westman et al. konnten in einer kleinen prospektiven Studie eine Assoziation zwischen höheren sNfL Werten und schlechtem kognitiven Outcome sowie der Entwicklung von sekundären Anti-NMDA-Rezeptor-Antikörpern zeigen. [1] Insgesamt könnte Serum NfL als zusätzlicher Parameter bei (1) der Differenzierung zwischen HSE/BM/AM; (2) der Prognoseabschätzung; (3) der Risikobeurteilung der Entwicklung einer sekundären Autoimmunität hilfreich sein.

DGKL: 08. Infektion, Infektionsserologie, Mikrobiologie und Mikrobiom

P-07-08

Humoral SARS-CoV-2 Immune Response in COVID-19 Recovered Vaccinated and Unvaccinated Individuals Related to Post-COVID-Syndrome

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Introduction: The duration of anti-SARS-CoV-2-antibody detectability up to 12 months was examined in individuals after either single convalescence or convalescence and vaccination. Moreover, variables that might influence an anti-RBD/S1 antibody decline and the existence of a post-COVID-syndrome (PCS) were addressed. As cfDNA is released into the bloodstream from dying cells, it might provide information on organ damage in the late recovery of COVID-19. Therefore, we evaluated cfDNA concentrations of COVID-19 recovered participants as potential predictor of a PCS.

Methods: Forty-nine SARS-CoV-2-qRT-PCR-confirmed participants completed a 12-month examination of anti-SARS-CoV-2-antibody levels and PCS-associated long term sequelae. Cell-free DNA (cfDNA) was isolated and quantified from EDTA-plasma. In the context of antibody dynamics, a random forest-based logistic regression with antibody decline as the target was performed and internally validated.

Results: The mean percentage dynamic related to the maximum measured value was 96 (±38)% for anti-RBD/S1 antibodies and 30 (±26)% for anti-N antibodies. Anti-RBD/S1 antibodies decreased in 37%, whereas anti-SARS-CoV-2-anti-N antibodies decreased in 86% of the subjects. Clinical anti-RBD/S1 antibody decline prediction models, including vascular and other diseases, were cross-validated (highest AUC 0.74). Long-term follow-up revealed no significant reduction in PCS prevalence but an increase in cognitive impairment, with no indication for cfDNA as a marker for a PCS.

Conclusion: Long-term anti-RBD/S1-antibody positivity was confirmed, and clinical parameters associated with declining titers were presented. A fulminant decrease in anti-SARS CoV-2-anti-N antibodies was observed (mean change to maximum value 30 (±26)%). Anti-RBD/S1 antibody titers of SARS-CoV-2 recovered subjects boosted with a vaccine exceeded the maximum values measured after single infection by 235 ± 382-fold, with no influence on preexisting PCS. PCS long-term

prevalence was 38.6%, with an increase in cognitive impairment compromising the quality of life. Quantified cfDNA measured in the early post-COVID-19 phase might not be an effective marker for PCS identification.

DGKL: 03. Biobanking

P-07-09

Identification of patients at risk for a systemic inflammatory response syndrome (SIRS)

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Introduction: The Systemic inflammatory response syndrome (SIRS) is a common complication after surgery that in severe cases leads to multiple organ dysfunction and even death. An uncontrolled activation of the immune system with release of damage associated patterns is a characteristic feature of SIRS. Especially in cardiac surgery with cardiopulmonary bypass, SIRS is a phenomenon with an incidence of up to 60%. It remains elusive why some patients develop SIRS while others survive without complications. In this project, we hypothesize that a hyperactivity of the immune system can trigger cascades of pro-inflammatory events that lead to SIRS. We developed a whole blood in-vitro assay to evaluate and quantify the immune reactivity of patients. In a prospective study with over 100 patients scheduled for an open heart surgery, we interrogated if our whole blood assay has the potential to identify patients with an hyper-reactive immune system that could lead to SIRS in response to the surgical trauma.

Methods: To identify patients at risk for SIRS, we have set up a standardized whole-blood ‘in-tube’ culture and stimulation system, using a broad spectrum of stimulants of innate immune receptors (TLR4, TLR7, TLR8, STING and T-cell activation) found to stimulate neutrophils, monocytes and T cells. This assay is performed in culture tubes prefilled with medium containing the respective stimulus. Hirudin anti-coagulated peripheral blood is filled into the reaction tubes and incubated for 4hrs at 37°C. After incubation, supernatants are harvested for cytokine quantification. The remaining cell pellet is lyzed for total mRNA isolation and subsequent RNA sequencing analysis.

Results: Over 30 patients were included into the study of which 15 patients developed a SIRS after open heart surgery. During the pre-clinical screening, none of these patients showed deviations in clinical parameters or inflammatory markers, except slight differences in cell counts for CD19+ B cells and CD4+ T cells in patients who developed a SIRS. Analysis of RNA sequencing data from untreated and stimulated whole blood assays showed a clear separation of transcriptional profiles from patients who developed SIRS to patients without complications. Moreover, genes participating in immune signalling pathways were stronger enriched and expressed in the group of future SIRS patients in comparison to patient who did not develop any complications after surgery.

Conclusion: Our data reveal for the first time that the phenomenon of SIRS is most likely a consequence of an hyperreactive immune system which is actived by the trauma during open heart surgery. Further, we propose our whole blood immune assay as a prospective test to identify patients at risk for SIRS which could be treated by anti-inflammatory therapeutics in preparation of the surgery to prevent life-threating complications like SIRS.

DGKL: 03. Biobanking

P-07-10

Kultur-basierte Differenzierung von Erregern stationär erworbener Pneumonie mittels Ionenmobilitäts- und Massenspektrometrie

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Einleitung

Die nosokomiale Pneumonie gilt als die häufigste Todesursache unter den im Krankenhaus erworbenen Infektionen. Daher ist eine schnelle und zuverlässige Diagnose entscheidend für die Einleitung einer gezielten Antibiotikatherapie. Bei kulturbasierten Diagnoseverfahren dauert es jedoch oft bis zu 48 Stunden, bis der ursächliche Erreger identifiziert ist. Daher muss eine Technologie für den gezielten Nachweis von nosokomialen Infektionen entwickelt werden. Das hier vorgestellte Projekt zielt darauf ab, anhand der mikrobiellen, flüchtigen organischen Verbindungen (mVOCs) in der Ausatemluft auf den verursachenden Erreger zu schließen. Um diese mVOCs aus der menschlichen Atemluft direkt am Patientenbett zu messen, wurde ein mobiles Ionenmobilitätsspektrometer in Kombination mit einer gaschromatographischen Vortrennung (GC-IMS) aufgebaut und muss validiert werden.

Methoden

Zur Bestimmung spezifischer mVOC-Profile und Markerverbindungen wurde der Gasraum von Referenzstämmen (Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, Legionella pneumophilia, Acinetobacter baumannii complex und Escherichia coli) mit dem mobilen GC-IMS beprobt. Da die Probenahmekammer einen Inkubator enthält, können außerdem frisch beimpfte Agarplatten während ihrer Wachstumsphase kontinuierlich gemessen werden.

Da die Identifizierung der einzelnen mVOCs unerlässlich ist, um Rückschlüsse auf den spezifischen Stoffwechsel der Erreger zu ziehen, wird zur Validierung ein Thermodesorptions-Gaschromatograph gekoppelt mit einem Massenspektrometer (TD-GC-MS) eingesetzt. Für den parallelen Nachweis der mVOCs wurde ein IMS als zweiter Detektor in gleicher Flusslinie unter Verwendung eines Splitters eingeführt. Die Kopplung aus TD-GC-MS-IMS ist in der Literatur noch nicht beschrieben.

Ergebnisse

Erste Messungen von ausgewählten Bakterienkulturen zeigen, dass insbesondere die hohe Detektionsleistung des IMS den Nachweis von erregerspezifischen VOC-Mustern ermöglicht. Die Identifizierung einzelner mVOCs erfolgt mittels MS und einer etablierten IMS-Datenbank mit mehr als 30 relevanten Substanzen. Indol wurde beispielsweise in Escherichia coli gefunden, das nachweislich ein Produkt seines Tryptophan-Stoffwechsels ist. Es konnte außerdem gezeigt werden, dass spezifische mVOCs bereits nach sechs Stunden Inkubation nachgewiesen werden können.

Diskussion und Schlussfolgerung

Der nächste Schritt wird die Durchführung einer Studie im klinischen Umfeld sein. Bei Erweiterung der mVOC Datenbank könnte die MS und IMS eine vielversprechende Technik für den komplementären Einsatz bei der Diagnostik von nosokomialen Pneumonien sein. Das Einsatzgebiet könnte auch auf weitere Krankheitsbilder erweitert werden, bei denen eine Änderung des VOC Profils zu erwarten ist.

DGKL: 04. Bioinformatik, Digitalisierung und Medizininformatik

P-08-01

Advanced automation in liquid chromatography-tandem mass spectrometric methods (LC-MS/MS) using liquid-liquid extraction

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Introduction: Performing manual liquid-liquid extractions for sample processing of high-throughput parameters e.g. vitamin D by LC-MS/MS is laborious and time-consuming. Automation can significantly reduce the manual processing time. Usually in clinical chemistry, simpler protein precipitations in terms of process technology are carried out as sample preparations that can be easily automated.1 Liquid-liquid extraction, in opposite, presents a challenge for automation. Here we report on the automation of a liquid-liquid extraction for the analysis of vitamin D in human serum using a robotic system and its compatibility with manual sample processing.

Materials and Methods: For manual pretreatment 110 µl serum and 5 ml hexane were added in 2 pipetting steps to a primary tube, mixed by vortexing and centrifugated. In a third pipetting step 4 ml from the upper phase were transferred to a secondary tube, followed by evaporation and reconstitution in 100 µl mobile phase and injecting in the LC-MS/MS system. To process 96 samples 2 glass test tubes, 1 microtiter plate and 2h hand on time are required. For automated handling both pipetting and mixing steps but not centrifugation were transferred to a Tecan EVO® robotic system (Tecan, Maennedorf, Switzerland). Performance of the automated pretreatment was evaluated by comparison of precision and accuracy of measurement results (2 levels of quality control (QC) samples, 20 single runs on separate days) to results obtained by the manual procedure as well as by parallel analysis of patient samples. In addition, saving of time and material costs by the automated handling were evaluated.

Results: Imprecision and inaccuracy evaluated using the QC samples were < 6% and < 2% with the robotic pretreatment vs. < 6% and < 4% with the manual pretreatment. No significant differences between results generated with the two procedures were found using patient samples (Pasing-Bablok regression, n=16; mean Bias -5.95%). The new developed Tecan EVO®-based process required 2 deepwell plates, 1 ml hexane and ~1h hand on time for 96 samples.

Discussion and Conclusions: Automation of the vitamin D pretreatment resulted in reduction of hand on time and material costs by ~50 % each. Particularly solvent requirement was decreased by 80%. Disadvantages of automation compared to the manual procedure were a higher sample dead volume and the requirement of uniform sample tubes for automation. Crucial for the realization of the liquid-liquid extraction were strong consideration and control of different viscosities of the solvents applied. Well comparable analytical precision and accuracy were demonstrated. In conclusion, automation of liquid-liquid sample pretreatment by a robotic system is a feasible alternative for high-throughput parameters and allows for substantial saving of resources and conserving the environment.

DGKL: 09. Leitlinien und Diagnostische Pfade

P-08-02

Laboratory diagnostic of acute kidney injury and its progression: Risk of underdiagnosis in female and elderly patients

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Introduction: Acute kidney injury (AKI) is a common disease, with high morbidity and mortality rates. In this study, we investigated the potential influence of sex and age on laboratory diagnostics of AKI and patient outcomes. It is known that serum creatinine (SCr) has limitations as a laboratory diagnostic parameter for AKI due to its dependence on muscle mass, which may lead to an incorrect or delayed diagnosis for certain patient groups, such as women and the elderly.

Methods: Overall, 7592 cases with AKI, hospitalized at the University of Leipzig Medical Center (ULMC) between 1st January 2017 and 31st December 2019, were retrospectively analyzed. The diagnosis and staging of AKI were performed according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, based on the level and dynamics of SCr. The impact of sex and age was analyzed as follows: each SCr was converted to an eGFR using the CKD-EPI equation and then backcalculated to SCr using the constants for the opposite sex (female to male) or an age of 30 years (old to young). General characteristics, comorbidities and AKI occurence and progression were analyzed for all subgroups.

Results: In our study cohort progressive AKI occurred in 19.2% of all cases (n = 1458). Female cases with AKI were underrepresented (40.4%), with a significantly lower first (–3.5 mL/min) and last eGFR (–2.7 mL/min) ($p < 0.001$). The highest incidence proportion of AKI was found in the [61–81) age group in female (49.5%) and male (52.7%) cases. Females with progressive AKI were underrepresented ($p = 0.04$). By defining and staging AKI on the basis of relative and absolute changes in the SCr level, it is more difficult for patients with low muscle mass and, thus, a lower baseline SCr to be diagnosed by an absolute SCr increase. AKIN1 and AKIN3 can be diagnosed by a relative or absolute change in SCr. In females, both stages were less frequently detected by an absolute criterion alone (AKIN1 ♀ 20.2%, ♂ 29.5%, $p < 0.001$; AKIN3 ♀ 13.4%, ♂ 15.2%, $p < 0.001$).

Conclusion: A recalculated SCr for females (as males) and males (as young males) displayed the expected increase in AKI occurrence and severity with age and, in general, in females. Our study illustrates how SCr, as the sole parameter for the diagnosis and staging of AKI, bears the risk of underdiagnosis in vulnerable patient groups such as Women and the elderly, most likely due to low muscle mass. A sex- and age-adapted approach to laboratory diagnostics of AKI might offer improvements.

DGKL: 10. Lernen und Lehren

P-08-03

Evaluation der Nutzung kursbegleitender digitaler Angebote im Fach Klinische Chemie/ Humanmedizin

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Einleitung

Während der Pandemie-Zeit entwickelte moodle-Räume wurden bei der Lehre der klinischen Chemie für Medizinstudenten als digitale Kursbegleitung zur Präsenzlehre im Wintersemester 2022/2023 angeboten. Individuelle Kommentare zeigen, dass dies für die Studierenden eine hilfreiche Ergänzung darstellt, eine Analyse zur allgemeinen Akzeptanz der moodle-Räume mit digitalen Angeboten in der klinischen Chemie fehlt.

Methoden

Die Rate an Einschreibung und Aktivitäten (Download von Kursunterlagen oder Vorlesungsfolien, obligate und freiwillige Micro-Learning-Einheiten mit Fragen, virtuelle Mikroskopie, relevante Fragensammlung in Amboss) wurde für die

kursbegleitenden moodle-Räume für das 5. Semester, das 7. Semester und das 9. Semester für das Fach Humanmedizin anonym ausgewertet.

Ergebnisse

Generell besteht eine hohe Einschreibungsrate in die begleitenden moodle-Kursräume (87-99% der Kursteilnehmer). Obligate Aktivitäten besitzen eine deutlich höhere Bearbeitungsrate (82%-95%) als die Auswahl freiwilliger Aktivitäten (10%-60%). Letztere werden gerade von Kursteilnehmern im 7. und 9. Semester verstärkt genutzt (Mittelwert 70% bzw. 55% für Vorlesungsfolien bzw. Microlearning-Einheiten. Ebenso zeigt die virtuelle Mikroskopie (Integration des virtuellen Histokastens der Anatomie II, Universitätsklinikum Jena) eine hohe Aktivitätsrate (Mittelwert 44%).

Fazit und Ausblick

Die Nutzungsraten weisen auf eine gute Akzeptanz des digitalen Angebots hin und auch freiwillige digitale Lehrangebote scheinen für einen großen Teil der Studierenden eine hilfreiche Ergänzung darzustellen. Eine Limitation der Auswertung ist, dass bezüglich der freiwilligen Aktivitäten nur das Auswählen der Aktivität registriert wurde. Im Rahmen einer zielgerichteten Weiterentwicklung der digitalen Kursbegleitung sind daher zukünftig ergänzende Instrumente (z.B. Umfragen) zur genaueren Akzeptanz- und Bedarfsanalyse geplant.

DGKL: 10. Lernen und Lehren

P-08-04

Digital Competence in Laboratory Medicine: Results from a Survey Among Young Scientists

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BACKGROUND-AIM

Innovative digital health technologies, such as virtual and augmented reality, artificial intelligence including machine learning, big data analytics and telemedicine are at the centre of current and future developments in healthcare. Digital competences are needed to understand these technologies and to use them in the best interests of patients. We aimed to assess digital competence of young scientists (< 40 years old) and to evaluate the needs of young scientists in laboratory medicine in terms of important digital skills.

METHODS

A global web survey was conducted in 2022 by two young scientists who are members of national societies: the DGKL (Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin) from Germany and the SFBC (Société Française de Biologie Clinique) from France. The 13 questions were disseminated to young scientists on the lists of three large networks: the national federation of Germany, the EFLM Task Group-Young Scientists, and the IFCC Task Force-Young Scientists and its corresponding members.

RESULTS

A total of 119 young scientists participated in that survey, representing 40 countries from Europe, Africa, Asia, and North and South America. 59% declared using digital skills in their work, but 80% reported no further education during their academic qualification. Of these, 96% consider it necessary for digital skills to be taught as part of academic education. One-third of participants have colleagues in their institution who have pronounced digital skills. Most young scientists would like to better understand the concrete applications of digital tools in their daily work in the laboratory.

CONCLUSIONS

At worldwide proportions, young scientists need and demand greater education and training in the field of digital competence. In 2023, we will establish an international working group on digital competence to build a learning environment and propose common international resources (tutorial articles, videos, exercises and technical articles) to strengthen digital competence in laboratory medicine. Our aim is to support the next generation of specialists in laboratory medicine in mastering the upcoming professional challenges.

DGKL: 17. Qualitätssicherung und Management

P-08-05

Peer-Review-Verfahren in der Laboratoriumsmedizin: Interprofessioneller Dialog auf Augenhöhe

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Einleitung: Das Peer Review Verfahren bietet die Möglichkeit einer kritischen Reflexion der eigenen Abläufe und Prozesse in einem strukturierten und vertrauensvollen Dialog mit FachkollegInnen. Kennzeichnend für das Peer Review ist die interprofessionelle Durchführung mit sowohl technischem als auch akademischem Personal auf Augenhöhe aus unterschiedlichen Einrichtungen. Die Teilnahme ist freiwillig. Seit 2019 hat das Peer-Review-Verfahren als Alternative zu internen Audits Einzug in die Rili-BÄK gehalten. Die Bundesärztekammer bietet seit 2013 ein Curriculum zum ärztlichen Peer-Review an, das selbstverständlich auch von technischem Personal absolviert wird, um dem Charakter des Peer Reviews gerecht zu werden. Ziel eines Peer-Reviews in der Laboratoriumsmedizin ist die Verbesserung der PatientInnensicherheit durch inhaltliche und qualitative Weiterentwicklung der Prozesse. Die hier vorgestellten Peer Reviews erfolgten im Rahmen der curricularen Ausbildung durch die ÄK Niedersachsen. Die jeweiligen Peer-Teams setzten sich dabei paritätisch aus technischem und akademischem Personal zusammen.

Material und Methoden: Für den strukturierten Ablauf eines Reviews wurde ein Fragenkatalog für die Selbst- und Fremdbewertung mit etwa 90 Einträgen entwickelt. Dieser umfasst die wesentlichen Themen aus allen Bereichen (Organisation, Führung/Mitarbeitende, PatientInnen, EinsenderInnen, Analytik, Qualitätsindikatoren, Validation und Abrechnung). Die Selbstbewertung erfolgte jeweils im Vorfeld gemeinsam mit technischem und akademischem Personal. Ein Merkmal des Peer Reviews ist weiterhin, dass die besuchten Labore für sie wichtige Themen zur Begutachtung während des Besuches auswählen können. Es fallen lediglich Reisekosten an.

Ergebnisse und Diskussion:

Während des Peer Reviews erfolgt die Fremdbewertung durch das interprofessionelle Peer Team. Dazu gehört sowohl eine Dialogphase als auch ein Laborrundgang. Insbesondere für das besuchte Labor werden Lösungsvorschläge und Reflexionen gemeinsam erarbeitet. Die Ergebnisse werden im Rahmen einer SWOT-Analyse zum Abschluss des Peer Reviews mündlich vorgestellt und im weiteren Verlauf als schriftlicher Bericht zur Verfügung gestellt. Nach etwa einem halben Jahr erfolgt eine Evaluation der abgeleiteten und der ggf. bereits umgesetzten Maßnahmen.

Zusammenfassung: Das Peer-Review-Verfahren ist ein nützliches Instrument zur Qualitätssicherung in medizinischen Laboren. Die Erstellung eines Fragenkatalogs für die Selbst- und Fremdbewertung erleichtert hierbei den strukturierten Ablauf und erhöht die Effizienz. Insbesondere die enge Einbindung von technischem Personal sowohl in die curriculare Ausbildung als auch in die Durchführung ermöglicht einen fruchtbaren Dialog und führt zudem zu einem guten Austausch über Best practices zwischen den Laboratorien.

DGKL: 09. Leitlinien und Diagnostische Pfade

P-08-06

HbA1c Messung in K2EDTA und in Blutentnahmeröhrchen mit Glukosestabilisation

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Zielsetzung

Für die Diagnostik und Verlaufskontrolle eines Diabetes mellitus ist unter anderem die Bestimmung der HbA1c Konzentration unerlässlich. Die Bestimmung erfolgt in der Regel aus EDTA Vollblut. Im selben Kontext ist es notwendig, die Glukosekonzentration zuverlässig zu bestimmen. Um die in-vitro Glykolyse zu inhibieren werden Röhrchen zur Glukosestabilisierung verwendet, die als Additiv Fluorid und Citrat enthalten. Eine gleichzeitige Analyse von Glukose und HbA1c in diesen Röhrchen wird von den Herstellern nicht ausgewiesen. Hauptziel der Arbeit war zu prüfen, ob die Bestimmung von HbA1c unter Verwendung unterschiedlicher Primärröhrchen vergleichbar und somit zuverlässig erfolgen kann.

Methoden

In die Studie wurden 13 Patienten mit und 17 Patienten ohne diagnostizierten Diabetes mellitus eingeschlossen. Die Sammlung von Probenmaterial erfolgte in vier verschiedene Blutentnahmegefäße: ein K2EDTA Röhrchen (Beckton-Dickinson) und drei Fluorid+Citrat Röhrchen (Greiner, Kabe, Sarstedt). Die Bestimmung der HbA1c Konzentration erfolgte mittels HPLC. Die Ergebnisse wurden mittels TOST-Äquivalenztest und Passsing-Bablok Regression ausgewertet.

Ergebnisse

Die HbA1c Messwerte in den drei untersuchten Fluorid+Citrat Röhrchen (Greiner, Kabe, Sarstedt) sind äquivalent zu den K2EDTA basierten Messwerten (TOST p-Werte für alle Vergleiche < 0.05). Weiterhin führt eine 24-stündige Lagerung der Röhrchen bei 4°C zu keiner maßgeblichen Veränderung der HbA1c Messwerte.

Diskussion und Schlussfolgerung

Im Sinne des Patient-Blood-Managements hat die Bestimmung von Glukose und HbA1c, der beiden wichtigsten Messgrößen in der Diagnostik und Verlaufskontrolle eines Diabetes mellitus, einen hohen Stellenwert. Die Abnahme nur eines Röhrchens zur simultanen Messung von Glukose und HbA1c Konzentration kann zu einer Kostensenkung und einer logistischen Vereinfachung in der Praxis führen. So kann bei einem erhöhten Glukosewert innerhalb der Nachmeldefrist die HbA1c

Bestimmung aus demselben Material nachgefordert werden. Das bedeutet keinen weiteren Aufwand für den Arzt und für den Patienten.

DGKL: 11. Molekulare Diagnostik

P-09-01

Establishment of a workflow for sputum samples for microbiome research in long COVID patients

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Introduction:

Due to COVID-19 and its pulmonary complications, the lung microbiome has come back into the focus since it is associated with various respiratory diseases and the immunity. Many studies have shown that the microbiome of patients suffering from respiratory diseases changes compared to healthy ones. The goal of my study is to develop a workflow for isolation, PCR, sequencing and analysis to study the lung microbiome of post-COVID patients. As the lung microbiome may contain diagnostic or prognostic information, it could therefore become a clinical parameter for long COVID.

Methods:

The first step is to find a method for the isolation of bacterial DNA from sputum samples to perform 16S PCR. The isolation method is of crucial importance as the quality, quantity and purity of the isolated DNA is responsible for the success of the long-range PCR. Three Kits were tested: QIAamp DNA Microbiome Kit (QIAGEN), Prepito NA Body Fluid Kit (PerkinElmer) and ZymoBIOMICS DNA Microprep Kit (Zymo Research). The subsequent 16S PCR (16S Barcoding Kit 1-24, Oxford Nanopore Technologies) and sequencing (MinION, Oxford Nanopore Technologies) should not pose any problems if the quantity and quality of the DNA is optimal.

Results:

The ZymoBIOMICS DNA Microprep Kit (Zymo Research) performed best among the methods and kits tested for isolating bacterial DNA from sputum samples. All samples except one were successfully amplified. Even in the microbiology no pathogenic germs or normal lung flora could be detected in the failed one. Compared to the previous experiments, this isolation method was also performed using samples from the current day, which wasn´t always the case before (problem of sample availability - dependent on external submitter).

Conclusion:

DNA isolation and downstream applications like long-range PCR from sputum are challenging e.g., due to the complex matrix, its heterogeneity and the variability of sputum quality, which is the current opinion in literature and was confirmed by the results.

In summary, the freshness of the samples (sampling and isolation on the same day) is important for the success of the PCR and the clinical condition of the patient, if present, should be noted. The success of sequencing requires optimal amplification quality, which should be achieved by optimizing the isolation/ PCR method.

DGKL: 11. Molekulare Diagnostik

P-09-02

Use of multiplex PCR for detection of resistance genes in multiresistant gram negative strains

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Multiresistant-bacteria are a global problem, and the increase in antibiotic resistance leads to limited treatment options, increased complications, and higher mortality rates. These resistant bacteria pose a serious threat to public health as they are difficult to treat and can result in higher healthcare costs. Therefore, rapid and appropriate antimicrobial treatment, along with early diagnosis, is crucial to contain the spread of antibiotic resistance. The use of multiplex PCR tests to detect these antibiotic resistances might be helpful in facilitating early diagnosis.

We apply a qualitative in-vitro diagnostic test, utilizing multiplex real-time PCR that enables simultaneous detection of five carbapenemase genes, extended-spectrum beta-lactamase genes, and vancomycin resistance genes in swabs or bacterial colonies. Within just three hours, from sample extraction to final result, the test can provide detection of three important antibiotic resistances (involving a total of eight resistance genes) in a single reaction, surpassing the time required for culture-based detection.

The presented results are comparative analyses between the conventional culture-based approach and the multiplex PCR for antibiotic resistance determination. Following the culture-based detection, identification is carried out using MALDI-TOF technology, along with a rapid test (NG-Test Carba-5, NG Biotech) for determining possible resistances. The PCR has clear advantages over the culture-based method due to its short processing time (3 hours) compared to culture-based findings (2 days). The detection of Klebsiella pneumoniae carbapenemase (KPC) and Verona integron-encoded metallo-beta-lactamase (VIM) resistance can be fully confirmed with PCR. Discrepancies with the rapid test were observed for approximately 10% of presumed positive OXA-48 carbapenemase and New Delhi metallo-beta-lactamase (NDM) findings. Furthermore, in almost 5 % of the samples, an additional finding for the resistances just mentioned was detected with the help of the PCR method. The data shown suggests that PCR exhibits higher specificity and sensitivity compared to the rapid test.

The multiplex PCR test for the detection of antibiotic resistance is a fast and efficient approach in routine practice to support the culture-based detection and enable rapid treatment decisions through simultaneous screening and subtyping. Since the resistance genes in the PCR can be detected directly from the original patient sample, whereas the culture-based approach only provides results 24 hours later, the PCR is a sensible alternative and allows higher throughput.

DGKL: 11. Molekulare Diagnostik

P-09-03

Round robin trial for whole exome sequencing (WES) within the MIRACUM consortium

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Introduction: Molecular tumor boards enable genetically stratified, innovative and personalized treatment of cancer patients based on next-generation sequencing (NGS) of tumor tissue. As this is a highly complex diagnostic workflow, one goal of the MIRACUM - Medical Informatics in Research and Care in University Medicine – consortium was to establish a unified bioinformatic pipeline for analyzing WES data from cancer patients treated by the molecular tumor boards. To assess the degree of harmonization of the analytical and bioinformatics WES workflow within the different MIRACUM sites, a round robin trial was conducted.

Methods: Lyophilized genomic DNA from tumor tissue, along with the appropriate germline control from a cancer patient, was pre-characterized by the laboratory organizing the program and distributed to participating institutions. All consortium laboratories were asked to use their routine standard operating procedures (SOPs) and the MIRACUM pipeline or, if this was not standard practice, to additionally use their own bioinformatics pipeline. All participants were required to submit back technical and analytical details, raw sequencing data, and .vcf files generated. All results were evaluated by the program organizers.

Results: Seven consortium sites participated in this study, with two sites additionally using their own bioinformatics pipeline for data analysis. Overall, quality metrics were comparable between participants, with some exceptions. High variability in the wet lab part was observed, with almost every lab using a different analytical workflow. This led to a high diversity of sequencing results, just as the use of different bioinformatics pipelines contributed to the variability of results. The level of variability was significantly reduced by the introduction of a harmonized pipeline within the consortium, but some limitations also became apparent that need to be addressed in the future.

Conclusion: This round robin revealed a high degree of diversity in sequencing results caused by both the wet lab part and the use of different bioinformatics pipelines. The results show that the use of a harmonized pipeline can help to reduce diversity and represent an important step towards a standardized and harmonized workflow in molecular tumor boards – thus this trial proves the clinical value of this MIRACUM use case. Nevertheless, aspects that should receive more attention in the future became apparent trough this round robin trial.

DGKL: 11. Molekulare Diagnostik

P-09-04

No signs of chronic infection and CNS damage in patients with long COVID

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Introduction: Long COVID (LC) is a condition characterized by persistent symptoms that last over weeks or months after acute SARS-CoV-2 infections and usually occur in at least 10% of severe acute infections. Patients experience various symptoms across multiple organs including fatigue, chest pain, cardiovascular disease onset, myalgia, breathlessness, persistent headaches, brain fog, etc. The exact causes of LC are still not well understood, but findings from various biomedical research findings suggest a persisting viral reservoir in the body after acute infection is cleared. In this study, we investigated the presence of circulating viral particles, neurological and immunological markers in the blood of patients with acute COVID-19 compared to LC patients with persisting symptoms with up to 12 months after infection and controls.

Methods: A total of 108 samples from patients, from two separate studies were analysed with highly sensitive immune assays from Meso Scale Diagnostics (Rockville, MD, USA) and Quanterix (Billerica, MA, USA). SARS-CoV-2 N-protein, α-SARS-CoV-2 IgG antibodies and their neutralization capacity in sera were quantified using the analysis platforms of Meso Scale Diagnostics. Markers for neuroinflammation Abeta 40 (Aβ40), Abeta 42 (Aβ42), Glial Fibrillary Acidic Protein (GFAP™) and Neurofilament light (Nf-L) were quantified using the Simoa multiplexing assays (Quanterix). To investigate the inflammatory status of LC patients, classical pro- and anti-inflammatory cytokines were additionally quantified using Simoa multiplexing assays.

Results: We observed similar concentrations for Aβ40, Aβ42, GFAP and NF-light between the LC patients and LC controls. In comparison to patients suffering from acute and severe COVID-19 levels of GFAP and NF-light were diminished in patients with LC. Ab titers against the Spike protein of different SARS-CoV-2 variants in LC patients were elevated up to eight months after infection and decreased over the observation period of 12 months. We identified elevated levels of circulating IL10 in the blood of LC patients in the first four months after acute infection, compared to patients with longer persisting symptoms than 4 months.

Conclusion: Our data show that there is no persisting circulation of viral particles in patients with LC as sign of a chronic SARS-CoV-2 infection. Additionally, in contrast to severe COVID-19, we did not detect serological signs of CNS damage or reactive astrogliosis in patients presenting symptoms like headache or fatigue. Further investigations are needed, to pinpoint the aetiology of persisting symptoms after clearing the initial SARS-CoV-2 infection, thus opening a potential door for treatment.

DGKL: 12. Neugeborenen-Screening und seltene Erkrankungen

P-09-05

LC-MS^3 yields unparalleled diagnostic specificity in primary newborn screening for congenital adrenal hyperplasia

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Introduction: Newborn screening for congenital adrenal hyperplasia (CAH) by immunoassay (IA) suffers from high numbers of false positive results (frequently up to 1%). Culpable analytical limitations of IAs, like lack cross reactivity and matrix effects can be overcome by application of mass spectrometry (MS). LC-MS/MS has been established as 2nd tier analysis, reducing recall rates below 0.1%. Its use as primary screening method, however, is limited due to long turnaround times. We applied a novel, rapid LC-MS^3 method for CAH screening and determined 17-OHP cutoff values depending on gestational age (GA) as well as birthweight (BW). The aims of this study were to compare the diagnostic efficiency of LC-MS^3 and IA for primary CAH newborn screening and to evaluate the measureable variable BW as a substitute for GA regarding dependency of cutoff values.

Methods: 1730 samples from residual dried blood cards from routine newborn screening at the Leipzig University Medical Center were subjected to LC-MS^3 based quantitation of 17 OHP. Methanolic extracts from 3.2 mm dried blood spots of calibrators, controls, and samples were analyzed, utilizing a Sciex QTRAP® 6500+ (Framingham, MA, USA) coupled to a Shimadzu Nexera (Kyoto, Japan). Total run time including online SPE was 1.5 min. MS detection was achieved by two-stage fragmentation (mass transition for 17 OHP m/z 329/285/123). Cutoff values were calculated as 99.5th quantile within distinct maturity groups ((1) extreme premature, (2) premature, (3) late premature, (4) mature).

Results: Physiological conditions of premature birth necessitate maturity dependent cutoffs, even when using LC-MS^3. 17-OHP concentration was inversely correlated to both GA (ρ = 0.679) and BW (ρ = -0.646). Determined cutoffs based on GA and BW were similar (GA/BW: 136/135 nmol/L (1), 103/94 nmol/L (2), 40/46 nmol/L (3), 15/18 nmol/L (4)). Compared to IA measurement, the number of false positives was reduced from 731 to 31/27, resulting in an increase in specificity from 58 % to 98 %, while very clearly identifying seven confirmed cases of CAH.

Conclusions: High recall rates in newborn screening cause mental strain on the affected population while also consuming ample amounts of resources for follow-up analysis. By application of the presented LC-MS^3 approach, diagnostic specificity in CAH screening can be vastly improved, due to elimination of virtually all impairment. Leaving pathological or extreme physiological conditions as only sources of high 17-OHP concentrations. Using birthweight might be better suited as dependency for cutoff determination as well as evaluation since it represents a hard, measurable value and avoids the calculated variable gestational age.

DGKL: 12. Neugeborenen-Screening und seltene Erkrankungen

P-09-06

Establishing in vivo and ex vivo working tools for defining the therapeutic potential of somatic gene repair in Hereditary Spastic Paraplegia

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Introduction: Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of rare monogenic movement disorders. The main clinical feature of HSP is a progressive lower limb spasticity. The pathological correlate of HSP is an isolated degeneration of upper motoneuron axons in the central nervous system. Currently, no causative treatment exists to prevent, retard or reverse disease progression in patients with HSP.

Aims and Methods: The main goal of the study was to elucidate the molecular requirements for therapeutic interventions in the selective HSP type SPG15. Therefore, we generated a novel SPG15 rescue-knockout mouse model (in the following named condZfyve26-Null) in which a knockout of murine SPG15 gene (Zfyve26) can be conditionally repaired. We aimed at the functional characterization and validation of the novel condZfyve26-Null mouse line as a prerequisite for testing and defining the therapeutic potential of somatic gene repair in HSP. Current characterization includes phenotyping, histological and molecular studies.

Results: We conditionally inactivated the Zfyve26 gene in mice using the Cre-loxP system, which consists of a floxed stop cassette, resulting in animals resembling a classical knockout. Genotyping and sequencing analyses confirmed the correct insertion of the stop cassette into intron 2 of the murine Zfyve26 gene. Zfyve26 mRNA levels were reduced in brain lysates of homozygous condZfyve26-Null animals. Null mice were viable and fertile. Compared to wildtype littermates, a higher body weight and impaired movement performance were observed in homozygous condZfyve26-Null mice. However, no metabolic correlates were detected by indirect calorimetry. Histological analyses of the central nervous system suggest neuronal loss.

Conclusion: Preliminary characterization of the novel condZfyve26-Null mouse model confirmed its relevance to better understand the genetic background of HSP type SPG15. Further experiments address the consequences of Zfyve26 (i.e., Spastizin protein) inactivation on subcellular compartments. Once characterization and validation are completed, the novel condZfyve26-Null mouse model can be used as a sophisticated tool to control Zfyve26 gene expression in vivo, temporally and spatially. Thus, the novel condZfyve26-Null mouse model provides the experimental basis as well as a valid working tool for defining the therapeutic potential of somatic gene repair in HSP.

DGKL: 12. Neugeborenen-Screening und seltene Erkrankungen

P-09-07

Application of anticoagulated blood on dried blood cards increase the rate of false positive SCID and SMA newborn screening results

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Introduction:

Newborn screening (NBS) is a valuable public health program that detects infants at risk of developing genetic and metabolic disorders in the early stages of life. The most common method of NBS is the collection of dried blood spot (DBS) samples on

filter paper cards, which are then analyzed using various techniques, including real time polymerase chain reaction (RT-PCR). A heel prick is the recommended way of blood collection. An alternative site for blood collection from newborns on intensive care units (ICUs) is from a peripheral venous catheter. However, this carries the potential risk of applying anticoagulated blood on the filter paper cards. In this study, we aimed to investigate the impact of pre-analytical errors, specifically the effect of using EDTA blood and lithium heparin (LH) blood for NBS on the accuracy of RT-PCR analysis.

Methods:

Three different types of DBS cards were prepared using (1) EDTA-, (2) LH-, and (3) capillary blood without anticoagulant as a control. All three DBS types were prepared and analyzed as regular NBS samples in the newborn screening laboratory of the University Medicine Greifswald (UMG). The DBS were tested for severe combined immune deficiencies (SCID) and Spinal muscular atrophy (SMA) using the SPOT-it TREC & SMN1 Screening Kit (ImmunoIVD, Sweden) on a QuantStudio 5 Dx Real-Time PCR System (Thermo Fisher Scientific, USA). The copy number of TRECs, ACTIN B and the ct-value for the survival motor neuron 1 (SMN 1) were quantified and statically evaluated between all three tested DBS card types.

Results:

The application of LH blood on DBS cards leads to a decrease of TREC copy numbers as well as to increased ct-values for the SMN 1 gene. Both circumstances will, depending on the detected TREC copies and/or ct-values, either result in a repeated sample taking or even in an emergency admittance of the newborn in a specialized center for further evaluation. Furthermore, LH blood as well as EDTA blood on DBS cards decrease the copy number of ACTIN B, which is quantified in all samples as an internal quality control, resulting in a second sample taking and therefore in an additional heel prick for the newborn.

Conclusion:

Our study emphasizes the crucial importance of heel prick-based DBS sample collection. Mishandling of samples, such as sample taking from peripheral venous catheters can result in pre-analytical errors that compromise the quality of NBS results. This, in turn, may lead to false-positive RT-PCR analysis results. Therefore, it is imperative to provide adequate training to sample collectors, ensuring that they follow the proper procedures for DBS sample collection.

DGKL: 12. Neugeborenen-Screening und seltene Erkrankungen

P-09-08

Deciphering molecular checkpoints and therapeutic targets in Hereditary Spastic Paraplegia

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Introduction: Hereditary Spastic Paraplegias (HSP), a group of rare monogenic movement disorders, are caused by the degeneration of upper motoneuron axons in the central nervous system (CNS). More than 60 genes and more than 80 genetic loci (spastic paraplegia gene loci; SPG1-80, plus other) have been found to be associated with HSP. Many of the HSP associated genes are also expressed in organ systems outside the CNS, but their functional link primarily to axon biology remains unknown. Currently, there is no causative therapy for HSP available.

Aims and Methods: The main goal of the study was to examine the subcellular mechanisms underlying for the clinically similar HSP forms SPG11, SPG15 and SPG48. The respective proteins play a role for trafficking of endosomes, recycling of lysosomes as well as process of autophagy and autophagic lysosome reformation. Applying cell-based ex vivo analyses we aimed at deciphering the molecular basis for therapeutic interventions in SPG11, SPG15 and SPG48. Current characterization includes expression studies of mechanistically related genes and proteins, which are presumed to function along the molecular endo-/lyso-/autophagosomal axis.

Results: Western blot based analyses showed an upregulation of the WASH protein complex subunit WASHC5 (HSP type SPG8) in SPG15 mouse embryonic fibroblasts (MEF). Subsequent qPCR based investigations revealed a higher amount of WASH complex (WASHC1, WASHC2, WASHC4, WASHC5) transcripts in SPG15 MEF compared to the wildtype. Moreover, qPCR based gene expression analyses indicate an upregulation of a direct WASHC5 interacting partner (VCP), while the transcript amount of an indirect WASHC5 interacting partner (VPS35) remained comparable to wildtype cells.

Conclusions: Preliminary characterization of SPG15 MEF points to potential molecular compensatory mechanisms of mechanistically related genes/proteins in murine HSP models. Subsequent experiments address the consequences of SPG11 and SPG48 gene/protein inactivation on mechanistically related molecules/compartments. Further OMICS based (transcriptome, proteome) studies should confirm obtained results and identify additional pathomechanistically as well as therapeutically relevant HSP target(s) and/or cell signaling pathway(s).

DGKL: 13. Omics – Technologien (Metabolom, Lipidom, Proteom, Glykom)

P-09-09

Influence of urine sample volume on the quality of NMR measurements

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Introduction

Scarcity of material may be a crucial issue in both research and clinical routine, e.g. in biobanking or when taking specimen from children. In such cases, measurements have to be performed with limited material. On the other hand, manufacturer's specifications regarding the sample volume have to be complied. In our study, we investigated the influence of urine sample volume on the quality of Nuclear Magnetic Resonance spectroscopy (1H NMR) measurements.

Methods

Aliquots with varying sample volume were prepared from urine samples from two obvious healthy probands (sample A and B) according to manufacturer specifications (90% urine sample + 10% buffer). The aliquot filling levels varied from a total of 400 µL up to 600 µL in steps of 20 µL; three aliquots were prepared per filling level. Measurements were performed on a 600 MHz NMR spectrometer from Bruker Bio Spin GmbH (Bruker Biospin, Ettlingen, Germany). NMR spectral quality parameters and sample preparation quality parameters were evaluated for each filling level if their values were lying within the target value ranges. Quantified metabolites were considered if less than 20% missing values, i.e. measured concentration below limit of detection, occurred. Outliers were identified as lying above or below the mean concentration +/- 2*SD (SD: standard deviation). Spectra and scatter plots of concentration levels of the quantified metabolites were plotted color-coded by the sample volume.

Results

Evaluation of NMR spectral quality and sample preparation quality parameters showed different requirements towards the filling level, e.g. 460 µL were sufficient for parameter LineWidth whereas the parameter Residual Water Signal needed a filling level of at least 500 µL. Sufficient quality for all parameters was achieved for 500 µL and more. NMR spectra showed high concordance for filling levels of a total volume of 500 µL or more (450 µl urine + 50 µl buffer). Nineteen and five quantified metabolites for sample A and B, respectively, were considered. Outliers were found for measurements with a total sample volume of 420 µL (n=27), 440 µL (n=6) and 460 µL (n=1), respectively. In the scatterplots, no ordering of measured concentrations according to the filling level could be observed.

Conclusion

Our results imply, that a filling level of 500 µL (450 µL urine + 50 µL buffer) is sufficient for reliable NMR measurements of urine samples. Compared to the manufacturer recommendations to use 600 µL, the filling level could be reduced by a total of

100 µL (90 µL urine + 10 µL buffer), thus saving 90 µL of urine sample, which might be advantageous regarding samples with only scarce material, e.g. as can be found in biobanking or when dealing with children's specimen.

DGKL: 16. POCT

P-10-01

Comparison between a new point-of-care (POC) CRP test and a routine laboratory method

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Introduction:

In healthy subjects, C-reactive Protein (CRP) is a trace protein, but during inflammation, levels rise dramatically. Therefore, CRP levels can be used to detect and monitor inflammation in patients. In patients with bacterial infection or sepsis, rapid CPR test results are of great value to reduce the time until therapeutic intervention.

Point-of-Care Testing (POCT) is defined as medical diagnostic testing near the patient, providing rapid results and allow for timely clinical decision making at the point of care. The POCT method contrasts with the standard model of sending specimens out tot he lab. The advantages of POCT have been widely reported. However, these can only be achieved when its corresponding method delivers a sufficient analytical and clinical performance.

Methods:

In this clinical evaluation of the LumiraDx CRP Test, we tested the diagnostic performance of the measurements of CRP concentrations obtained from the POCT device LumiraDx Platform by using samples from 242 patients in comparison to the results from a central lab-based CRP determination.

CRP concentration was quantitatively determined from EDTA plasma samples.

The analyzers in this evaluation use immunofluorescence in combination with next-generation microfluidics architecture (LumiraDx CRP) and dual radius enhanced latex turbidimetry (Roche® cobas 8000 CRPL3 assay). The Test results generated on the LumiraDx Platform are quantitative and appear on the instrument display within 4 minutes from sample application.

Results:

The values of the 242 analyzed tests on the Roche analyser ranged from 0,3 – 35,6 mg/dL with LumiraDx values ranging from 5 – 250 mg/L.

Linear regression analysis yielded a linear correlation coefficient of r = 0,99, which signifies a strong correlation of the two methods. The Passing-Bablock fit yielded a correlation of r = 0,99 (7,3 – 249,90 mg/L, Intercep -4,95, Slope 1,02) In the Bland-Altman-Plot, a small positive bias was found for the Roche method, only a small percentage of samples were outside the 1,96 standard deviation (SD) boundaries on both sides.

The overall performance of the LumiraDx CRP Test was comparable to the Roche® cobas 8000 CRPL3 laboratory test. The regression analysis showed a strong correlation of both methods. The Bland-Altman plot showed a small positive bias, with an acceptable number of samples outside the standard deviation boundaries.

Conclusion:

With these results, the LumiraDx CRP Test has proven to show a lab-comparable performance within its measuring range and can be recommended for use in any POCT setting.

DGKL: 16. POCT

P-10-02

Substantial Risk of Overlooking Myocardial Infarction Using Point of Care-Testing for Cardiac Troponin Detection

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Substantial Risk of Overlooking Myocardial Infarction Using Point of Care-Testing for Cardiac Troponin Detection

Abstract:

Background: Cardiac troponin (cTn) is the gold standard biomarker for the diagnosis of acute coronary syndromes, including myocardial infarction (MI). High-sensitivity cardiac troponin (hs-cTn) assays (e.g. Roche Elecsys Troponin T hs STAT, reference: < 14 ng/L) have further improved the accuracy and precision of troponin measurements, leading to earlier and more accurate diagnosis of MI. Point of care-testing (POCT) for cardiac troponin T (cTnT) with low turnaround time expedites triage and diagnosis of patients with chest pain, but its use may be limited by lower analytical sensitivity (Roche CARDIAC POC Troponin T, reference: < 50 ng/L).

The aim of this work is to highlight the risk of missing MI in patients admitted to an emergency department (ED) with chest pain when triaged with POCT. It shall be shown that a substantial number of patients with MI have cTnT elevations between 14 ng/L and 50 ng/L.

Methods: Correlation (ρ 0.97, $p < 0.0001$) and concordance of cTnT values from blood samples of patients admitted to the ED of the University Hospital Schleswig-Holstein (UKSH) from January 2018 to December 2021 were analysed. Determination of cTnT on admission was performed with Roche's POCT assay (h232, Roche Diagnostics) and Roche's platform-based assay (cobas 8000, Roche Diagnostics).

In addition, cTnT levels of hospitalised patients with confirmed MI were scrutinised to determine whether all patients had elevated troponin levels and, if so, whether the levels surpassed the threshold of 14 ng/L and additionally 50 ng/L. All cTnT determinations were performed with Roche's platform-based assay. To account the characteristics of the data, only the maximum values for cTnT in a patient case were included in the analysis.

Results: 2375 patients were hospitalised at UKSH under the diagnosis MI.

More than 99% (n= 2358) of patients with confirmed MI had cTnT elevations above 14 ng/l. Adjusting the threshold to 50 ng/L, as used in Roche's POCT method, the detection rate for MI drops from 99 to 75 percentage points. 25% of patients (n= 594) did not have cTnT values above 50 ng/l at any time point.

Discussion: Evaluation has shown that a considerable number of patients with MI have cTnT elevations between 14 ng/L and 50 ng/L. The use of POCT for cTnT carries a significant risk of missing cases of MI, with potentially fatal consequences for patients. If used at all, POCT should be used with caution. In particular, if the result is below 50 ng/L and clinical suspicion persists, the more sensitive method should be performed.

Keywords: Myocardial Infarction, Elecsys Troponin T hs STAT, POCT of Troponin

DGKL: 16. POCT

P-10-03

QuikRead go HbA1c test fulfils the German quality requirements for laboratory measurements

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Introduction

Measurement of glycated hemoglobin (HbA1c) concentration is important in the diagnosis of diabetes and identification of patients at risk for developing diabetes mellitus (1). Measuring at the point-of-care (POC) brings valuable aspects to the treatment process: HbA1c result can be immediately discussed with the patient which leads to improved over-all result of care with increased patient satisfaction and self-motivation. Importantly, the possible treatment modification can be performed during the same visit (1).

Aidian QuikRead go HbA1c test is an immunoturbidometric in vitro diagnostic test performed with the QuikRead go instrument for quantitative measurement of HbA1c from finger prick capillary blood or anticoagulated (EDTA or heparin) venous whole blood samples.

The aim of the study was to evaluate the precision of the QuikRead go HbA1c assay in customer use in Germany and monitor whether the RiliBÄK quality requirement for precision (2)(maximum CV% value is 3) was met. The study was performed according to the CLSI EP05-A3 protocol. In addition, the method comparison between the Tosoh version G8 HPLC analyzer (IFCC and NGSP certified method) and QuikRead go HbA1c test system was performed with fresh patient samples distributed around the clinically relevant range.

Material and methods

QuikRead go HbA1c controls (including two different HbA1c levels, material of human origin) were measured in the core laboratory of the Klinikum rechts der Isar by laboratory personnel as four replicates in one run a day for 10 days using two QuikRead go -instruments. Results were analyzed by calculating the between-run, within- and between-day, and within laboratory precision as coefficient of variation (CV%) according to CLSI EP05-A3. The accuracy of the QuikRead go HbA1c assay was evaluated by a method comparison to Tosoh G8 analyzer (cation-exchange HPLC, Tosoh Bioscience) according to the CLSI EP09C-ED3:2018 protocol.

Results

The coefficient of variation within laboratory (total CV%) was 2.9% for HbA1c Control (range 40-52 mmol/mol) and 2.3% for HbA1c Control High (range 66-90). The results of the method comparison will be used for investigation of bias between Tosoh G8 and QuikRead go HbA1c after the study has been completed and for showing the accuracy of QuikRead go HbA1c test.

Conclusions

Fast HbA1c testing at the POC improves diabetes care and disease management leading to better clinical outcomes. HbA1c POC also increases patients' quality of life, satisfaction, and adherence to the treatment (3). However, HbA1c POC tests must fulfil accuracy and precision requirements before implementation in different healthcare settings. The results show that the QuikRead go HbA1c, as a representative of POC test for HbA1c, fullfills the RiliBÄK quality requirements. QuikRead go HbA1c is suitable for use in the diagnosis of diabetes and successful management of diabetic patients.

DGKL: 18. Therapeutisches Drug-Monitoring und Pharmakogenetik

P-10-04

Entwicklung einer schnellen HPLC-MS/MS-Methode zur Quantifizierung von Piperacillin und Meropenem im Serum

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Zielsetzung

Der Erfolg einer antiinfektiven Therapie mit Betalactam-Antibiotika hängt von der Zeit ab, in der die Serumkonzentration des Antibiotikums über der minimalen Hemmkonzentration (MHK) des Erregers liegt. Idealerweise soll die MHK um das Vierfache für mindestens 40% bzw. 50% der Behandlungsdauer (fT > 4MHK) überschritten werden (1,2). Empirische Dosierungen können bei septischen oder dialysepflichtigen Patienten versagen (3,4). Ein Therapeutisches Drug Monitoring scheint für diese Substanzen von klinischer Bedeutung zu sein. Die Methodenlaufzeiten kommerzieller Kits liegen bei 8,5 Minuten oder mehr.

Ziel der vorliegenden Arbeit ist es eine schnelle (< 3 Minuten) und einfache HPLC-MS/MS-Methode zur Bestimmung von Piperacillin- und Meropenemserumspiegeln zu erstellen und zu validieren.

Methoden

Die Probenaufbereitung beinhaltet Zugabe von zwei deuterierten Standards, Proteinfällung mit Acetonitril und Probenverdünnung der Überstandes mit einem Methanol-Wasser-Gemisch nach dem Zentrifugieren. Die Laufzeit pro Probe beträgt nur 2,5 Minuten. Es wurde eine MassTox® TDM MasterColumn® Series A von Chromsystems und ein Xevo TQ MS von Waters unter Anlage einer Gradientenelution zur chromatographischen Trennung verwendet. Die Laufmittel stammen aus dem MassTox®-Kit von Chromsystems. Das Massenspektrometer wurde im MRM-Modus betrieben. Die Detektion beider Analyten und ihrer Internen Standards findet im ESI positive mode statt und umfasst einen Messbereich von 3,435 – 188 mg/ L für Piperacillin (PIP) und 1,675 – 83,3 mg/ L für Meropenem (MER). Die LOD für PIP und MER liegt bei 0,05 und 0,24 mg/ L. Die LLOQ liegt jeweils bei 3,435 mg/ L und 1,675 mg/ L.

Ergebnisse

Die Methode ist im angegebenen Messbereich linear. Die Richtigkeit und Präzision der Methode liegt innerhalb der von der EMA geforderten 15% Abweichung(5). Matrixeffekte wurden mittels Post-Column-Infusion Test ausgeschlossen. Eine relevante Probenverschleppung findet nicht statt. Gegenüber gespiketen Proben zeigte sich eine systemische Abweichung von im Mittel 18,06% für PIP und 2,35% für MER. Eine Probenmessdauer von unter 3 Minuten wurde erreicht.

Diskussion und Schlussfolgerung

Die Probenaufbereitung ist einfach. Die Methode liefert reproduzierbare Ergebnisse in einer sehr kurzen Methodenlaufzeit. Nach Messung der Kalibratoren und Kontrollen kann die erste Patientenprobe somit bereits nach 20 Minuten gemessen werden. Sollte die vierfache MHK in der gemessenen Probe unterschritten worden sein, ist bereits vor der zweiten Gabe eine Dosisanpassung möglich. Kritische Unterdosierungen können so mit minimalem Verzug erkannt werden. Die systematische Abweichung in der Wiederfindung bedarf weiterer Untersuchung, kann aber durch einen Korrekturfaktor ausgeglichen werden.

DGKL: 18. Therapeutisches Drug-Monitoring und Pharmakogenetik

P-10-05

5-FU-Toxizität: DPD-Phänotypisierung und 5-FU-Monitoring als effiziente Alternative zur DPD-Genotypisierung

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Seit ihrer Markteinführung 1962 sind 5-FU-Präparate Bestandteil der Standardtherapie vieler bösartiger Tumoren. Der Abbau von 5-FU erfolgt normalerweise rasch durch die Dihydropyrimidin-Dehydrogenase (DPD). Bei einem Mangel oder verminderter DPD-Aktivität besteht jedoch das Risiko einer Akkumulation mit schwerwiegenden Nebenwirkungen bis hin zum Tod. Bis zu 9 % der europäisch-stämmigen Bevölkerung sind von einem partiellen DPD-Mangel betroffen, der eine

TDM-basierte Steuerung der 5-FU-Therapie nötig macht. Bei bis zu 0,5 % der Patienten besteht sogar ein vollständiger DPD-Mangel, der die Verabreichung von 5-FU-Präparaten kontraindiziert.

Um die zuvor beobachtete Therapie assoziierte Letalität von 0,2 - 1,0 % zu senken, haben EMA und DGHO eine DPD-Typisierung vor Therapiebeginn angeraten. Diese erfolgt bislang meist als Genotypisierung. Alternativ dazu bietet sich die DPD-Phänotypisierung durch die Bestimmung des Uracil-Spiegels als schnelle und effiziente Methode an.

In unserer Arbeit zeigen wir, wie in der klinischen Routinediagnostik die Implementierung der DPD-Phänotypisierung und des 5-FU-Drug-Monitoring mittels HILIC-MS/MS als behandlungsbegleitende Maßnahmen etabliert werden kann und zur Verbesserung der Therapie beiträgt.

DGKL: 18. Therapeutisches Drug-Monitoring und Pharmakogenetik

P-10-06

Cefazolin-Therapie bei kritisch kranken Patienten - Ein LC-MS-basierter Ansatz für das Cefazolin-Monitoring auf den Intensivstationen

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Cefazolin ist ein Cephalosporin-Antibiotikum der 1. Generation, das zur parenteralen Therapie akuter und chronischer bakterieller Infektionen unterschiedlicher Lokalisationen oder zur perioperativen Prophylaxe bei infektionsgefährdeten Operationen eingesetzt wird. In der Behandlung von Blutstrominfektionen mit Staphyloccus aureus, die mit einer hohen Krankenhaussterblichkeit assoziiert sind, zeigt Cefazolin neben Flucloxacillin den besten Erfolg. Eine TDM-gesteuerte Optimierung der Antibiotika-Therapie ist Teil der S3-Leitlinie der Deutschen Gesellschaft für Infektiologie zum Antibiotic Stewardship. Dies setzt zunächst die adäquate Bestimmung des wirksamen Cefazolin-Spiegels voraus.

Die meist angewendete Vorbereitung der Serum-Proben mittels Proteinfällung ist einfach, führt allerdings auch zur Denaturierung der Transportproteine (Albumin) und Freisetzung des gebundenen Cefazolins. Da allerdings nur der freie (ungebundene) Anteil eines Wirkstoffs pharmakologisch wirksam ist, geben die so bestimmten Spiegel keineswegs die tatsächlich aktiven in-vivo-Konzentrationen wieder, sondern bilden deutlich höhere Werte ab. Dies ist insbesondere problematisch, da Cefazolin eine hohe Plasmaprotein-Bindung (PPB) von ca. 80 % aufweist. Versuche, den wirksamen Anteil mithilfe der PPB aus der Gesamtkonzentration zu berechnen, können jedoch fehlerbehaftet sein, insbesondere bei schwer- und kritisch kranken Patienten mit einer gestörten PK/PD. Wir präsentieren eine Methode zur routinemäßigen Bestimmung des freien Anteils an Cefazolin mittels LC-MS nach Ultrafiltration.

DGKL: 18. Therapeutisches Drug-Monitoring und Pharmakogenetik

P-10-07

Therapeutic drug monitoring in epilepsy patients: Do plasma concentrations outside the respective reference range of brivaracetam, lacosamide and perampanel lead to dose adjustment?

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Introduction

Epilepsy is one of the most common neurological diseases worldwide. To reduce the probability of seizures, anti-seizure medications (ASMs) are administered as baseline therapy. For these agents, therapeutic drug monitoring (TDM) is widely used, despite limited evidence of benefit from routine monitoring of ASMs in general. As there is little evidence for established reference ranges, particularly for the newer third-generation ASMs, the question of their clinical relevance arises. We analyzed whether plasma concentrations outside the respective reference range led to dose adjustment. For this purpose, we examined the plasma concentrations of the third generation anti-seizure medication brivaracetam (BRV), lacosamide (LCM) and perampanel (PER).

Patients and methods

In 300 patients treated with either BRV (n = 100), LCM (n = 100), or PER (n = 100), the respective plasma concentrations were determined by Chromsystems-MassTox Antiepileptic Drugs (Chromsystems Instruments and Chemicals GmbH, Graefelfing, Germany) using liquid chromatography combined with mass spectrometry (LC-MS/MS). The patients visited the Department of Epileptology, University Hospital of Bonn, a tertiary epilepsy center. For LCM and PER, the reference range of the AGNP consensus guideline, and for BRV, the national guidelines for Norway were applied.

Results

35.3% (n = 106) reported seizure freedom, 58% (n = 174) were responders (≥ 50% reduction of seizure frequency) and 42% (n = 126) were non-responders (< 50% reduction of seizure frequency). In total, 142 measurement results (47.3%) were outside the reference range.

36.7% (n = 110) were above the respective reference range, 10.7% (n = 32) were below. In 5.6% (eight patients), the dose was adjusted (> reference range: dose reduced/ or BRV, LCM or PER discontinued; < reference range: dose increased), while in 94.4% (n = 134), the dose was not adjusted. In 5.5% (six of 110 pt.), whose plasma levels were above the reference range, adverse events (AEs) were reported, while in n = 2 cases, the dose was reduced and in one case the medication was discontinued. 12.1% (23 of 190 pt.) with plasma values ≤ reference range reported AEs.

Conclusion

In our collective, there was no relationship between plasma concentrations outside the reference range and dose adjustment. Efficacy and tolerability are the most important decision criteria for dose adjustment. Dose adjustment is carried out based on the individual case, separate from the reference range. As dose adjustment outside the reference range was rarely performed in our collective, larger studies are needed to confirm these results.

DGKL: 18. Therapeutisches Drug-Monitoring und Pharmakogenetik

P-10-08

Therapeutisches Drug Monitoring (TDM) der antiretroviralen Therapie bei HIV-Erkrankung

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Einleitung

Die medikamentöse Therapie der HIV-Erkrankung als Kombinationstherapie mit meist drei antiretroviralen Wirkstof-fen ermöglicht die weitestgehende Hemmung der HIV-Replikation und damit die Eindämmung der chronischen Ent-zündungsreaktion des Körpers auf das Virus. In den letzten 30 Jahren wurden dazu über 30 Wirkstoffe zugelassen. Das primäre Therapieziel ist die Senkung der HI-Viruslast, dementsprechend ist die Bestimmung der Viruslast auch der primäre Marker zum Monitoring der Therapie. Die antiretrovirale Medikation muss lebenslang eingenommen werden, denn die Wirkstoff-Versorgung muss lebenslang auf wirksamem Niveau vorhanden sein.

Dabei kann sich der Bedarf eines TDM ergeben, um bei sich verändernden Einflussfaktoren z.B. die Dosis anzupassen oder um die Umstellung auf andere Wirkstoffe zu begleiten. Zu niedrige Wirkstoffkonzentrationen können eine unzu-reichende virale Suppression und eine Resistenzentwicklung bewirken, zu hohe Konzentrationen dagegen zu uner-wünschten Arz-neimittelwirkungen bis hin zum Abbruch der Therapie führen. Einflussfaktoren können z.B. eine nach-lassende Adhärenz des Patienten, andere Erkrankungen bzw. deren Therapie oder sich altersgemäß verändernde Organ-funktionen insbes. von Leber und Niere sein.

Methoden

Seit einigen Jahren wird vom Labor Lademannbogen ein TDM der HIV-Medikation angeboten. In einer retrospektiven Auswertung wurde für 16 Wirkstoffe ermittelt, wie große der Anteil der Proben unterhalb bzw. im therapeutischen Be-reich bzw. darüber war, und ob sich Zusammenhänge zwischen den Messwerten und klinischen Angaben finden.

Ergebnisse

In die Auswertung konnten 3454 Spiegelbestimmungen einbezogen werden. Typische klinische Angaben waren z.B. Verdacht auf Nichteinnahme bzw. schlechte Bioverfügbarkeit oder Verdacht auf Nebenwirkungen, oft zusammen mit dem Hinweis auf eine zu hohe Viruslast. Nur sehr selten standen individuelle Basalwerte des Patienten aus der Zeit nach erfolgreicher Etablierung der Therapie zur Verfügung. Für jeden Wirkstoff wurde ermittelt wie groß der Anteil der Spiegel unterhalb / innerhalb / oberhalb des Referenzbereiches war. Der höchste Anteil von Spiegeln unterhalb des Referenzbereiches fand sich bei Darunavir (32 %) und Amprenavir (26 %). Der höchste Anteil von Spiegeln oberhalb fand sich bei Etravirin (57 %) und Lopinavir (54 %).

Diskussion und Schlussfolgerungen

Bezogen auf alle Messwerte lagen 13 % der Spiegel unterhalb und 20 % oberhalb der Referenzbereiche. Gemessen an der großen Zahl der im gleichen Zeitraum durchgeführten Viruslastbestimmungen und der hohen Quote nicht adäquat dosierter Patienten erscheint die Anzahl der TDM-Analysen gering. Als Hauptziel des TDM der aktuell verwendeten Wirkstoffe kann die Erkennung subtherapeutischer Spiegel der antiretroviralen Medikation abgeleitet werden.

DGKL: 18. Therapeutisches Drug-Monitoring und Pharmakogenetik

P-10-09

Analytical performance evaluation of a liquid chromatography-tandem mass spectrometric method (LC-MS/MS) for the determination of cenobamate concentrations in human serum

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Introduction: Cenobamate is a new anticonvulsant drug that has been approved 2021 in Germany for the adjunctive therapy of focal-onset seizures in adults without adequate treatment results with at least two other antiepileptic drugs. It is metabolized via numerous polymorphic enzymes (UGT2B7, CYP2E1, CYP2A6, CYP2B6, CYP2C19, CYP3A4/5) and also possesses inhibitory and inductive effects on various metabolizing enzymes (CYP2C19, CYP2C8, CYP3A4, CYP2B6). Lack of therapeutic efficacy, toxic symptoms, suspected lack of adherence, impaired renal or hepatic function as well as drug combination therapies with risk of pharmacokinetic interactions represent indications for therapeutic drug monitoring. This study reports on the development and validation of a LC-MS/MS method for quantification of cenobamate in human serum.

Methods: Sample pretreatment included protein precipitation with methanol followed by liquid-liquid-extraction by hexane. Gradient based chromatography was performed in 5.8 minutes on a Reprosil- Pur Basic C8 column (3 µm;60 x 2 mm, Dr. Maisch GmbH, Ammerbuch, Germany) using ammonium acetate/formic acid (3 mmol/0.05%) and acetonitrile as mobile phases and followed by detection via electrospray interface coupled QTRAP® 5500+ mass spectrometer (AB SCIEX, Darmstadt, Germany) in the positive multiple reaction monitoring mode. Topiramat-d12 (TRC, Toronto, Canada) was used as

internal standard (IS). Recorded mass transitions included (268/155 and 268/137 amu) for cenobamate and 369/270 amu for the IS. Both serum spiked with cenobamate and native patient samples were used for evaluation of the analytical performance.

Results: The lower limit of quantification for cenobamate was 0.5 mg/L and the method was linear up to 60 mg/L (n=2; r>0.998). Imprecision was < 5% (within-run, n=10, 2 concentration levels) and < 6.5% (between-run, n=15, 4 concentration levels). The bias ranged between -4 – 13 % (n=15, 4 concentration levels). Measurement results were not affected by carry-over or matrix effects. Serum samples were stable for at least 14 days at 4°C and 24h in the injector (10°C) after extraction. Comparison of cenobamate concentrations (n=4) measured with the proposed method and a second LC-MS/MS method performed in an external laboratory demonstrated good agreement with a bias of 1-13 %.

Conclusion: The presented LC-MS/MS method covers the clinically relevant measurement range for cenobamate and allows accurate, precise, and rapid quantification of the drug in serum samples for the purpose of therapeutic drug monitoring.

DGKL: 19. Varia

P-11-01

CRISPR/Cas9 induced compound heterozygote B3GALT6 knockout in neonatal human dermal fibroblasts as a promising model system for the analysis of the molecular mechanisms of spondylodysplastic Ehlers-Danlos syndrome

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Introduction: For the synthesis of functional proteoglycans, a tetrasaccharide linker consisting of four monosaccharides is required to bind the glycosaminoglycans (GAG) to the core protein. The corresponding glycosyltransferases are encoded by the genes XYLT1, XYLT2, B4GALT7, B3GALT6 and B3GAT3. Pathological mutations in these genes result in GAG linkeropathies inter alia characterized by altered ECM remodeling. Spondylodysplastic Ehlers-Danlos syndrome (spEDS) is an autosomal recessive skeletal dysplasia with a variety of symptoms, such as short stature, hypermobility of joints and skin and developmental delays. Genetic causes can be various mutations in the genes B4GALT7, B3GALT6 and SLC39A13. Due to the low availability of patient fibroblasts, the aim of this study is to establish a CRISPR/Cas9-based B3GALT6 knockout in neonatal human dermal fibroblasts (NHDF) and to subsequently validate the in vitro model by comparison with B3GALT6-deficient patient fibroblasts.

Methods: CRISPR/Cas9 based B3GALT6 knockout: NHDF were revers transfected with 10 nM of a ribonucleoprotein-complex targeting the B3GALT6 gene. After 24 h the cells were sorted using FACS technology, genomic DNA was isolated and afterwards analysed using sanger sequencing.

TA cloning: Taq polymerase amplified PCR-products were ligated into the vector pCR 2.1. After replication of the resulting vector using E.coli Top10 cells, the plasmid DNA was analysed by sanger sequencing.

Analysis of relative mRNA expression: relative mRNA expression levels were determined using quantitative real-time PCR.

Sulphated GAG (sGAG) analysis: After cultivation of NHDF as a confluent monolayer for 144 h, the total amount of sGAG was measured using the Blyscan assay (Biocolor, UK) according to the manufacturer’s instructions.

Protein expression analysis: Expression levels of different ECM-associated proteins were determined either via western blot or using an ELISA-assay, specific for the respective protein.

Results: A compound heterozygous B3GALT6 knockout could be established showing mutations within the coding region of the catalytic protein domain. This is similar to the compound heterozygous genotype of the spEDS-patient fibroblasts comparatively used in this study. The mRNA analysis of the linker glycosyltransferases show similar expression patterns

between spEDS- and knockout fibroblasts. Furthermore, patients and knockout cells show a significant decrease concerning sGAG amounts. The expression pattern of ECM-associated proteins was comparable.

Conclusion: An isolated B3GALT6 knockout was successfully established in NHDF which, comparable to spEDS patient fibroblasts, exhibits a compound heterozygous mutation within the catalytic domain. The analysis of mRNA- and protein expression levels showed similarities in knockout and patient fibroblasts. Thus, using a CRISPR/Cas9-based knockout of B3GALT6 in NHDF, an in vitro model could be created for the analysis of the molecular mechanisms of spEDS.

DGKL: 19. Varia

P-11-02

The Impact of Inflammatory Stimuli on Xylosyltransferase-I Regulation in Primary Human Dermal Fibroblasts

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Instruction: Inflammation plays a vital role in regulating fibrotic processes. Beside their classical role in extracellular matrix synthesis and remodeling, fibroblasts act as immune sentinel cells participating in regulating immune responses. The human xylosyltransferase-I (XT-I) catalyzes the initial step in proteoglycan biosynthesis and was shown to be upregulated in normal human dermal fibroblasts (NHDF) under fibrotic conditions. Regarding inflammation, the regulation of key enzyme XT-I remains elusive. This study aims to investigate the effect of lipopolysaccharide (LPS), a prototypical pathogen-associated molecular pattern, and the damage-associated molecular pattern adenosine triphosphate (ATP) on the expression of XYLT1 and XT-I activity of NHDF.

Methods: We used an in vitro cell culture model with NHDF and mimicked the inflammatory tissue environment by exogenous LPS and ATP supplementation. The cellular XYLT1 mRNA expression and XT-I activity of NHDF was assessed by quantitative real-time polymerase chain reaction and ultra-performance liquid chromatography/electrospray ionization tandem mass spectrometry, respectively. Other cellular and protein biochemical characteristics were analyzed using classical molecular biological methods such as the WST-1-reagent based cell proliferation, the bicinchonic acid assay and targeted gene silencing with small interfering RNA molecules.

Results: In the present study, we demonstrated for the first time that LPS has a regulatory effect on the relative XYLT1 mRNA expression of NHDF that is independent of the cell culture density and type of serum supplementation used. The XYLT1 mRNA expression decrease observed in both low- and high-cell-density-culture models is consistent with previous cross-tissue examinations of fibroblasts that highlighted shared fibroblast phenotypes across a spectrum of inflammatory and fibrotic diseases. Furthermore, we found a hitherto unknown mechanism involving the inflammasome pathway components cathepsin B (CTSB) and caspase-1 in XT-I regulation. The suppressive role of CTSB on the expression of XYLT1 was further validated by the quantification of CTSB expression in fibroblasts from patients with the inflammation-associated disease Pseudoxanthoma elasticum.

Conclusion: We conclude that the decreased XYLT1 mRNA expression can be utilized as a predictive tool for the involvement of inflammatory responses within primary fibroblasts. Altogether, this study further improves the mechanistic understanding of inflammatory XT-I regulation and provides evidence for fibroblast-targeted therapies in inflammatory diseases.

DGKL: 19. Varia

P-11-03

A Novel 2-in-1 UPLC-MS/MS-Based Assay for the Selective Quantification of Xylosyltransferase-I and -II Activity

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Introduction: Xylosyltransferase-I and -II (XT-I, -II) are the initial enzymes of the proteoglycan (PGs) biogenesis, catalysing the formation of a β-O-glycosidic bond between a xylosyl-residue and the hydroxyl side chain of a serine within the core-protein of a PG. Thereafter, further glycoslytransferases lead to the generation of a tetrasaccharide linker, which connects the PG core protein to the glycosaminoglycans. The isoform-selective quantification of XT-I and -II activity is of clinical interest because changes in the expression of both XT-isoforms are associated with fibrotic processes.

Methods: We detected the enzymatic activity of XT-I and -II through mass-spectrometry based measurement of the xylosyltation of isoform-selective acceptor-peptides (AP1 and AP2) using a Xevo TQ-S massspectrometer (Waters). Quantification was performed using a 2-dimensional chromatographic system composed of an C8 in-line extraction column and a C18 chromatography column. Multi-reaction monitoring after electrospray ionisation was used to distinguish products of AP1- and AP2-xylosylation. A standard prepared by quantitative enzymatic conversion was used for quantification. A xylosylated peptide with comparable physicochemical properties to AP1 and AP2 was used as an internal standard. XT-I and -II activity was calculated from AP1 and AP2 turnover.

Results: The developed method allows for the quantification of xylosylated AP1 and AP2 within a range of 7.9 to 1000 µg L-1. Measurements within this range show good linearity and high intra- and interassay reproducibility. Intra- as well as interassay precision and accuracy were validated. Measurements of samples containing predefined ratios of XT-I to XT-II showed a strong correlation between the quotient of AP1 to AP2 turnover and the XT-I to XT-II ratio.

Conclusion: The assay presented allows for sensitive as well as isoform-selective XT-I and -II quantification. As this method allows for high-throughput quantification of XT-I and -II activities in a variety of biological sample-types, it will be a promising approach to obtain further insides into the patho-biochemical roles of XT-I and -II within fibrotic diseases.

DGKL: 19. Varia

P-11-04

Role of the complement system in pathogenesis of Pseudoxanthoma elasticum

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Background: Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder which is mainly 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. Especially, angioid streaks which lead to choroidal neovascularization (CNV) could be seen in PXE patients. CNV is also characteristic for age-related macular degeneration (AMD). Beside CNV, patients which were affected by PXE or AMD show development of drusen which are located under the retina. In AMD drusen contains different factors of the complement system. The complement system belongs to the innate immune system and plays an important role in host defense. Deregulation of the complement system results in incorrect remove of pathogens or inflammation as seen in AMD. Because of similarities in clinical characteristics of

PXE and AMD, the complement system could also play an important role in PXE pathogenesis. Thus, the aim of this study was to analyze different complement factors in dermal fibroblasts and in sera from PXE patients for local and systemic complement activation.

Methods: Normal human dermal fibroblasts (n=4) and fibroblasts from PXE patients (n=4) were seeded with a final density of 177 cells/mm2. Medium was changed after 24 h to medium with lipoprotein-deficient serum. After additional 72 h, the gene expression of different complement system factors and complement system inhibitors were analyzed by quantitative real-time polymerase chain reaction. Also, protein concentration of complement factor 3 (C3) and 3a (C3a) were examined in supernatants of PXE fibroblasts and sera of PXE patients.

Results: Gene expression analysis of factors belonging to the C1 complex of the classical pathway as well as protein concentration of complement factor 1r in supernatants were significantly increased in PXE fibroblasts compared to control fibroblasts. Complement factor B was significantly upregulated, while regulatory factor H was significantly downregulated in PXE fibroblasts in contrast to control fibroblasts. The mRNA expression and protein level in supernatants of C3 and C3a was significantly increased in PXE fibroblasts compared to controls. Analysis of systemic C3 protein level revealed an increased C3 protein level in sera of PXE patients compared to sera from healthy control donors.

Conclusions: Our data indicate high expression and protein level in supernatants of different complement factors as well as high activity of complement system in PXE fibroblasts. Further we found high C3 protein level in sera from PXE patients, indicating not only local but also systemic activation of complement system. In conclusion, complement system seems to play a role in pathogenesis of PXE. Further investigations on its regulation seems necessary not only for understanding the pathobiochemistry of PXE.

DGKL: 19. Varia

P-11-05

Investigations on ABCC6-deficient human hepatocytes generated by CRISPR Cas9 genome editing

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Introduction: Patients diagnosed with the disease pseudoxanthoma elasticum (PXE, OMIM #264800) show ocular, dermal and vascular calcification of elastic fibers. Mutations in the ATP-binding cassette transporter subfamily c member 6 (ABCC6), whose substrate is unknown, cause these symptoms. Paradoxically, ABCC6 is mainly expressed in liver tissue, leading to the hypothesis that PXE is a metabolic disease. We developed an ABCC6 genome editing system for human immortalized hepatocytes (HepIm) to address this hypothesis. Furthermore, we performed an initial analysis of the generated clones with a focus on elements of PXE pathobiochemistry.

Methods: HepIm were transfected with an ABCC6-specific clustered regulatory interspaced short palindromic repeat (CRISPR-Cas9) genome editing plasmid and selected using fluorescence associated cell sorting. Individual clones were subsequently genetically characterized. ABCC6-deficient HepIm were analyzed for various markers of PXE pathobiochemistry. These included lipid homeostasis and extracellular matrix (ECM) remodeling, as well as inflammatory and senescent status. For this purpose, gene expression was determined by quantitative real-time PCR (qRT-PCR) and protein expression by enzyme-linked immunosorbent assay or immunofluorescence-based detection.

Results: Using the developed CRISPR Cas9 genome editing system, a heterozygous (htABCC6HepIm) and a compound heterozygous (chtABCC6HepIm) clone of HepIm were generated. The hABCC6 mRNA expression of these clones was significantly reduced. ABCC6 deficiency of HepIm resulted in diminished expression of the low-density lipoprotein receptor

inhibitor proprotein convertase subtilisin/kexin type 9 (PCSK9), reflecting an impairment of lipid homeostasis. Furthermore, they showed impaired ECM remodelling as indicated by lowered expression of collagen I (COL1A1). Regarding the inflammatory and senescene phenotype, a decreased expression of the complement component C3 and the cell cycle inhibitor p21 were detectable.

Conclusion: Our ABCC6 knock-out model of HepIm provides a tool for essential research on the metabolic features of the disease PXE in vitro. An initial analysis of the ABCC6-deficient clones indicates an impaired lipid homeostasis and an altered ECM remodeling. In addition, the clones exhibit a less senescent and anti-inflammatory phenotype compared to wild-type HepIm.

DGKL: 19. Varia

P-11-06

The influence of ABCC6 on senescence and calcification of human vascular smooth muscle cells

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Background: Calcification and fragmentation of elastic fibres in the vessel walls due to mutations in the ATP-Binding cassette subfamily C member 6 (ABCC6) gene are hallmarks of the autosomal-recessive disorder Pseudoxanthoma elasticum (PXE, OMIM #264800). Alterations in the vessel walls may induce atherosclerosis, leading to peripheral artery disease (PAD). Vascular smooth muscle cells (VSMCs) represent the main cell type in the arterial wall and display phenotypic plasticity. The calcification-prone osteogenic phenotype of VSMCs is considered to be crucial for the development of atherosclerosis and can be induced by different stimuli, including hyperphosphatemia and oxidative stress. It has been shown that fibroblasts from PXE patients exhibit a pro-calcifying and senescence-like phenotype with elevated β-galactosidase activity.

Methods: To induce acute senescence following oxidative stress, VSMCs were incubated with 750 µM hydrogen peroxide for one hour. Induction of senescence was confirmed using quantitative β-galactosidase assay, immunofluorescence and quantitative real-time PCR (qRT-PCT). Calcification of VSMCs was induced by incubation with 3 mM inorganic phosphate for 21 days and evaluated using alizarin red S (ARS) staining and qRT-PCR. Involvement of ABCC6 in the processes of senescence and calcification was investigated using qRT-PCR.

Results: Oxidative stress in VSMCs was successfully induced as shown by increased activity of senescence-associated β-galactosidase by 45 %. Expression of ABCC6 was not measurable in both the treated and untreated VSMCs. In contrast to other cell types, like fibroblasts, ABCC6 mRNA-expression was not elevated in senescent VSMCs. Induction of calcification resulted in a 2,8 fold stronger ARS staining compared to the control. qRT-PCR showed a tendentially lower ABCC6 mRNA--expression in calcified VSMCs compared to untreated VSMCs. After 21 days of cultivation, ABCC6 mRNA-expression was measurable in both the control and the calcified cells, indicating a time-dependent expression of ABCC6 in untreated VSMCs. Therefore, the mRNA-expression of ABCC6 was measured at different times after seeding, revealing a significant induction of expression after seven days of culture in association with matrix calcification and remodelling.

Conclusion and outlook: Time-dependent mRNA-expression of ABCC6 was detected, potentially triggered by the formation of extracellular matrix (ECM) remodelling, oxidative stress and calcification but not by senescence in VSMCs. To elucidate possible effects of ABCC6 function on calcification of VSMCs, ABCC6-knockout should be performed in VSMCs. Additionally, other phenotypic switches of VSMCs e.g. to macrophage-like cells, should be investigated to further understand the role of ABCC6 in PXE.

DGKL: 19. Varia

P-11-07

MARCKS as an essential regulator of monocytic ROS production

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Introduction – MARCKS (myristoylated alanine-rich C-kinase substrate) is a ubiquitously expressed, unstructured protein involved in multiple effects in various cells, e.g., actin crosslinking, signal transduction, and intracellular transport processes. However, MARCKS' specific function in monocyte/macrophages has not been addressed yet. Thus, this study aimed at elucidating the influence of MARCKS on cellular key processes in human monocytic cells.

Methods - We generated THP-1-derived MARCKS wildtype and knockout (KO) cells using the CRISPR/Cas9 technique. MARCKS levels were assessed by Western blot and cycle sequencing. Differentiation towards the monocytic cell type was induced by calcitriol. Reactive oxygen species (ROS) generation was detected in time course experiments using a luminometer. Proliferation, differentiation, phagocytosis, and cytokine mRNA expression were analyzed via cell counting, FACS-based detection of CD14 expression, FACS-based detection of phagocytosed labeled bacteria, and qPCR, respectively. (Phosphorylated) Akt levels were measured by Western blot.

Results - In the absence of MARCKS, both total and intracellular ROS production were virtually abolished, while other cell functions such as proliferation, differentiation, phagocytosis, or cytokine expression remained unaffected. Transient retransfection of MARCKS resulted in the restoration of ROS production in MARCKS KO cells. MARCKS deficiency also affected intracellular signal transduction cascades as represented by reduced basal phosphorylation and delayed re-phosphorylation of Akt. In MARCKS WT cells, a TNF long-term pre-incubation significantly elevated ROS production, an effect also completely suppressed in MARCKS KO cells.

Conclusion – Our data indicate that MARCKS is an essential contributor to monocytic ROS production. Further, MARCKS appears to be a part of signal cascades regulating ROS generation.

DGKL: 19. Varia

P-12-01

Serum-Magnesium: Zeit für einen einheitlichen, evidenzbasierten unteren Grenzwert für die Definition einer Hypomagnesiämie

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Zielsetzung: Ein Magnesiummangel gilt heute als erheblicher Risikofaktor für zahlreiche Erkrankungen. Ein Mangel wird derzeit fast ausschließlich über die Serum-Magnesiumkonzentration definiert. Allerdings existieren keine einheitlichen Grenzwerte für eine Hypomagnesiämie.

Methoden: Literaturrecherche im Zeitraum 2000-2023 mit den Suchbegriffen „reference interval“, „reference range“, „diagnostics“, „status“, „serum“, „plasma“, „hypomagnesemia“, „deficiency“.

Ergebnisse: Aktuell werden in Deutschland unterschiedliche Referenzbereiche für das Serum-Magnesium angewandt. Der untere Grenzwert liegt je nach Labor bei 0,60-0,85 mmol/l. Der häufig verwendete Referenzbereich von 0,75-0,95 mmol/l basiert auf mehr als 40 Jahre alten epidemiologischen Daten aus den USA.

Epidemiologische Untersuchungen zeigen einheitlich eine kontinuierliche Risikozunahme für verschiedene Erkrankungen (z. B. kardiovaskuläre Morbidität und Mortalität, Diabetes mellitus Typ 2 und Folgeerkrankungen) bei einer Serum-Magnesiumkonzentration unter 0,85 mmol/l.1,2 Als Hinweis auf ein vorbestehendes Defizit ergibt sich aus Bilanzuntersuchungen eine Netto-Retention von Magnesium bei Serum-Magnesiumkonzentrationen unterhalb von 0,85 mmol/l.

Ein strukturierter Konsensusprozess internationaler Magnesiumexperten führte 2022 zu der Empfehlung, den unteren Grenzwert des Referenzbereichs für die Serum-Magnesiumkonzentration auf 0,85 mmol/l festzulegen.3

Hämolyse kann falsch hohe Serum-Magnesiumwerte verursachen und eine bestehende Hypomagnesiämie überdecken.

Diskussion und Schlussfolgerung: Aufgrund der vorliegenden aktuellen Daten sollte daher der Referenzbereich für die Serum-Magnesiumkonzentration neu definiert und vereinheitlicht werden. Magnesium sollte wie die anderen Serum-Elektrolyte (Natrium, Kalium, Calcium) in der Routine-Labordiagnostik mit bestimmt werden unter Verwendung eines einheitlichen unteren Grenzwertes von 0,85 mmol/l.

DGKL: 19. Varia

P-12-02

A new LC-MS/MS screening method for the determination of a group of 739 illicit drugs, and other compounds in biological matrices

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Introduction

Nowadays it is increasingly important from a pharmacological, toxicological, and clinical point of view to have rapid screening tests available for the analysis of many compounds in a short time. Here, we discuss a rapid screening procedure in LC–MS/MS for the qualitative evaluation of 739 compounds in biological samples (blood, post–mortem blood, and urine) including New Psychoactive Substances (NPSs) and other illicit substances.

Methods

Blood samples were protein precipitated while urine samples were subjected to glucuronidase enzymatic reaction to hydrolyse any metabolites present. Chromatographic separation was carried out using a Restek Allure PFP Propyl (5 µm,60Å,50×2.1 mm) column in gradient elution mode. The deuterated internal standards were d9-methadone and d3--monohydroxycarbazepine. Mobile phases are M1: 2mM NH_4HCO_2 +0.2% CH2O2; and M2: acetonitrile +2mM NH_4HCO_2 +0.2% CH2O2. The method results in a rapid elution gradient and a change in flow rate during the run. The initial conditions are 90% M1 and 10% M2. Method includes two separate analyses in positive and negative ionization mode, without changing the instrumental parameters and run time was 18 min. Analyses were conducted on an ABSciex API 4500 QTrap instrument in MRM mode on 697 specific transitions.

Results

Thanks to the instrumental configuration the conventional analysis proceeds until the signal exceeds a threshold value, the mass spectrometer automatically begins to monitor the specific MRM transition and the entire fragmentation spectrum to obtain a comparable data with the mass spectra present in the database. Following the "match" process, the correct

identification of the compound is then provided following the retention time and the MS/MS fragmentation spectrum. Our method is simple with minimal sample handling and immediate comparison of the acquired MS/MS spectra with spectra in the database. This allowed to obtain the correct identification of the compound. This screening method was tested on 150 samples; all the samples were also analysed with confirmation test, to check the obtained screening results.

Conclusions

The comparison gives 100% correct identification of the positives and the type of substance. “Polyconsumers” were also identified.

DGKL: 19. Varia

P-12-03

Differential contribution of MARCKS to ROS production in monocytes and neutrophils

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Introduction

The myristolated alanine-rich C-kinase substrate (MARCKS) is a ubiquitous expressed protein that was initially identified as a regulator of cytoskeletal organization and dynamics that cross-links actin filaments. Later, MARCKS was shown to be involved in a variety of cellular processes, including protein secretion and cytokine expression in monocytic cells. Previously, we demonstrated that in THP-1-derived monocytic cells, MARCKS appears to be a key regulator in the production of reactive oxygen species (ROS), which play a role in pathogen clearance. In these cells, CRISPR/Cas9-mediated knockout (KO) of MARCKS resulted in a significant reduction of both total and intracellular ROS production in response to artificial (PMA) or physiological stimulation (opsonized bacteria), which was restored upon transient MARCKS re-transfection. Nevertheless, the molecular details of how MARCKS regulates ROS production in the myeloid lineage remains to be elucidated.

Methods

To study the function of MARCKS in ROS production, we used the MARCKS N-terminal sequence (MANS) peptide, which resembles the first 24 amino acids of MARCKS, as MARCKS inhibitor. Reactive oxygen species were detected in luminometer-based time course experiments. MARCKS WT and KO PLB-985 cells were generated using the CRISPR/Cas9 technique. Subsequently, MARCKS levels were assessed by Western blot analysis and cycle sequencing. Cell morphology was observed by light microscopy and proliferation by cell counting. Monocytic differentiation of THP-1 cells was induced by calcitriol and neutrophilic differentiation of PLB-985 cells by DMSO treatment. Cell differentiation was confirmed by flow cytometry-based detection of CD14 and CD11b.

Results

Inhibition of MARCKS by MANS treatment reduced ROS production of THP-1-derived monocytes significantly in comparison to cells treated with the scrambled control peptide (RNS) confirming our previous results obtained in THP-1 MARCKS WT and KO cells. In contrast, ROS production of PLB-985-derived neutrophils was delayed, but not significantly reduced by MANS treatment, suggesting different roles of MARCKS in ROS production depending on the cell type. MARCKS deficiency in PLB-985-derived neutrophils also reduced bacteria-induced ROS production though to a lesser extent than previously shown in the monocytic cell type. No significant effect of MARCKS deficiency was determined on cell proliferation, morphology, and differentiation capacity.

Conclusion

Our results indicate a differential function of MARCKS in monocytic and neutrophilic ROS production. Moreover, MANS appears to be a suitable tool to study the role of MARCKS in ROS production in various cell types.

DGKL: 19. Varia

P-12-04

Effect of anticoagulants and heparin contaminants on the expression of the biomarker MMP 9

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Introduction: Matrix metalloproteinase (MMP-) 9 can be assessed in blood samples as a prognostic biomarker for stroke. We have shown before that in contrast to other widely used anticoagulants, e.g., citrate and low-molecular weight heparin (LMWH), high-molecular weight heparin (HMWH) enhances the expression of MMP-9. This effect is based on stimulated T-cells, which - in response to HMWH - produce cytokines that induce monocytic MMP-9 expression. In our present study, the influence of further anticoagulants (i.e., hirudin, alteplase, and Fondaparinux) and the known heparin contaminants hyaluronic acid (HA), dermatan sulfate (DS), chondroitin sulfate (CS), and over-sulfated CS (OSCS) was analyzed to assess the suitability of MMP-9 as a biomarker under various conditions of anticoagulation.

Methods: Starved Jurkat T-cells were stimulated with anticoagulants or heparin contaminants. HMWH and LMWH were used as positive and negative controls, repectively. Subsequently, starved monocytic THP-1 cells were incubated with the conditioned Jurkat supernatant and MMP-9 mRNA levels were monitored via pPCR. Cytokines secreted by stimulated Jurkat cells were measured using proteome profiler arrays.

Results: While LMWH, Fondaparinux, HA (low concentration), and DS had no effect, the conditioned medium of HMWH-, hirudin-, alteplase-, HA- (high concentration), CS-, and OSCS-treated Jurkat cells significantly elevated MMP-9 mRNA levels in THP-1 cells. In this context, a basal significant MMP-9 induction appears to be provoked by soluble intercellular adhesion molecule (sICAM-) 1, which may cooperate with different interleukins (e.g., IL-16 or IL-5; in part supplemented by additional factors) to further enhance MMP-9 expression.

Conclusion: Our results suggest that in dependency of charge and molecular weight (but independent of an anticoagulatory activity) of the respective substance, different anticoagulants and heparin contaminants provoke the expression of T-cell-derived cytokine(s) subsequently resulting in the induction of monocytic MMP-9 expression. This effect may impair the diagnostic validity of MMP-9 under certain conditions.

DGKL: 19. Varia

P-12-05

XYLT1 Knockdown Impairs the Expression Profile and Functionality of Human Osteoclasts

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Bone homeostasis is a strictly regulated process in which bone formation and bone resorption are in balance. Proteoglycans (PGs), which are an important component of the bone matrix, also have important regulatory functions in extracellular matrix (ECM) organization, cell differentiation and signaling. The initial step of PG biosynthesis is catalyzed by the human xylosyltransferase isoforms XT-I and XT-II by transferring xylose to specific serine residues of the PG core protein. Mutations in the human XT-I coding gene XYLT1 cause Desbuquois dysplasia type II, a rare autosomal recessive, skeletal dysplasia characterized, inter alia, by joint laxity and facial dysmorphism. In comparison, the Spondyloocular syndrome, another rare

autosomal recessive disorder, is the result of XYLT2 mutations that leads, inter alia, to osteoporosis. Since, in relation to bone homeostasis, primarily osteoblasts and chondrocytes synthesize PGs, the research focus to date has been particularly on the study of XT-I in bone formation. To obtain a better overview of bone metabolism in Desbuquois dysplasia type II patients, the aim of this study was to analyze the effects of a XYLT1 deficiency on the differentiation and functionality of human osteoclasts. Human osteoclast progenitor cells were differentiated into multinucleated mature osteoclasts (OCs) by culturing with m-CSF and RANKL. On day 5 of differentiation, cells were transfected by lipofection with XYLT1 or control siRNA. At 48 and 96 h after transfection, XYLT1 knockdown (XYLT1-KD) was verified on mRNA expression level by qPCR and on protein level by a XT-I selective mass spectrometric activity assay. At 48 h after transfection, the mRNA expressions of NFATc1, a master transcriptional factor of osteoclast differentiation, collagen recognition receptor OSCAR and matrix metallopeptidase 9 were significantly increased in XYLT1-KD OCs in comparison to control. After 96 h of transfection, the analysis of the osteoclast marker protein α5β3 by immunofluorescence staining revealed no evident differences between XYLT1-KD and control, but at 48 h the mRNA expression of the integrin α subunit was significantly increased in the XYLT1-KD OCs. In comparison, no significant changes were detected for the mRNA expression of the TRAP coding gene ACP5, but TRAP-positive multinucleated XYLT1-KD OCs showed a larger cell area as well as changes in TRAP expression in comparison to control. In contrast, the resorbed bone matrix by XYLT1-KD OCs was decreased in comparison to control. The data from this study provide preliminary evidence that Desbuquois dysplasia type II patients with XYLT1 mutations not only have impairments in bone formation, but also in bone resorption caused by alterations in osteoclastogenesis. Since the data from mRNA expression analysis and TRAP staining are consistent with increased osteoclast differentiation, but bone resoprtion capacity is reduced in XYLT1-KD OCs, further investigations are needed.

DGKL: 19. Varia

P-12-06

Extracellular vesicle lipid functions in inflammation

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Introduction

Extracellular Vesicles (EV) are phospholipid bilayer-enclosed particles that are secreted by a variety of cell types, including macrophages. EVs can convey different effects in physiological cell homeostasis but also in diseases: EVs are involved in inflammation, but also in malignancies by promoting tumor growth and metastasis. These EV functions depend on the respective cargo, which itself depends on type and state of the originating cells. While EV nucleic acids and EV proteins have been studied extensively in current literature, characterization and function of EV lipids is still not sufficiently understood. Particularly, bioactive lipids such as PUFAs and eicosanoids may contribute to EV functions.

In our study, we first aimed at identifying changes of EV lipid profiles during inflammation. Secondly, we investigated the functional relevance of the identified changes.

Methods

EVs derived of differently polarized THP-I macrophages were purified by Sequential Filtration. EV size distribution and surface marker expression were assessed by Fluorescent Nanoparticle Tracking Analysis and by Flow Cytometry. PUFA and Eicosanoid profiles in the EV preparations were assessed by means of targeted quantitative mass spectrometry (LC-MS/MS). The functional relevance of the identified differences in eicosanoid profiles was investigated by means of RT-qPCR, TEER, cell adhesion assays and cell migration assays. 12-LOX was inhibited by ML355.

Results

The lipid profiles of macrophage-derived EVs differed largely depending on the polarization of the originating macrophages. Particularly, we observed major changes in the arachidonic acid pathway. M1 macrophage-derived EVs showed decreased levels of arachidonic acid but increased levels of ARA-derived 12-LOX and 15-LOX products, particularly 8-HETE/12-HETE. Both M1 EVs and 8/12 HETE induced expression of pro-inflammatory chemokines in target cells. After 12-LOX inhibition, M1 macrophage-derived EVs induced significantly lower changes. M1 EVs also induced monocyte adhesion, monocyte migration, and endothelial barrier dysfunction. These effects were significantly lower in 12-LOX inhibited M1 macrophage-derived EVs.

Discussion

EVs derived of differently polarized macrophages have different eicosanoid profiles. These changes are functionally relevant: 12-LOX inhibition of pro-inflammatory macrophages reduces pro-inflammatory effects of their EVs. In order to confirm these findings, further downstream in vivo investigations are necessary.

DGKL: 19. Varia

P-12-07

Identification of heparin-binding receptors on human T-cells

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Introduction: The anticoagulant high-molecular weight heparin (HMWH) has been shown to induce the mRNA and protein expression of the diagnostic/prognostic biomarker matrix metalloproteinase (MMP-) 9 in human blood samples. Mechanistically, HMWH-challenged T-cells secrete cytokines that induce monocytic MMP-9 expression. In this study, we aimed at identifying the respective HMWM-binding T-cell receptors/surface proteins driving the expression of MMP-9-inducing mediators.

Methods: Jurkat T-cells were lysed and the surface protein-containing membrane fraction was isolated by ultracentrifugation in a sucrose buffer. Purity of the fraction was assessed by detecting membrane-bound CD4 and intracellular Hsp90 using the Western blot technique. Afterwards, the membrane fraction was incubated with biotin-coupled HMWH. Proteins binding to HMWH were isolated by a pull-down assay using streptavidin-coupled magnetobeads and subsequently identified via mass spectrometry (MS; n=3). Proteins with a significant ($p < 0.01$) and at least 5-fold enrichment compared to bead control were classified as interaction partners. Subsequently, cellular localization and molecular function of these candidates were determined via Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) term analyses.

Results: The detection of CD4 and Hsp90 demonstrated an enrichment of membrane proteins and a depletion of cytosolic proteins in the isolated fraction. Among the proteins identified via MS, three membrane proteins were significantly enriched, i.e., the “don’t eat me” signal molecule CD47 and the solute carrier (SLC) transport proteins SLC3A2 (an ancillary subunit of several nutrient transporters) and SLC19A1 (the reduced folate carrier). The detection of residual intracellular proteins known as HMWH interaction partners (e.g., hnRNP A1 and the ribosomal proteins L7, L22, and L29) served as a positive control.

Conclusion: Our results indicate that HMWH directly binds to surface proteins on T-cells. The association with CD47 suggests a heparin-driven induction of CD47-dependent signaling, potentially resulting in T-cell activation. Moreover, the enrichment of SLC superfamily proteins suggests the possibility that transport and exchange processes of organic substance may also contribute to the HMWH-induced induction of cytokine expression in T-cells.

DVTA: 01 Aus Ausbildung in Theorie und Praxis

P-13-01

Wissensstruktur für Selbst- und patientennahe Diagnostik – Das 5 C-Modell im ersten Bachelor-Studiengang “Biomedizinische Labordiagnostik” in der Schweiz

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Fragestellung

Der erste Bachelorstudiengang “Biomedizinische Labordiagnostik” startete im Herbst 2022. Wie sieht eine Wissensstruktur für den Bereich Selbst- und patientennahe Diagnostik aussehen, die die fachbereichsübergreifende Ausrichtung zwischen “akademischen Gesundheitsberufe mit naturwissenschaftlichem Hintergrund” und “Naturwissenschaftler:in im Gesundheitswesen und in der Forschung” abbildet und eine Grundlage für die Umsetzung des akademischen Studiengangs darstellt?

Methode

Auf der Grundlage einer Berufsfeldanalyse durch den Berufsverband Labmed wurde das Curriculum von einer Projektgruppe entwickelt und anschließend akkreditiert. Die endgültigen Kompetenzen wurden auf der Grundlage der CanMEDs-Rollen formuliert. Auf der Grundlage dieser Vorarbeiten wurde eine Wissensstruktur entwickelt.

Die folgenden fünf Stichworte sollen die longitudinale Verankerung der Selbst- und patientennahen Diagnostik im Curriculum verdeutlichen:

CARE (pflegen), CONNECT (verbinden), COMMUNICATE (kommunizieren), COLLABORATE (zusammenarbeiten), CREATE (kreieren).

Das Modell wird laufend kommunikativ mit Studierenden und Lehrenden evaluiert.

Resultate

Den 5 Schlüsselwörter wurden den entsprechenden Rollenkompetenzen und exemplarischen Modulen aus dem Curriculum sowie strategischen Leitfragen zugeordnet.

Die dichotome Ausrichtung des Curriculums auf den Forschungsprozess (Planen, Durchführen, Auswerten) und den labordiagnostischen Prozess (Präanalytik, Analytik, Postanalytik) bilden die Grundlage. Die Analyse kann manuell durchgeführt werden, halbautomatisch oder vollautomatisch durch biomedizinische Labordiagnostiker:innen (Labordiagnostik), aber auch als patientennahe Diagnostik durch anderes medizinisches Fachpersonal (Point-of-Care-Testing) oder von Laien (Selbsttestung) durchgeführt werden. Hier müssen verschiedene Daten für die Gesundheitsversorgung vernetzt, kollaborativ verarbeitet und kommuniziert werden. Die Studierenden sollen grundlegende Kompetenzen für die Entwicklung neuer Tests in Zusammenarbeit mit anderen Berufsgruppen und Patienten erwerben und diese evidenzbasiert umsetzen können.

Diskussion

Die vorliegende Wissensstruktur ist ein erster Entwurf und muss mit Expert:innen aus den Gesundheits- und Naturwissenschaften sowie mit Patient:innen diskutiert werden.

Take-Home-Message

Für die Implementierung eines neuen Bachelor-Studiengangs in der Schweiz wurde ein 5-C-Modell entwickelt, das auf dem Labordiagnostik und dem Forschungsprozessmodell, den CanMEDs-Rollen und grundlegenden Testansätzen (Labor, POCT, Selbsttest). Dieses soll die Dualität zwischen Gesundheitsfachleuten und Naturwissenschaftlern visualisieren, um ein neues akademisches Profil in der Labordiagnostik zu verankern für die aktuelle und zukünftige Gesundheitsversorgung zu verankern.

DVTA: 01 Aus Ausbildung in Theorie und Praxis

P-13-02

Von der Vision zur Mission?

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Eine empirische Untersuchung zur Ergründung der Relevanz und des Potenzials der Kompetenzfacette Lerncoaching als unterstützende Maßnahme zur Professionalisierung der pädagogischen Handlungskompetenzen Lehrender in Gesundheitsberufen.

Ziel:

Die Novellierung der Gesetze in den Gesundheitsberufen mit dem Ziel, ebendiese zukunftsfähig und attraktiv zu gestalten, wird insbesondere für die MT-Berufe ab 2023, zur Herausforderung. Allgemeine Lernziele wie die Stärkung der Selbstwirksamkeit, die Fähigkeit zum transformativen Lernen und die Anerkennung des lebenslangen Lernens geraten neben der Vermittlung der Fachkompetenz vermehrt in den Fokus zukünftiger Lehr-Lernprozesse.

Die Vision, dass die Vermittlung von Lerncoachingelementen die Lehrenden zukünftig optimal bei der Begleitung der Lernenden zur Erreichung der o.g. Lernziele unterstützen und somit zur Professionalisierung der pädagogischen Handlungskompetenzen beitragen kann, habe ich in meiner Masterarbeit untersucht.

Ziel dieser Arbeit war, die Relevanz und das Potenzial der Kompetenzfacette Lerncoaching als unterstützende Maßnahme zur pädagogischen Professionalisierung der Handlungs-kompetenzen Lehrender in Gesundheitsfachberufen zu ergründen und mögliche Auswirkungen, die Lerncoachingkompetenz im Berufsalltag ebendieser erzielen kann, aufzuzeigen.

Methode:

Die Forschungsfragen zur Masterarbeit wurden zum einen mittels strukturierter Literaturrecherche beantwortet. Zum anderen unterstützte die Auswertung und Analyse leitfadengestützter Expert:inneninterviews mit Hilfe der qualitativen Inhaltsanalyse nach Mayring (2010) bei deren Beantwortung.

Ergebnisse:

Es zeigte sich, dass eine Lerncoachingausbildung unterstützende Auswirkungen auf die professionellen pädagogischen Handlungskompetenzen Lehrender in Gesundheitsberufen hat. Konkret zeigten sich die Auswirkungen in der Stärkung des Bewusstseins der Lehrer:innenrolle, der Haltung und der Selbststeuerungsfähigkeit. Des Weiteren berichteten die Lehrenden von einem enormen Zuwachs des Methodenrepertoires. Darüber hinaus konnte festgestellt werden, dass die Lerncoachingkompetenz eine Schlüsselkompetenz in der Professionalisierung der pädagogischen Handlungskompetenzen darstellt.

Schlussfolgerung und Diskussion:

Die Vision, Lerncoachingelemente bereits im Studium zukünftiger Lehrender in Gesundheitsberufen zu implementieren, sollte wegen seines enormen Potenzials zur Förderung der Professionalisierung der pädagogischen Handlungskompetenzen, weiterhin verfolgt und demnach zu einer Mission werden.

Zu bedenken ist, individuelle und wirksame Lernbegleitung braucht Zeit und bindet personelle Ressourcen. Dies sollte in den Ausbildungsplanungen Berück¬sichtigung finden.

Letztendlich ist das Outcome der Lernenden von entscheidendem Interesse. Trotz erfolgsversprechender Hinweise stehen die Untersuchung zur tatsächlicher Wirksamkeit von Lerncoaching noch aus. Gelingt es Lehrenden mit der neuausgestatteten Lerncoachingkompetenz tatsächlich ihre Lernenden zu selbständigem, verantwortungsbewussten und sozialkommunikativem Handeln zu befähigen? Gelingt es ihnen, das Feuer für lebenslanges Lernen zu entfachen?

Ebendiese Fragen bleiben unbeantwortet und stellen lohnende Herausforderungen für die Zukunft dar.

DVTA: 03. Aus Wissenschaft und Forschung

P-13-03

Streptococcus-Biofilme auf 3D-gedruckten Oberflächen für die Implantologie

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Durch die fortschreitende Alterung der Bevölkerung nehmen orthopädische Eingriffe wie zum Beispiel Zahnimplantate einen immer größer werdenden Stellenwert in der Medizin ein. Technologien wie computer-aided-design (CAD) und Computer-tomographie (CT) kommen bereits heute in der Implantologie zum Einsatz und könnten zur Herstellung von Zahnimplantaten mittels 3D-Druck verwendet werden (1). Zu den wesentlichen Risikofaktoren für den Verlust eines Zahnimplantates zählen die Mucositis und Periimplantitis, die durch Biofilme hervorgerufen werden (2). Das orale Mikrobiom wird hauptsächlich von Streptokokken gebildet. Beispiele sind sowohl der physiologisch vorkommende Streptococcus sanguinis als auch der Kariesbildner Streptococcus mutans (3, 4).

Ziel der Bachelorarbeit war es, die Biofilmbildung auf verschiedenen 3D-gedruckten Materialien für Zahnimplantate zu testen. Als Testkeime wurden S. mutans und S. sanguinis herangezogen.

In einem Biofilm-Assay wurde mit jeweils einem der beiden Bakterienstämme auf verschiedenen 3D-gedruckten Materialien die Biofilmbildung induziert und diese nach 24 Stunden geerntet. Anschließend wurde das Ausmaß der Biofilmbildung mittels Vitalfärbung mit flowzytometrischer Auswertung und Proteingehalt-Bestimmung mit spektrophotometrischer Auswertung bestimmt.

Sowohl in der Vitalfärbung als auch in der Proteingehalt-Bestimmung konnten für S. mutans deutlich höhere Messwerte als für S. sanguinis ermittelt werden. Die höchsten Zellzahlen für beide Bakterienstämme konnten auf hirtisiertem Titan Grade 2 festgestellt werden. Auf dem polierten PEEK wurden die niedrigsten Zellzahlen für beide Bakterienstämme festgestellt.

Das Keramikmaterial VIT105 eignet sich am besten als Material für Zahnimplantate, da sich im Vergleich zu den anderen Materialien mehr S. sanguinis-Biofilm aber weniger S. mutans-Biofilm bilden konnte. Weitere Tests mit den Materialien in Flow Chamber-Systemen oder mit anderen Bakterienstämmen des oralen Mikrobioms sollten durchgeführt werden.

DVTA: 02. Aus Qualitätssicherung und Labormanagement

P-13-04

Validierungsstrategie für NGS-basierte Panelsequenzierung zirkulierender Tumor-DNA (ctDNA)

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ZIELSETZUNG: Das Liquid Profiling gewinnt als Alternative zur gewebsbasierten Diagnostik insbesondere für die Companion Diagnostik, d.h. für den Nachweis tumorspezifischer genetischer Veränderungen als Voraussetzung für die Administration zielgerichteter Therapeutika, zunehmend an Bedeutung. Die kontinuierlich steigende Anzahl zugelassener zielgerichteter Pharmaka erfordert aus analytischer Sicht eine zeitgleiche Analyse einer immer größer werdenden Vielfalt genetischer Varianten. Um dieser Erfordernis gerecht zu werden, ist der Einsatz von hochsensitiven NGS-basierten Assays erforderlich. Da diese in der Regel nicht für die Krankenversorgung zugelassen sind, ist eine aufwendige laborinterne Validierung erforderlich, die u.a. auch die Herstellung eigener Kontrollmaterialien notwendig macht.

METHODEN: Die Validierung eines lab-developed Tests (LDTs) in der Molekulargenetik erfordert neben einer ggf. erforderlichen Assayoptimierung, eine Validation des Untersuchungsmaterials, des gesamten präanalytischen Arbeitsablaufes, der Bestimmung der Nachweis- sowie Quantifizierungsgrenze, des Linearitätsbereiches, der analytischen Spezifität, einer möglichen Verschleppung, potentieller Störsubstanzen, von Präzision und Richtigkeit sowie schlussendlich der diagnostischen Sensitivität und Spezifität. Weiterhin besteht die Herausforderung, dass häufig kein kommerzielles Referenzmaterial bzw. interne Kontrollen erhältlich sind.

ERGEBNISSE: Exemplarisch wird die Validierungsstrategie für einen kommerziell erhältlichen research-use-only (RUO) Test zur Panelsequenzierung aus zellfreier DNA dargestellt. Im Laufe des Validierungsprozesses wurden sowohl kommerziell erhältliche Referenzmaterialien getestet als auch eigene Kontrollen hergestellt.

DISKUSSION UND SCHLUSSFOLGERUNG: Für das Liquid Profiling kommen zunehmend Panelsequezierungen zum Einsatz. Neben der Präanalytik und Bioinformatik stellt der hohe Anteil von RUO Tests eine analytische Herausforderung dar, der insbesondere im Hinblick auf die IVDR eine zielgerichtete und optimierte Validierungsstrategie erfordert.