Congress Abstracts

German Congress of Laboratory Medicine: 20th Annual Congress of the DGKL and 7th Symposium of the Biomedical Analytics of the DVTA e. V.

Leipzig, Germany, October 22 – 24, 2025

Under the auspices of
International Federation of Clinical Chemistry and Laboratory Medicine
European Federation of Clinical Chemistry and Laboratory Medicine

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DGKL: 01. Qualitätsmanagement und Qualitätssicherung in der Labormedizin

P-01-01

Resilience of Blood Analytes During Repeated Drone Transport: A Preanalytical Validation Study

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Background

Uncrewed aerial vehicles (UAVs), commonly known as drones, are gaining momentum as innovative tools in medical logistics, especially for improving access in remote or underserved regions. While their utility for single-trip sample transport has been established, the potential cumulative effects of repeated aerial transport on blood sample integrity and diagnostic parameters remain insufficiently explored. This study evaluates whether multiple drone flight cycles influence analyte stability across common blood matrices.

Methods

Serum, EDTA plasma, lithium-heparin plasma, and citrate plasma samples were transported via a rotor-based hybrid drone over ten consecutive 30-minute flight cycles. Across all samples, 35 routine laboratory analytes were quantified both before and after transport using standard clinical chemistry, hematology, and coagulation assays. In-flight conditions such as temperature and vibration were continuously monitored with high-resolution data loggers and accelerometers. Statistical analyses included paired t-tests to assess significance, Passing-Bablok regression for systematic bias detection, and Bland-Altman plots to visualize variability trends.

Results

The majority of analytes remained analytically stable throughout the repeated transport. For instance, bilirubin levels showed a negligible mean bias of +0.1%, while alkaline phosphatase exhibited a minimal decrease of −0.7%. Potassium demonstrated slightly increased variability with a Passing-Bablok slope of 1.07 and a Bland-Altman mean bias of +2.5%. Thrombocyte counts rose by an average of +3.2%. Coagulation markers showed more pronounced changes: Quick values increased from 91.05% to 107.12% (mean bias: +15.0%; slope: 1.177), while INR values decreased modestly from 0.94 to 0.90 (mean bias: −4.3%; slope: 0.957). The aPTT showed the most significant shift, increasing from 20.32 to 24.78 seconds (slope: 1.220; mean bias: +18.0%). All remaining analytes remained within clinically acceptable deviation limits.

Conclusion

Repeated drone transport does not significantly compromise the integrity of most blood analytes, supporting its use in extended or multi-leg medical logistics operations. Nonetheless, the observed variability in potassium, thrombocyte counts, and coagulation-related parameters (Quick, INR, aPTT) highlights the importance of analyte-specific preanalytical validation. These findings emphasize the potential of drone-based transport as a robust and scalable solution for modern healthcare systems, provided quality assurance protocols are adapted to account for sensitive diagnostic markers.

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P-01-02

Impact of Aerial Transport by Drone on HIL Indices in Blood Samples

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Background

Accurate laboratory diagnostics depend on the integrity of transported blood samples, particularly with regard to pre-analytical quality markers such as the hemolytic, lipemic, and icteric (HIL) indices. These indices are essential for detecting sample degradation due to hemolysis, lipemia, or icterus. With drones gaining prominence in healthcare logistics—especially in rural or traffic-congested regions—this study assessed whether drone-based transport affects the stability of HIL indices in four common blood matrices.

Methods

A total of 25 samples per blood type (serum, EDTA whole blood, lithium-heparin plasma, citrate plasma) were transported using a medical-grade hybrid drone over a 25-kilometer route. Flights lasted 30 minutes at a cruising speed of 100 km/h and altitude of 100 meters. Blood samples were secured in insulated containers designed to limit vibration and thermal fluctuations. In-flight conditions were monitored continuously using environmental data loggers and accelerometers. HIL indices were measured before and after transport using spectrophotometry on a Roche Cobas system. Paired t-tests ($p < 0.05$) were used to evaluate statistical significance.

Results

No statistically significant changes were observed in HIL indices for any blood matrix. In serum, the hemolytic index decreased slightly by −0.15 ($p = 0.19$), and the lipemic index declined by −0.20 ($p = 0.38$). EDTA whole blood exhibited a comparable decrease in the hemolytic index (−0.15; $p = 0.42$) and a mild, non-significant increase in the lipemic index (+0.25; $p = 0.23$). Lithium-heparin plasma showed complete stability in the hemolytic index (0.00; $p = 1.00$) and only minimal variation in the lipemic and icteric indices (+0.05; $p = 0.79$). Citrate plasma demonstrated consistent results across all markers, with no statistically or clinically relevant deviations. All p-values exceeded the threshold of 0.05, confirming overall index stability.

Conclusion

The findings indicate that drone-based transport preserves HIL index integrity across multiple blood matrices, supporting its use in routine medical logistics. The drone system effectively minimized preanalytical variability, offering a reliable and environmentally friendly alternative to traditional transport methods. Given their ability to reduce delivery times and overcome geographic barriers, drones hold promise for wider integration into healthcare networks. Future studies should investigate the impact of drone transport on additional analytes and explore scalability in diverse clinical settings.

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P-01-03

Comparison of two different systems for the measurement of residual WBC in platelet and red blood cell concentrates

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Introduction

German guidelines for haemotherapy demand a limit of no more than 106 residual white blood cells (rWBC) per unit of platelet concentrates (PC) and red blood cell concentrates (RBC). Transfusion services must therefore provide an accurate and reliable system for the measurement of rWBC. Here, we present validation results of two different systems: a bead-based flow cytometric assay (BD Leucocount on BD FACSLyric cytometre) was used as a reference in this study. Further, a fully automated microscopic analyser (ADAM-rWBC, NanoEntek) and a haematology analyser (Sysmex XN-1000, blood banking mode) were used.

Material and Methods

In order to determine linearity of the three systems, plasma was spiked with whole blood to a concentration of 20 WBC/µl and then diluted serially down to 0.3125 WBC/µl. Each dilution series was prepared and measured at three different times. Precision was determined by 10 consecutive measurements of RBC, pooled PC and apheresis PC that were spiked with whole blood to a final concentration of 3, 5 and 10 WBC/µl, respectively. These very low concentrations are closest to the limit of 106 WBC per unit.

Results

All three systems provided clear linear slopes. The correlations with the dilution series were above R^2=0.995 for all investigated systems. In pooled PC and aphaeresis PC only ADAM-rWBC was within a range of 40% at all concentrations. Sysmex XN-1000 was above this range with higher counts at concentrations 3 and 5 WBC/µl. The relative standard deviation (RSD) was below 0.18 for all three systems for spiked RBC and at all three low concentrations. For spiked pooled and aphaeresis PC RSD was up to 0.24. However, the RSD for aphaeresis PC in the same concentration was 0.09 for Leucocount and 0.1. for ADAM-rWBC but wider for Sysmex XN-1000 (0.31).

Discussion

Measuring spiked routine blood products, the investigated systems can be regarded as suitable for routine use. While Leucocount and ADAM-rWBC provided more comparable data, the fully automated ADAM-rWBC provided the most consistent results. Higher counts with the Sysmex XN-1000, blood banking mode in the critical range are a nuisance but not grave in contrast to an underquantification. In a high throughput setting, however, this may lead to a higher amount of out-of-specificity (OOS) results. While an advantage of the Sysmex XN-1000 may be its speed a combination with another system that is applied for the control of OOS can be discussed.

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P-01-04

Weighted Sliding Window method for Age-dependent Reference Interval Estimation in Laboratory Medicine

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Reference intervals are essential for interpreting medical laboratory results by providing a range of values that encompass normal variations in measurements taken from healthy populations. Determining age- and time-dependent reference intervals is crucial in medical diagnostics, particularly for children and adolescents and for analytes with marked diurnal variations. Reference intervals are traditionally calculated within fixed age or time periods. However, these static reference intervals are limited when the underlying data indicate continuous progression across different age groups or time periods.

We have modified the reflim algorithm to create a new, promising approach: the Weighted Sliding Window method. This method uses a moving window to analyze data in overlapping segments in order to calculate locally adjusted reference values.

The Weighted Sliding Window method was implemented in the R programming language and is used in a user-friendly Shiny application called reflimR_Sliding. The Sliding Window method involves dividing data into overlapping segments ("windows") that gradually move along a covariate axis, such as age or time. For each window, a subset of the dataset is selected, and reference intervals are calculated. There are two approaches to the Sliding Window method: one with a fixed window size and step, and one with a flexible window requiring a minimum number of data points. The fixed approach may lack sufficient data at the start for reliable reference intervals. The flexible approach avoids this but can produce overly wide, less accurate intervals. In a Weighted Sliding Window method, data points are assigned weights according to a weighting function for the window, such as Gaussian or triangular.

On synthetic datasets, the Weighted Sliding Window method demonstrated smooth transitions between consecutive reference intervals and accurately reflected the underlying data trend. For real clinical datasets, the method successfully generated age-dependent reference intervals that closely followed known trends from the laboratory measurements.

The results indicate that the Weighted Sliding Window method provides a useful tool for estimating continuous reference intervals in medical laboratory datasets that account for age- or time-dependent changes. The computational complexity increases with larger datasets, particularly when smaller windows are used or when our method is applied to datasets with a high proportion of pathological values and/or unusual distributions. Future work will focus on further optimizing the method's computational efficiency and exploring its applicability to other types of clinical measurements with more pathological values, different data distributions and parameter settings. Laboratory physician expertise will continue to be required to select the appropriate parameters for the Weighted Sliding Window method and the covariate-dependent course of the laboratory values.

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P-01-06

Improvement of the Peer Review Process in Laboratory Medicine by an intuitive Digital Approach

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Introduction

Peer-review in medical laboratories is a voluntary, structured, and collegial evaluation process aimed to improve patient care and safety. Unlike bureaucratic audits, it emphasizes a supportive, non punitive exchange among equally qualified clinical peers through on site visits and confidential feedback. The interprofessional peer teams comprise technicians, natural scientists, It-professionals and physicians, The guidelines from the German Medical Association (Rili-BAEK)

introduced the peer-review process as an alternative to internal audits in 2019. While this efficient procedure is more and more established in enhancing the overall quality of medical laboratories, its current implementations will be further improved by an enhanced digital support, which will include data-driven graphical analyses for further developments.

Methods

We present the development and implementation of a digital tool (an online Shiny app) designed to support the peer-review process in laboratory medicine. The app supports both, the self-assessment as well as the external assessment. It comprises a comprehensive questionnaire (approximately 80 entries) developed by INQUAM e.V. covering key areas such as organization, leadership, management, employees, patient care, referrers, analytics, quality indicators, and validation. The Peer review process begins with a self-assessment, followed by a peer dialogue during an onsite visit, which includes an external assessment and a detailed report. The app can be used by laboratory staff and external peers for both self- and external assessments. It enables the external peers to submit responses online which are then aggregated and visualized based on the data of all colleagues involved in the peer review process.

Results and Discussion

By digitizing the peer-review process, the app transforms subjective, time-intensive evaluations into structured, data-driven assessments. This enhances both efficiency and reliability. It further provides insights into the quality and safety indicators and highlights a deviation between self- and external assessments. This fosters the dialog between the Peers and leads to a higher quality of the discussions, because the data of self and external assessments are analysed simultaneously, showing uniform and discrepant results in an intuitive manner.

Conclusion

The digital peer-review tool enhances quality assurance in medical laboratories by enabling structured, data-driven, and collaborative assessments. It streamlines the review process and supports continuous improvement in patient care.

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P-01-05

When robots join the team: a report about the implementation of a hybrid-robotic system in a tertiary care hospital laboratory

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Introduction:

The implementation of automation systems and robotics into routine laboratory diagnostics is seen as a promising strategy to shortage of qualified medical laboratory professionals and to rising diagnostic requirements. However, a broad experience in integrating complex laboratory robotic systems is still lacking and numerous challenges exist: limited space, infrastructural restrictions, compatibility with laboratory analyzers and laboratory information systems, high investment costs, and the need for a sensitive change management.

This project aims to establish a hybrid-robotic system into a pre-existing tertiary care hospital laboratory to improve workflow efficiency, error reduction and personnel workload.

Methods:

Infrastructural planning and continuous functional re-evaluation of the robotic system were performed by an interdisciplinary laboratory management and medical team. The hybrid-robotic system was installed to support and conduct pre-analytical steps prior to routine laboratory testing and to perform sample archiving. Patient samples intended for emergency testing were excluded from the robotic line and processed manually by laboratory technicians.

Key-performance indicators such as the number of processed samples, operating-time and turn-around-time were monitored. Periodic error surveillance and regular interviews with laboratory technicians were established to enable continuous diagnostic quality optimization and improve the user experience.

Results:

The commercially available robotic system contains of three automated robotic arms. Two static robotic arms carry out pre-analytic sample preparation including centrifugation, decapping and sample-to-rack-placement. A third dynamic and axis-guided robotic arm is in charge of sample positioning between the pre-analytic unit and the analyser systems for clinical chemistry, haematology, hemostaseology, and the sample archive unit.

The hybrid-robotic system demonstrates a stable and constant performance during the routine operating hours and the sample-peak times leading to an increased walk-away-time and workload reduction. The continuous involvement of the laboratory technician team into the transformative technical process was essential for the implementational success and cultural integration of this new technology.

Conclusion:

Our findings underline the strategic values of individual robotic systems for routine laboratory diagnostics. However, lacking of technical system standardization still requires individual and therefore work-intensive laboratory answers.

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P-01-07

Wertigkeit der Bestimmung von Akanthozyten im Urin

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Zielsetzung

Akanthozyten im Urin werden als Marker für glomeruläre Schädigung in vielen Laboren mehrmals täglich bestimmt, entweder per automatisierter oder manueller Mikroskopie. Im Labor der Universitätsmedizin Mannheim (UMM) werden Proben mit der Fragestellung nach Akanthozyten gemäß einer internen Vereinbarung zwingend manuell mikroskopiert. Ob die Probenverarbeitung effizienter gestaltet werden kann, ohne dabei die Patientensicherheit zu gefährden, indem mittels automatisierter Mikroskopie eine Vorauswahl mit erhöhter Vortestwahrscheinlichkeit getroffen wird, wurde in dieser Arbeit untersucht.

Methoden

Die aus einer Literaturrecherche übernommene Messgenauigkeit des an der UMM verwendeten Urin-Analyzers wurde hierfür zugrunde gelegt. Parallel wurden alle Urinproben mit dem Auftragskommentar „Akanthozyten“ aus dem

Klinikbetrieb im Jahr 2024 in Bezug auf ihre in der Automation gemessene Erythrozytenzahl sowie den manuell festgestellten Akanthozytenanteil ausgewertet. Anschließend wurden bei akanthozyten-positiven Fällen mit unauffälliger Erythozytenzahl weitere Parameter betrachtet, die eine mögliche Alternative zum Akanthozytenanteil darstellen, und geprüft, ob bestehende Leitlinien mit einer neuen Vorgehensweise eingehalten würden.

Ergebnisse

Nur in einem marginalen Teil der Proben mit dem Auftragskommentar „Akanthozyten“ und einer Erythrozytenzahl von 0-2/HPF wurden tatsächlich Akanthozyten nachgewiesen. Da es sich bei diesen Proben in keinem Fall um Notfallanforderungen handelte, sich z.T. auch Verlaufsbeurteilungen darunter fanden und weitere, standardmäßig erhobene Parameter auf eine glomeruläre Nierenschädigung hinwiesen, ist es nicht zwingend nötig, alle im digitalen Sediment analysierten und erythrozyten-negativen Proben auch auf Akanthozyten zu untersuchen.

Diskussion

Diese Beobachtung wirft zwangsläufig die Frage nach der Vereinbarkeit des Vorgehens mit den Leitlinien auf, in denen jedoch keine allgemeingültige Definition der Begriffe Hämaturie und Mikrohämaturie existiert, obwohl beide in zahlreichen auch internationalen Empfehlungen und den deutschen Leitlinien im Bereich der Urindiagnostik verwendet werden, aber verschieden definiert sind. So sind der Diskurs und die Etablierung einrichtungsübergreifender Standards erschwert. Auch innerhalb des Labors wurde die Fehleranfälligkeit des „Faktor Mensch“ sichtbar, sei es durch Unachtsamkeit beim Übertragen der Werte ins System oder Verwechselung von Referenzbereichen ähnlicher Parameter und Methoden.

Schlussfolgerung

Die Turn Around Time kann durch Selektion der Proben sinnvoll verkürzt werden.

Weiterhin sollte auf die Wichtigkeit einer sinnvollen Indikationsstellung der Akanthozytenbestimmung hingewiesen, sowie ein Bewusstsein für die bisher definitorisch und methodisch eingeschränkte Standardisierung der Urindiagnostik geschaffen werden.

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P-01-08

Effect of ‘TEMPUS600® Vita’ on diagnostic samples and transport time

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Introduction

Ten years ago, we introduced the innovative pneumatic tube system (iPTS) ‘TEMPUS600®’ (at that time TIMEDICO A/S, Bording, Denmark, now Sarstedt) in the University Medicine Greifswald. The iPTS is characterized by a fast sample transport and a very high reliability. It connected the Emergency Department (ED) directly with the lab automation system (LAS) ‘FlexLab’ (Inpeco, Turin, Italy). Samples from the ED could be sent to the LAS within 30 s. After the samples were loaded into the TEMPUS600, no other staff had to touch the samples as the LAS is processing the samples automatically and analysis of specimens could start at different analyzers connected to the LAS. In 2019 the ED moved into a new building, whereas the old ward was transformed into a Stroke Unit (SU). The iPTS now connected the SU with the LAS of the lab. The direct connection

from the ED to the LAS was no longer available. This resulted in increased hands on time for sample handling and in extended transport times. The turn-around time (TAT) was significantly increased and therefore the results of lab analyses were available later to the physicians in the new ED in comparison to the previous constellation. In 2024 a second iPTS 'TEMPUS600® Vita' (Sarstedt, Nümbrecht, Germany) could be installed in the new ED – again with a direct connection to our LAS.

We investigated effects on sample integrity of the new iPTS 'TEMPUS600® Vita', and the already established transport systems.

Methods

Three measurands (potassium, free hemoglobin (fHb) and lactate dehydrogenase (LDH)) - known to be very sensitive to hemolysis - were measured and compared in 14 blood sample quartets transported by courier, TEMPUS600®, TEMPUS600® Vita and a conventional pneumatic tube system.

Results

Samples transported by TEMPUS600® and TEMPUS600® Vita were sent to the lab within 30 to 60 s. The hemolysis measurands showed no or only marginal adverse effects on the sample integrity, with no clinical relevance. The measured results are fully comparable to the courier transport.

Conclusion

Both iPTS reduce TAT by a fast transport and reducing hands-on time. No significant differences in the measurements were found for the investigated analytes between courier and iPTS transports. Based on these findings the TEMPUS600® Vita was cleared for clinical use.

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P-01-09

Method comparison – a tooling chain from preparation to graphical analysis

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Introduction

Ensuring the quality of diagnostic results necessitates method comparisons in clinical chemistry. These comparisons are crucial for evaluating the performance of new or modified measurement procedures against established methods. Such method comparisons are required by the guidelines of DIN EN ISO 15189 (Medical laboratories – Requirements for quality and competence) and the German Medical Association's guideline on quality assurance for medical laboratory examinations (Rili-BAEK). They are performed to judge whether differences in measurement results have a significant impact on clinical interpretation.

Methods

We present a comprehensive software framework designed for conducting and evaluating such method comparisons.

This framework offers an integrated chain of software tools that cover the entire process - from data acquisition to statistical analysis, graphical representation, and reporting. The framework supports various statistical analysis methods, including Bland-Altman analysis and regression analyses, enabling a transparent and traceable evaluation of measurement systems.

Results

It has currently been used for over 130 comparisons since 2023. By automating and standardizing the evaluation processes, it significantly enhances efficiency and minimizes potential sources of error.

Discussion

Our goal is to provide laboratories with a robust and user-friendly tool that reliably meets the high-quality demands in clinical chemistry in order to further improve patient safety. Due to the standardization of the process the time needed set-up and evalution could be decreased.

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P-01-10

Potassium Preanalytics – An Inconvenient Truth

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Introduction: Potassium is one of the most frequently requested laboratory measurands, with a tightly regulated physiological range in vivo, but a limited whole blood stability in vitro. It is therefore widely acknowledged that preanalytical factors, including hemolysis, delays in centrifugation, sample material and storage conditions, have a significant impact on the quality of analytical results. The 2023 revision of the German Medical Association's guidelines (Rili-BAEK), which mandated the utilization of lithium-heparin (Li-Hep) for potassium measurements, reinforced the debate about the optimal sample material. The present study offers a comprehensive evaluation of different preanalytical factors, with a particular focus on sample material and storage temperature. The investigation also addressed delayed centrifugation, stability post-centrifugation, and the impact of platelet count.

Methods: The study was conducted on a total of 120 healthy adults. A set of 29 blood samples were collected from each participant (14 pairs of Li-Hep/Serum and one EDTA). Four pairs of samples were subjected to centrifugation 0.5 hours after blood collection and consisted of tubes from four manufacturers (Sarstedt, Greiner, BD, Kabe). Moreover, five pairs of samples were stored at 4°C and five pairs were stored at 20°C for the subsequent timepoints of centrifugation (2h, 4h, 6h, 8h, 24h). The samples were maintained at the same storage temperature until the final measurement. Potassium measurements were taken directly following the aforementioned timepoints of centrifugation, as well as 3, 5 and 8 days post blood collection using indirect ion-selective electrodes on a cobas pro analyzer (Roche Diagnostics).

Results: Immediately after blood collection, the mean potassium concentration in the serum samples was significantly higher than in the Li-Hep samples (4.08 mmol/L, 95% CI [4.04, 4.12] vs. 3.86 mmol/L [3.82, 3.90]). The mean difference was found to be 0.22 mmol/L, with individual differences ranging from -0.01 mmol/L to 0.51 mmol/L. When stored at 4°C potassium concentrations increased up to 7.0 % in serum (4.37 mmol/L [4.40, 4.44]) and 6.3 % (4.1 mmol/L [4.02, 4.19]) in Li-Hep after two hours and up to 30.9% in serum (5.35 mmol/L [5.22, 5.48]) and 36.3% in Li-Hep (5.26 mmol/L [5.14, 5.38]) after 8h. Platelet counts ranged from 151 to 447/nL and correlated moderately with elevated potassium concentrations in serum (r=0.524).

Conclusion: In accordance with previous findings, serum demonstrated significantly elevated potassium concentrations in comparison to Li-Hep, especially when stored at 4°C. Manufacturer's recommendation to centrifuge within 2h after sampling lead to average potassium increase below 7%. When centrifuged as late as 8h after sampling potassium results are highly impaired and inadequate for patient care.

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P-01-11

Short analytical evaluation of the new Beckman Coulter DxC 500i clinical analyzer

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Background

The new DxC 500i clinical analyzer from Beckman Coulter combines two analytical units for general chemistry including ISE and for immunochemistry analysis. In this study we tested the analytical performance of this new consolidated system.

Methods

To evaluate the imprecision we used commercially available control material for 35 applications representing the majority of the assay menu (general chemistry, ISE, specific proteins and immunochemistry assays). All parameters were measured over a period of 20 days twice a day. In addition, for selected parameters we used samples of an external quality scheme and measured them in duplicate two times a day over a period of three days. For comparison studies samples from daily routine were used. The system used was a cobas pro C703/E801 (Roche Diagnostics, Mannheim, Germany). Time to first and last result was determined with different test panels.

Results

Imprecision CVs were between 1.11 and 1.45 % for ISE, between 0.77 and 3.26 % for general chemistry and specific proteins and between 2.45 and 8.69 % for immunoassays, respectively.

The accuracy of the commercial ring trials samples was within the acceptable range for all analytes. Method comparison showed slopes between 0.8301 and 1.1753 for ISE or general clinical chemistry with a coefficient of correlation between 0.8963 and 0.9996. Slopes were between 0.8023 and 1.5294 for immunoassays with r2 between 0.699 and 1.0, respectively.

Conclusions

This study demonstrates that the new DxC 500i system delivers accurate and precise results as well as a good method comparability.

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P-01-12

Ensuring Clinical Reliability of Neurofilament Light Chain as a Biomarker: Insights from a Pilot External Quality Assurance Study

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Neurofilament Light Chain (NfL) is a key protein for structural integrity in neurons, particularly enriched in axons. Upon neuronal injury, due to trauma or neurovegetative disease, NfL is released into the cerebrospinal fluid and blood stream in proportion to the extent of axonal damage. This makes NfL a promising biomarker for assessing and monitoring of central nervous system injury and diseases such as Multiple Sclerosis, Alzheimer's Disease and amyotrophic sclerosis.1,2

However, variability in pre-analytic handling, assay platforms and calibration standards can significantly impact measurement accuracy. External quality assurance (EQA) programs are essential to ensure inter-laboratory comparability, identify systematic biases and support assay harmonization.3 To ensure NfL becomes a reliable and clinically actionable biomarker, standardization therefore plays a critical role.

We therefore initiated a pilot EQA scheme using lyophilized human serum samples distributed to participating laboratories. Each lab analyzed NFL concentrations using their preferred method (choice of photometric detection, fluorescence detection, luminescence detection, immunoturbidimetric methods, magnetic particle mediated immunoassay, others) and reagent kits. Submitted results were evaluated for accuracy across all participants and with Method/kit per subgroups. The findings demonstrated strong agreement across platforms, supporting the feasibility and value of the proposed EQA scheme in enhancing the reliability of NFL as a biomarker for neurodegenerative processes.

DGKL: 01. Qualitätsmanagement und Qualitätssicherung in der Labormedizin

P-01-13

External Quality Assessment for Urinary Antigen Detection of Legionella pneumophila and Streptococcus pneumoniae

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Legionella pneumophila and Streptococcus pneumoniae are significant pathogens responsible for severe respiratory infections, including Legionnaires' disease and pneumococcal pneumonia. Infections can be life-threatening, particularly in vulnerable populations such as elderly or immunocompromised individuals. Rapid and reliable detection is crucial for timely treatment and effective outbreak management.

Urinary antigen tests (UTAs) enable early detection of both pathogens, typically within 1-3 days after symptom onset. Compared to PCR, UTAs offer a faster result, are cost-effective, require minimal laboratory infrastructure, and eliminate the need for respiratory samples, which can be difficult to obtain in some patients. These advantages make UTAs a valuable diagnostic tool in acute clinical setting.1

External quality assurance (EQA) programs are essential for identifying variability, promoting standardization and ensuring inter-laboratory comparability.2 The independent quality assurance contributes to the optimization of laboratory results by providing objective feedback and benchmarking, thus supporting the continuous improvement of diagnostic accuracy and drawing attention to possible procedural errors. Establishing an EQA scheme for urinary antigen detection of L. pneumophila serogroup 1 and S. pneumoniae thus improves diagnostic accuracy and strengthens public health surveillance.

To meet this need, we developed an EQA program enabling participants to detect one or both pathogens in four urinary samples using either immunoassays or rapid tests. Submitted results are assessed based on correct classification of each sample as positive or negative for the respective pathogen. The initial round robin included 167 participants and was well

received as participation increased by 12 % over one year. A broad variety of test systems was used. Across three rounds, good concordance among methods was observed with success rates ranging from 98-99 % for L. pneumophila and 82-97 % for S. pneumoniae.

These findings demonstrate both the feasibility and strong demand for the presented EQA scheme. Consistent external quality assessment of urinary antigen detection for Legionella pneumophila and Streptococcus pneumoniae may therefore significantly increase test reliability in a clinical practice, thereby reinforcing patient confidence in medical care.

DGKL: 01. Qualitätsmanagement und Qualitätssicherung in der Labormedizin

P-01-14

Interpretationsvariationen von Zlog Farben

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Herausforderungen und Anforderungen an eine einheitliche Farbskala für medizinische Befundinterpretation: Eine Analyse der Zlog Farbskala

In der medizinischen Praxis stellt sich die Frage, ob eine einheitliche Farbskala für die Darstellung von Laborwerten, wie der Zlog Farbskala, eine zuverlässige und verständliche Interpretation ermöglicht. Dabei wird untersucht, wie unterschiedliche medizinische Fachkräfte, beispielsweise eine onkologische Station im Vergleich zu einem Hausarzt auf dem Land, auf einen niedrigen Hb-Wert von 6 g/dl reagieren. Es wird die Frage aufgeworfen, ob die Zlog Farbskala bereits ausgereift ist oder nur auf bestimmte Labortypen zugeschnitten wurde. Zudem wird die Eignung der Zlog Skala als einheitlicher, deutschlandweit verwendbarer Standard im Kontext der elektronischen Patientenakte (ePA) und des darin vorgesehenen MIO Zlog bewertet. Das Ziel ist es, die Sicherstellung einer einheitlichen Interpretation zu diskutieren, um möglichen Fehlinterpretationen durch abweichende Farbskalen vorzubeugen und somit die Patientensicherheit sowie die klinische Kommunikation zu verbessern.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-01

Entfällt - Identification of serum biomarkers linking myocardial fibrosis, systolic dysfunction and outcomes in patients with severe aortic stenosis

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Introduction: Myocardial fibrosis (MF) is central to the development of heart failure in severe aortic stenosis (AS) and correlates with impaired systolic function and adverse outcomes post-transcatheter aortic valve replacement (TAVR).

Biomarker-based approaches may enable non-invasive assessment integrating MF, cardiac dysfunction, and risk stratification.

Methods: Serum levels of 184 cardiovascular biomarkers were measured in 133 AS patients prior to the TAVR procedure. These were linked to the degree of histological MF derived from left ventricular biopsies (available for n=100), and systolic function via echocardiographic left ventricular ejection fraction (LVEF) assessment (n=133). Associations with cardiovascular mortality following TAVR were analyzed using Cox regression.

Results: Twelve biomarkers were significantly associated with MF (> median), and 36 with reduced systolic function (LVEF < 50%). Six biomarkers - brain natriuretic peptide (BNP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), growth hormone (GH), fibroblast growth factor 23 (FGF23), growth/differentiation factor 15 (GDF15), and paraoxonase 3 (PON3) – demonstrated significant associations with both MF and reduced LVEF. All but PON3 showed odds ratios > 1. BNP (HR: 1.28, p=0.031), NT-proBNP (HR: 1.36, p=0.016), and GDF15 (HR: 1.65, p=0.048) were additionally found to have prognostic potential with respect to cardiovascular mortality post-TAVR. PON3 (HR: 0.60, p=0.087) and FGF23 (HR: 1.21, p=0.099) showed trends toward significance.

Conclusion: This study identified serum biomarkers linking MF, systolic dysfunction, and mortality in AS. Specifically GDF15 may contribute to a more comprehensive risk assessment. These findings support the integration of biomarker profiling into personalized diagnostic and therapeutic strategies for AS.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-02

OMICs-based Characterization of Disease Models for Hereditary Spastic Paraplegias: Mapping Molecular Targets for Diagnostic, Prognostic and Therapeutic Interventions

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Introduction:

Hereditary Spastic Paraplegias (HSP) are a group of rare genetically heterogeneous movement disorders. The major pathological correlate of the clinical symptoms is a distal-to-proximal degeneration of upper motor neuron cell axons in the central nervous system (CNS). Mutations in HSP-associated genes lead to the functional alterations of the corresponding proteins resulting in a disturbed molecular cell homeostasis. As is the case for many other congenital neurodegenerative disorders, there are no common molecular targets established for diagnostic, prognostic or therapeutic interventions in HSP.

Methods:

Our study aims to dissect the functional background for a particular group of HSP focusing on SPG11, SPG15 and SPG48. The goal is to identify common relevant molecular targets for these pathomechanistically related HSP forms. By analyzing valid murine models for SPG11, SPG15 and SPG48, our multiparametric approach includes OMICs-based characterization of various organ systems such as murine brain and immortalized embryonic fibroblasts (iMEF).

Results:

Comparative transcriptomic analyses of pre- and post-symptomatic SPG11, SPG15 and SPG48 murine brain tissue reveal differentially expressed gene patterns; but similar subcellular pathways are affected. Gene expression patterns in SPG11,

SPG15 and SPG48 iMEF comparing non-starved and starved cell conditions seem to be variable. Analyses of subcellular pathways in SPG11, SPG15 and SPG48 iMEF (non-starved and starved) suggest differences when compared to their brain tissue. Genes of Alzheimer's, Huntington's, Parkinson's, and Amyotrophic Lateral Sclerosis disease, as well as general pathways in neurodegeneration, are significantly altered in both iMEF and CNS models. In contrast, genes of the Mitogen-Activated Protein Kinase pathway were significantly altered only in the CNS model.

Conclusion:

Our multiparametric approach promises molecular insights and better understanding of HSP related pathomechanisms. With a potential for clinical applications, ongoing analyses and research collaborations aim to extend and validate current results, providing importance for the prospective diagnostic intervention and therapy of the underlying human disease.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-03

Influence of ABCC6-deficiency on senescence, reactive oxygen species generation and fatty-acid metabolism in mesenchymal stem cells

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Introduction: The autosomal-recessive disorder Pseudoxanthoma elasticum (PXE, OMIM #264800), characterized by calcification and fragmentation of elastic fibres in the skin, retina and vessel walls, is caused by mutations in the ABCC6 gene. This gene encodes for ATP-binding cassette subfamily C member 6 (ABCC6), a transporter mainly localized in the basolateral membrane of hepatocytes and kidney cells. It has been shown that fibroblasts from PXE patients exhibit a senescence-like phenotype (SASP) with elevated β-galactosidase activity and increased mRNA expression of the cell cycle inhibitor p21 and interleukin (IL) 6. An altered balance of ROS formation and degradation may contribute to the SASP of fibroblasts derived from PXE patients. Furthermore, signs of oxidative stress have been reported in the serum of PXE patients and in PXE fibroblasts. Due to recent reports indicating a role of the bone marrow in the pathogenesis of PXE, human mesenchymal stem cells (hMSCs) were chosen for these investigations.

Methods: An RNP-based CRISPR/Cas9-approach was used to introduce an ABCC6-deficiency in hMSCs. To induce oxidative stress, wildtype and knockout-hMSCs were incubated with 1 mM H_2O_2 for 1 h following a change of medium and 72 h of incubation. Senescence was analysed by measuring the β-galactosidase activity, evaluating the mRNA expression of senescence markers using qRT-PCR and immunofluorescence staining of p21. Staining of different types of reactive oxygen species and nitic oxide was performed using various molecular probes and fatty-acid uptake was evaluated using a fluorescence-labelled fatty-acid analog.

Results: Elevated β-galactosidase-activity was detected in knockout-hMSCs, further indicating a role of ABCC6 in the process of cellular senescence. The β-galactosidase activity of the wildtype and knockout hMSCs increased following treatment with H_2O_2 for 1 h with elevated mRNA expression of p21 and senescence-associated cytokines IL1β and IL8 confirming the induction of a senescent phenotype. The immunostaining of p21 revealed an increase in the portion of p21-positive cells to 100%. Our preliminary data show an inductive influence of ABCC6-deficiency on production of specific types of ROS in hMSCs whereas an altered senescence-associated phenotype in ABCC6-deficient hMSCs still needs to be elucidated. Furthermore, ABCC6-deficiency seems to accelerate fatty-acid uptake in hMSCs, an observation that requires further investigation.

Conclusion and outlook: Knockout of ABCC6 confirms an association of ABCC6 with the process of cellular senescence and oxidative stress by showing signs of an induced senescent phenotype along with an induction of ROS production. The pathomechanistic link between ABCC6 and oxidative stress will be investigated further by evaluating markers of senescence and investigating fatty-acid uptake in ABCC6-deficient hMSCs.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-04

Introduction of a novel LC-MS/MS analytical method for the quantification of cotinine and nicotine in serum and plasma, accompanied by a comparative evaluation against corresponding urinary concentrations

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Introduction:

Smoking significantly increases the risk of vascular constrictions, inflammation, and organ rejection in heart transplant patients. Nicotine and its metabolites impair blood circulation, increasing the risk of severe complications. Cotinine measurement in serum plays a crucial role in ensuring patient compliance, offering precise and timely data on tobacco use. Unlike urine tests, by determining cotinine levels in serum or plasma, patient compliance can be significantly improved, as this method cannot be manipulated or falsified. To assess potential differences in smoking status evaluation based on cotinine or nicotine measurements in serum versus urine, we analyzed 665 patient samples and determined their smoking status. Quitting smoking increases survival rates and promotes long-term health in transplant patients.

Methods:

A 50 µL volume of blank serum, calibrator standards, QC samples, and serum or plasma samples was mixed with 200 µL zinc sulfate solution (0.1 mol/L), followed by precipitation with 500 µL acetonitrile containing cotinine-D4 and nicotine-D4 as internal standards. After centrifugation, the supernatant was used for cotinine and nicotine measurement by UPLC-MS/MS, with a run time of 3.5 minutes per injection. In addition, nicotine and cotinine in urine were measured using our published method.

Results:

Cotinine and nicotine calibration curves were linear within 1.0 – 2000 µg/L and 1.0–1000 µg/L, respectively. Limits of detection in serum were 0.47 µg/L for cotinine and 0.69 µg/L for nicotine. Interassay CVs were ≤ 1.0% for cotinine and ≤ 7.2% for nicotine. Mean recovery was 83.0 ± 19.8% for cotinine and 114.8 ± 16.0% for nicotine. Serum and plasma cotinine levels showed strong correlation (R = 0.99), as did nicotine levels (R = 0.99). Comparisons between cotinine levels in serum and urine revealed urine concentrations averaging 3.7 times higher than serum levels, however, with good correlation (R = 0.80). Urine nicotine levels were 100 times higher than serum, with a weaker correlation (R = 0.51). Reference values were determined: non-smokers had cotinine < 2 µg/L, passive smokers 2 – 20 µg/L, and smokers > 20 µg/L. Nicotine thresholds were < 10 µg/L for non-smokers and > 10 µg/L for smokers. Among 665 patient samples, cotinine measurements in serum and urine showed a high agreement in determining smoking status, with only minimal differences: serum tests identified 101 smokers and 551 non-smokers, while urine tests reported 99 smokers and 542 non-smokers. Passive smoker counts varied, with 13 detected via serum and 24 via urine. Nicotine-based classification showed greater discrepancies: urine identified 85 smokers and 580 non-smokers, whereas serum identified only 43 smokers and 622 non-smokers.

Conclusion:

Our newly developed UPLC-MS/MS test has proven that cotinine measurement in serum or plasma provides a reliable assessment of smoking status, while nicotine measurement is unsuitable for this purpose.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-05

CircRNA Signatures Reflect Cell Differentiation States and are Biomarkers of Vascular Disease

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Introduction

Circular RNAs (circRNAs) are a class of non-coding RNAs underlying cell type- and developmental stage-specific expression. Due to their closed loop structure, circRNAs are relatively stable, and therefore promising candidates as biomarkers. Here, we characterize circRNA expression during the differentiation of human induced pluripotent stem cells (iPSCs) into endothelial cells (ECs) and vascular smooth muscle cells (SMCs), and evaluate their diagnostic potential in atherosclerotic vascular disease in vivo in a translational approach.

Methods

We performed high-throughput RNA sequencing using a de novo circRNA detection pipeline to profile circRNA expression daily during a two-week differentiation protocol of iPSC into ECs and SMCs. A total of 31,369 circRNAs were identified, of which 4,071 were robustly expressed and quantified at all time points. We analyzed changes in circRNA expression relative to their linear host genes and investigated correlations to mRNA levels of splicing factors and transcriptional regulators. To evaluate the potential of circRNAs as biomarkers, circRNA levels were profiled in atherosclerotic and healthy arterial tissue (n = 54/54) as well as in peripheral blood mononuclear cells (PBMCs) from patients with coronary artery disease (CAD) and healthy controls (n = 89/77). A support vector machine was trained on the circRNA expression levels of our iPSC-to-EC differentiation and tested for corresponding in vivo profiles from arterial tissue. In addition, circRNA levels of PBMCs were tested to discriminate CAD patients from healthy controls.

Results

Differentiation into ECs and SMCs resulted in a global increase in RNA circularization, with 397 circRNAs significantly upregulated in mature ECs compared to progenitor cells (>2-fold, adjusted $P < 0.05$). This correlated with downregulation of linear mRNA levels for specific splicing factors with known roles in circRNA biogenesis, such as SRSF1 and SRSF2. In contrast, circRNA levels were globally decreased in diseased arteries and a set of these circRNAs were also downregulated in PBMCs from CAD patients. Machine learning identified circRNA levels from COL4A1, COL4A2, HSPG2 and YPEL2 that were able to distinguish CAD from healthy tissue with an area under the curve (AUC) of 0.79. Furthermore, HSPG2- and YPEL2-derived circRNAs in PBMCs discriminated between patients with and without CAD with an AUC of 0.73.

Conclusion

Our results demonstrate a dynamic upregulation of circRNAs during vascular cell differentiation and a decrease of specific circRNAs in arteries and blood immune cells of CAD patients. Taken together, these results suggest the use of circRNAs as blood-based biomarkers for vascular disease and provide a framework for biomarker development using human stem cell models in combination with clinical sample validation, facilitating the translation of circRNA profiling into diagnostic applications.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-06

Development of a Biological Age Score from Urinary Metabolomics Using High-Resolution NMR

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Introduction:

The concept of biological age, in contrast to chronological age, reflects an individual's aging status based on a range of factors including genetics, lifestyle, and environmental influences. Metabolic profiling has emerged as a promising tool in this context. In a previous study, sex-specific metabolic age scores were derived from NMR-based metabolite profiles in urine samples collected from the Study of Health in Pomerania (SHIP), a large, population-based cohort, measured on a 400 MHz NMR platform. In our study, we aim to refine and validate a metabolic age score in the same cohort using an expanded metabolite panel and a higher-resolution NMR platform.

Methods:

Urinary samples from the SHIP cohort were measured on a 600 MHz NMR platform. Samples were available from baseline and three follow-up studies. Metabolic age was estimated by regressing chronological age on urinary metabolites. The metabolic age was validated by examining correlation to biological age and associations to a range of age-related diseases. Further, the predictive value was assessed via logistic regression.

Results:

The metabolic age score revealed high correlation to chronological age and significant associations to a range of clinical outcomes and diseases, e.g. diabetes. In longitudinal analysis, logistic regression showed the independent predictive value of the metabolic age score for some of the considered outcomes.

Conclusion:

Our findings show that a metabolic age score based on urinary metabolites measured via high-resolution NMR is associated to a range of age-related outcomes and adds prognostic value beyond chronological age. The planned analyses might help to improve the early identification of individuals at risk of developing age-related adverse outcomes and thus pave the way towards personalized medicine.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-07

Verification of ^{1}H-NMR Detection Limits for Ketone Bodies in Plasma

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Introduction: Ketone bodies such as 3-hydroxybutyric acid (3-HBA) play an important role in energy metabolism during fasting or starvation and are currently discussed as potential early disease markers for both acute and chronic heart failure. However, quantitative determination in plasma is hardly available, and established diagnostic methods (e.g., ELISA, enzymatic assays) often lack the sensitivity to reliably detect low physiological concentrations. ^{1}H-NMR (proton nuclear magnetic resonance) spectroscopy enables absolute quantification of multiple ketone body species in a single measurement and has demonstrated promising detection limits. The Plasma/Serum B.I. Quant-PS™ platform (Bruker BioSpin GmbH) specifies low limits of detection (LODs) of 10–20 µM for clinically relevant ketone bodies. This study aims to verify these limits in ^{1}H-NMR measurements of plasma using the IUPAC regression method, supplemented by bootstrapping calculations.
Methods: EDTA plasma samples (anonymized, leftover material) were pooled and homogenized. Aliquots of 300 µL were stored at −80 °C until usage. On each experimental day, pooled plasma samples were thawed and subjected to dialysis against phosphate-buffered saline (PBS) at 4 °C for 24 hours using a 3.5 kDa molecular weight cut-off membrane. This procedure aimed to remove low-molecular-weight metabolites, including ketones, while preserving the plasma matrix. The resulting de-metabolized plasma was spiked with in house stock solutions of 3-HBA, acetoacetate (AcAc), pyruvate (Pyr) and α-ketoglutarate (α-KG) to yield concentration levels from 5 to 750 µM. One-dimensional (1D) 1H-NMR spectra were acquired using a 600 MHz NMR spectrometer (Bruker). A weighted least-squares regression (weights = SNR^2, signal to noise based) of the peak areas yielded the slope s and the residual standard deviation σ. Limits of detection and quantification were calculated as LOD=3σ/s and LOQ=10σ/s. 95 % confidence intervals were obtained with a non-parametric cluster-bootstrap resampling approach.

Results: Estimated LODs (µM) [95 % CI] were: 3-HBA 13.2 [2.1–17.2], AcAc 10.5 [6.4–14.9], Pyr 13.3 [4.1–18.0] and α-KG 12.5 [8.7–17.4]. Corresponding LOQs were 39.9 [6.4–52.1], 31.7 [19.3–45.0], 40.3 [12.3–54.7] and 37.8 [26.3–52.9] µM, respectively. All point estimates confirm Bruker's claimed 10–20 µM LOD range; bootstrap intervals indicate that, under optimal conditions, true LODs may fall to ~5 µM while remaining below ~18 µM across days.

Conclusion: Our validation verifies existing high field NMR LOD claims for plasma ketone bodies. Bootstrap-derived confidence intervals indicate that, under optimized conditions lower LODs are possible. This supports its potential application in future clinical studies, where accurate metabolic profiling is essential.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-08

Untargeted metabolomics: Reproducibility and detection power across data-dependent acquisition, data-independent acquisition, and AcquireX

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Introduction: Untargeted metabolomics aims at the unbiased metabolic profiling and biomarker discovery but requires methods with high sensitivity and reproducibility. Here, we compare three acquisition modes—Data-Dependent Acquisition (DDA), Data-Independent Acquisition (DIA), and AcquireX —to evaluate performance and reproducibility in detecting low-abundance metabolites in a complex matrix.

Methods: Bovine total lipid extract (Avanti Polar Lipids Inc) was spiked with decreasing levels (10–0.01 ng/mL) of 15 eicosanoid standards, with known retention times and m/z values, to compare the detection power of each mode. Reproducibility was evaluated over three independent measurements, spaced one week apart. Chromatographic separation was performed on a C18-Kinetex Core-Shell column and HRAM-MS/MS data were acquired using an Orbitrap Exploris 480. Data processing was conducted using Compound Discoverer 3.3.

Results: DIA demonstrated superior reproducibility, with a coefficient of variation of 10% across detected metabolic features, compared to 17% for DDA and 15% for AcquireX. DIA consistently identified the highest number of metabolic features (average: 1036), outperforming DDA (18% fewer) and AcquireX (37% fewer). However, the MS^2-identification yield was comparable across methods. DIA accurately identified more spike-in standards and maintained high reproducibility even when annotations were incorrect, benefiting from its deterministic fragmentation pattern. AcquireX and DDA showed inconsistencies due to stochastic or sub-optimal MS^2 triggering, challenging the presumed superiority of DDA in precursor–fragment linkage. DIA provided better detection at moderate concentrations, while DDA and AcquireX were superior at lower concentrations due to shorter cycle times and sensitivity thresholds. Yet, none effectively detected eicosanoids at physiologically relevant low concentrations, highlighting critical trade-offs between MS^2 coverage and sensitivity.
Conclusion: DIA outperformed at moderate concentrations, while DDA and AcquireX were more sensitive at lower levels due to faster cycle times. However, none reliably detected eicosanoids at physiological levels, underscoring trade-offs between MS^2 coverage and sensitivity, and the need for acquisition-aware data strategies.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-02-09

Real-time Monitoring of Sample Stability in Plasma Samples using ^{1}H-NMR

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Introduction: Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy provides a rapid and non-destructive analytical method for metabolomics studies on biofluids, such as blood plasma. Unlike static systems, these complex biomatrices contain numerous reactants that can dynamically interact over time, even in the absence of cellular components. NMR enables real-time monitoring of potential ongoing (bio)chemical processes following sample collection and preparation. This study aims to assess time-resolved metabolic changes in plasma pool samples.

Methods: EDTA plasma samples from a hospital laboratory (1 litre anonymized, leftover material) were pooled and stored at -80 °C until use. Time-series experiments were conducted on n=4 replicates with measurements taken every three hours over a 72-hour period, using a 600 MHz NMR spectrometer (Bruker) to record water-suppressed 1D 1H-NOESY (Nuclear Overhauser Effect Spectroscopy) spectra. Between measurements, samples were stored at 6°C (SampleJetTM, Bruker). All spectra were aligned to a pH-independent glucose signature to visualise shift induction. Spectral assessment included signal assignment, chemical shift, peak area, and total intensity.

Results: Several changes in the spectrum were observed over time: The baseline intensity of the overall spectrum increased, while the signal of the internal reference signal from TSP (trimethylsilylpropionate) progressively broadened, resulting in apparent decrease in peak area. Additionally, several amphoteric metabolites (e.g. alanine, histidine) exhibited a systematic downfield shift, suggesting an increase in sample pH. The stoichiometric ratio between creatine (up) and creatinine (down) also changed, indicating a shift in chemical equilibrium likely due to re-hydrolysis of creatinine, which is promoted by

alkalization of the sample. Likewise, changes were observed in detectable intermediates of central energy metabolism, including clinically relevant parameters such as glucose, lactate, and citrate.

Conclusion: Real-time monitoring reveals multiple alteration processes in cell-free plasma samples – beginning from sample preparation – potentially influencing the results of metabolomics studies. Our findings underscore the importance of careful, metabolite-specific interpretation of delayed follow-up measurements.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-01

Impaired Conversion of Omega-3 Fatty Acids to Pro-Resolving Mediators in Acute Myocardial Infarction

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Introduction:

Specialized pro-resolving mediators (SPMs) are bioactive lipid derivatives of omega-3 polyunsaturated fatty acids (PUFAs), playing a key role in actively resolving inflammation. While SPMs have been implicated in atheroprotection and myocardial repair, their plasma profiles in patients with stable coronary artery disease (CAD) or acute myocardial infarction (AMI) remain incompletely characterized.

Methods:

We developed a high-throughput online SPE-LC-ESI(–)-MS/MS method enabling the simultaneous quantification of 31 SPMs and their precursors (EPA and DHA) within a 9-minute runtime. This method was applied to plasma samples of 499 participants from the LIFE-Heart study: 167 controls, 166 with stable CAD, and 166 with AMI. Clinical and demographic variables were recorded. SPMs with >20% missing values were excluded. Multivariable logistic regression models adjusted for relevant covariates (e.g., age, LDL-C, fasting, ALT) were used to examine associations of PUFA-derived metabolites and their ratios with CAD and AMI status.

Results:

Twelve metabolites were detectable in plasma; four SPMs—17,18-dihydroxyeicosatetraenoic acid (17,18-DiHETE), 14,15-DiHETE, 19,20-dihydroxydocosapentaenoic acid (19,20-DiHDPA), and 16-hydroxydocosahexaenoic acid (16-HDoHE)—met analytical criteria. While no significant differences in metabolite concentrations were observed between CAD patients and controls, AMI patients showed significantly higher plasma levels of EPA and DHA, but lower levels of 17,18-DiHETE and 14,15-DiHETE. These findings show a strong inverse associations with AMI risk. Conversely, EPA and DHA were positively associated with AMI status. Ratios of metabolites to precursors—especially 17,18-DiHETE/EPA and 14,15-DiHETE/EPA—were markedly reduced in AMI, indicating impaired metabolic conversion via cytochrome P450 (CYP) enzymes.

Conclusion:

Our LC-MS/MS method enabled sensitive detection of key SPMs in human plasma. In contrast to stable CAD, AMI patients exhibited altered omega-3 PUFA and SPM profiles, characterized by elevated precursor levels but reduced concentrations and conversion efficiency of CYP-derived diols. These findings suggest an acute dysregulation of inflammation-resolving pathways in AMI. The observed decrease in metabolite-to-precursor ratios points to impaired enzymatic resolution signaling and underscores the potential of SPMs as state-specific biomarkers for acute cardiovascular events. Further research is needed to evaluate their prognostic utility and therapeutic relevance in cardiovascular disease.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-06-05

Studying the Interaction between SARS-CoV-2 N Protein and G3BP1 and Their Influence on Stress Granule Formation with Cross-Linking Mass Spectrometry

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The nucleoprotein (N protein) is one of the four structural proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is involved in RNA packaging, mediates viral replication upon binding to host proteins involved in RNA processing, and interferes with the host's immune response. The N protein is highly abundant in the virion and in host cells after infection.

The N protein was structurally characterized with respect to its interactions with Ras-GTPase-activating protein-binding protein 1 (G3BP1). The SARS-CoV-2 N protein is comprised of two structured domains, flanked by three intrinsically disordered regions (IDRs). Among its many roles in modulating the host-immune response, the N protein was shown to impair stress granule (SG) formation. G3BP1 is one of the key factors in SG formation and consists of two structured domains and three IDRs. Upon infection of a host with SARS-CoV-2, the N protein interacts with G3BP1, leading to impaired SG formation. While the influence of N protein on SG formation has been confirmed in vivo and in vitro, the molecular details of the interactions between the N protein and G3BP1 are still elusive. It has been suggested that the N-terminal IDR1 of the N protein plays an important role in G3BP1 binding, involving an ITFG motif, which binds to the structured dimerization domain of G3BP1. Other studies indicate that the C-terminal region of the N protein might contribute to G3BP1 binding. Gaining detailed insights into the molecular mechanisms of the interactions between the N protein and G3BP1 is mandatory for better understanding the influence of a SARS-CoV-2 infection on SG assembly and disassembly. Cross-linking mass spectrometry (XL-MS), native polyacrylamide gel electrophoresis (native PAGE), and mass photometry were employed for a structural characterization of the interaction between the N protein and G3BP1, with a focus on the IDRs of both proteins. While XL-MS allowed identifying interaction sites, native-PAGE and MP gave valuable insights into the oligomeric states of the single proteins as well as the N protein–G3BP1 complexes. Combined information from all experiments served as basis for integrative modeling of the protein–protein interactions between N protein and G3BP1 using AlphaFold3. The obtained model provides valuable information on the interaction on the molecular level and aids to verify the already existing knowledge about the N protein–G3BP1 complex.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-02

Differential enrichment of membrane proteins by high- and low-molecular weight heparins reveals CD3/T cell receptor as a candidate receptor for HMWH-induced T cell activation

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Introduction

High-molecular weight heparin (HMWH-), but not low-molecular weight heparin (LMWH)treated T cells secrete cytokines that induce monocytic matrix metalloproteinase (MMP)9 expression thus enhancing MMP-9 levels in blood samples. To elucidate the underlying mechanism(s) in T cells, HMWM-binding membrane proteins on Jurkat T cells should be identified.

Methods

The Jurkat membrane fraction was isolated by ultracentrifugation and incubated with biotin-coupled HMWH and LMWH. Heparin-binding proteins were isolated using streptavidin-coupled magnetobeads and identified via mass spectrometry. Cellular localization and molecular function of significantly enriched proteins (≥ 5-fold; $p < 0.01$) were determined by UniProtKB and Gene Ontology analyses. The interaction network of identified cell membrane proteins was analyzed by k-means clustering using STRING.

Results

Among the cell surface proteins specifically binding to HMWH, a cluster of multiple proteins associated with T cell activation via the CD3/T cell receptor (TCR) complex was enriched including CD3E and TCRA. To assess the potential involvement of CD3/TCR in HMWH-induced T cell activation, Jurkat cells were treated with the zeta-chain-associated protein kinase 70 (ZAP 70) inhibitor ZAP-180013 before stimulation with HMWH. THP-1 monocytic cells that were exposed to the supernatant of HMWH-stimulated Jurkat cells pre-treated with ZAP-180013 exhibited significantly less increased MMP-9 mRNA levels than THP-1 exposed to HMWH-conditioned medium from Jurkat cells cultured without inhibitor.

Conclusion

Our data suggest an induction of CD3/TCR-dependent signaling in response to HMWH, resulting in T-cell activation and the generation of monocyte-activating soluble mediators.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-03

Characterization of a novel obesity-associated type 2 diabetes mouse model reveals circadian clock disruption in diabetic kidney disease

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Introduction:

A recently identified mouse model for obesity-associated type 2 diabetes is based on a homozygous mutation in the leptin receptor gene (C57BL/6N-LeprL536Hfs*6-1NKB; Obis) on chromosome 4. Obis mice exhibit phenotypic characteristics including hyperphagia, obesity, decreased energy expenditure and reduced locomotor activity, as well as hyperglycemia and

hyperinsulinemia, compared to C57BL/6N control mice. However, the effects of this mutation on kidney function in this model have not yet been investigated.

Methods:

We initially assessed the phenotype of 22-week-old female Obis and control mice. This was followed by a histopathological examination for characteristic features of diabetic kidney disease (DKD) and an analysis of circadian clock gene expression in the kidney.

Results:

Consistent with previous reports, female Obis mice showed a significant increase in body weight and blood glucose levels. Additionally, serum creatinine levels as well as increased kidney weight further indicated the presence of kidney dysfunction. Periodic acid-Schiff (PAS) staining demonstrated enlarged glomeruli with pronounced extracellular matrix accumulation, tubular hypertrophy, and loss of tubular epithelial brush border. Immunohistochemical staining for Wilms' tumor 1 (WT-1) indicated significant podocyte loss in Obis mice. In addition, nephrin expression, as assessed by immunofluorescence, was significantly decreased in diabetic Obis kidneys. Masson's trichrome staining showed significant tubulointerstitial fibrosis, and transcript analysis revealed increased expression of Kidney Injury Molecule-1 (Kim-1), a known marker of proximal tubular injury. Given prior observations of altered circadian gene expression in STZ (type 1)- and db/db (type 2)- diabetic models, we examined the protein expression of central clock genes in Obis mice. Notably, CLOCK and BMAL1 were moderately but significantly upregulated, whereas CRY1 expression was strongly downregulated. These results are consistent with previous data and support the hypothesis that DKD is associated with dysregulation of the peripheral circadian clock of the kidney.

Conclusion:

In summary, this initial characterization for the Obis model of type 2 diabetes highlights key structural, functional, and molecular features of DKD. Moreover, the marked downregulation of CRY1 indicates a significant disruption of the renal circadian clock in this model. These data make the Obis mouse a valuable tool for future investigations of the pathophysiological mechanisms linking circadian dysregulation to DKD. Ongoing studies aim to extend the phenotypic characterization and explore potential sex-specific differences in male Obis mice.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-04

Endothelial-specific deletion of the redox regulator p66Shc attenuates the progression of atherosclerosis and liver injury under high-fat diet conditions

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Background:

Atherosclerosis remains one of the main causes of cardiovascular morbidity and mortality worldwide. The redox protein p66Shc plays a key role in promoting oxidative stress and vascular damage, processes that are central to the development of atherosclerotic plaques. While studies in mice with systemic p66Shc deficiency have been linked to lower disease

burden, these models fail to differentiate the specific contribution of p66Shc in the endothelium. Investigating the effects of endothelial-specific p66Shc deletion would provide new insights into the role of p66Shc in vascular disease and may uncover new therapeutic strategies. Here we aim to investigate the effects of endothelial-specific p66Shc deletion on the progression of atherosclerosis and identify the potential mechanisms underlying this effect.

Methods:

An inducible, endothelium-targeted p66Shc knockout mouse model was generated using the CreERT2 system and newly generated p66ShcLoxP mice, allowing targeted deletion of the p66 splice variant of the Shc gene. Atherosclerosis was induced by adenoviral injections of the PCSK9 gain of function mutant (PCSK9D377Y.AAV), resulting in hypercholesterolemia, and mice were exposed to either a standard (Chow) or high-fat diet (HFD) for 16 weeks. Serum lipid parameters, atherosclerotic plaques and liver histopathology were assessed ex vivo.

Results:

Unexpectedly, mice lacking p66Shc in endothelial cells have significantly lower serum LDL, cholesterol and triglyceride levels. Consequently, mice lacking p66Shc in endothelial cells lack detectable plaques despite a high-fat diet and PCSK9 gain of function. Likewise, the liver tissue of these animals are protected against diet-induced tissue damage.

Conclusion:

Endothelial-specific deletion of p66Shc markedly reduces plasma lipids and hence protects against atherosclerosis. These results uncover a previously unknown function of p66Shc for lipid metabolism and indicate that p66Shc is a potential therapeutic target for metabolic and vascular diseases. Ongoing studies aim to define the mechanism through which p66Shc modulates plasma lipids.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-05

Ischemia related mitochondrial calcium loading and cell death is regulated by cellular energy, and is driven by IRE1 alpha

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Introduction:

Mitochondrial calcium loading, excessive ROS generation, cell death, and tissue injury are hallmarks of ischemia reperfusion injury (IRI). Previous studies have addressed these aspects individually, providing some mechanistic insights. However, overall, our insights into mechanisms leading to IRI are limited and we lack, in particular, druggable targets. Considering that both the endoplasmic reticulum and mitochondria, and their interaction regulate cell survival and death we evaluate in this project the role of the ER stress sensor IRE1alpha in IRI.

Methods:

We employed CRISPR-Cas9 to knock out (KO) IRE1alpha in immortalized murine tubular proximal cells. For functional studies, we generated various IRE1alpha mutants which were re-expressed in IRE1alpha KO cells. We also generated

inducible whole body or endothelial- or tubular-cell specific IRE1 alpha KO mice to evaluate the role of IRE1alpha in ischemic injury models such as stroke, kidney ischemia reperfusion injury, and cold ischemic injury associated organ transplantation. To gain mechanistic insights we additionally employed calcium imaging, co-immunoprecipitation, proximity ligation assay, several proteomic studies, and cell free experiments.

Results:

IRE1alpha drives mitochondrial calcium loading and cell death during ischemia. Mitochondrial calcium accumulation primes mitochondria for a 'burst' of activity during the initial phase of reperfusion injury when oxygen and glucose are re-introduced. These activities of IRE1alpha are independent of its canonical signaling. Rather, IRE1alpha directly senses intracellular ATP/ADP levels, inducing mitochondrial calcium accumulation at low ATP levels during ischemia. KO of IRE1alpha or mimicking ATP-binding to IRE1alpha prevents ischemic cell injury and cell death.

Conclusion:

Our data identify IRE1alpha as a central inducer of ischemia-induced injury through mitochondrial calcium loading. These data are in agreement with a model in which cells stressed by ischemia attempt to adapt to the low energy state by calcium loading through IRE1alpha aiming to sustain mitochondrial energy generation. However, excessive calcium loading primes cells for death. Targeting ATP-sensing by IRE1alpha may allow to modulate this pathway and thus to potentially suppress ischemic tissue injury.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-06

PAR4 engages with different G-proteins to induce thrombin-mediated podocyte injury in glomerulonephritis.

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Background: Glomerulonephritis (GN) is characterized by proteinuria and renal dysfunction. Increased thrombin generation is linked with GN and podocyte injury, but the mechanism underlying thrombin-induced renal dysfunction in GN and whether this can be pharmaceutically targeted is not well known. This study aims to elucidate the mechanisms through which thrombin causes renal injury in GN and to investigate whether targeting thrombin receptor, protease-activated receptor 4 (PAR4) can mitigate glomerular injury in GN.

Methods: To assess whether thrombin primarily targets PAR4 and the consequences thereof, we employed two approaches. First, we used PAR4 knockout (KO) mouse podocytes, and second, we exposed human podocytes to PAR1 and PAR4 inhibitors (P1pal-12 for PAR1, & BMS3 and P4pal-10 for PAR4). The therapeutic potential of the above inhibitors (P1pal-12, BMS3 and P4pal-10) were also tested in a mouse nephrotoxic serum (NTS) GN model. Finally, we silenced Gαq and Gα13 in human podocytes and stimulated the cells with thrombin, PAR1 activating peptides, and PAR4 activating peptides to elucidate the role of G-protein subunits involved in the pathways leading to podocyte injury.

Result: PAR4 inhibition alleviated thrombin-induced podocyte effacement and suppressed NLRP3 inflammasome activation in mouse and human podocytes, while PAR1 inhibition only provided partial protection. In the NTS model, targeting PAR4 alleviated NLRP3 inflammasome and albuminuria, while targeting PAR-1 (p1pal-12) failed to restrict NLRP3 activation. Finally, we observed that silencing Gα13 inhibited podocyte effacement whereas silencing both G-protein subunits (Gαq and Gα13) inhibited PAR4-thrombin mediated NLRP3 inflammasome activation.

Conclusion: These results demonstrate that PAR4 engages with different G-proteins to induce podocyte effacement (Gα13) and inflammasome activation (Gαq and Gα13) and GN. Additionally, our findings suggest that targeting PAR4 ameliorates thrombin-induced inflammasome activation and podocyte injury, providing mechanistic insights and suggesting a new potential therapeutic strategy for GN.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-07

Effect of direct oral anticoagulants on kidney in diabetic kidney disease

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Introduction

Diabetic kidney disease (DKD) is the primary cause of chronic kidney disease and end-stage kidney disease worldwide. Mechanisms of DKD are incompletely understood but thrombo-inflammation and abnormal coagulation activity are associated with DKD. Direct oral anticoagulants (DOACs) inhibit specific coagulation factors and are given to patients with diabetes mellitus with associated cardiovascular risk factors. Although DOACs are widely known for their anticoagulation properties, there is increasing evidence that they convey distinct cellular and anti-inflammatory properties. Whether DOACs target mechanisms independent of coagulation in DKD remains unexplored. We therefore aim to study the effects of fXa inhibition (using rivaroxaban) and fIIa inhibition (using dabigatran) in DKD.

Methods

C57/BL-6J mice were subjected to streptozotocin induced diabetes resulting in diabetic kidney disease. A sub-group of diabetic mice were treated with either DOACs alone or in combination with O1918, a GPR18 and GPR55 inhibitor. Albuminuria and histology were performed to characterize kidney damage. Multiple omics techniques like RNAseq, targeted plasma proteomics and lipidomics were conducted to identify possible mechanisms.

Results

Despite a comparable anticoagulant activity, fXai conveyed a stronger nephroprotective effect as fIIai. RNAseq profiling revealed differentially regulated genes (DEGs), with a larger number of changes in fXai compared to fIIai treated mice. fXai DEGs were associated with metabolic pathways including metabolism of polyunsaturated fatty acids (PUFA) and arachidonic acid. Plasma oxylipins, bioactive lipids generated by oxidation of PUFAs, were differentially regulated in fIIai-treated versus fXai-treated mice. Among these, Resolvin D2, a pro resolving lipid mediator, was significantly upregulated only by fXai treated mice. O1918, an inhibitor of resolvin D2-signalling, abolished the nephroprotective effect of fXai.

Conclusions

These results suggest distinct effects of DOACs targeting different coagulation proteases, with fXai being superior to fIIai in DKD. Nephroprotection by fXai is associated with changes in oxylipins, particularly an induction of resolvin D2. Nephroprotection by fXai is mechanistically linked to resolvin D2 signaling. These findings, if confirmed in humans, will have clinical implications when choosing a DOAC in patients with or at risk of DKD.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-08

Platelet-neutrophil interaction promote thrombo-inflammation and glomerular endothelial dysfunction in diabetic kidney disease

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Introduction

Diabetic kidney disease (DKD) is a major cause of end-stage renal failure and is associated with endothelial dysfunction, platelet hyperreactivity, immune-cell infiltration and dysfunctional glomerular filtration barrier. Mechanistic insights into the role of platelets and interaction with neutrophils in DKD progression are limited.

Objectives

We aim to scrutinize the mechanistic crosstalk between platelets and neutrophil and the ensuing renal thrombo-inflammation in DKD.

Methods

We evaluated plasma samples from a cohort of human patients (n=65) with and without DKD. In mice, streptozotocin induced type-1 diabetic mice model was used along with interventions targeting platelet activation (aspirin) or platelet-neutrophil interactions (P-selectin ligand blocking, PSGL-1). In vitro studies were performed using primary human glomerular endothelial cells (GENC), washed platelets and primary blood neutrophils exposed to high glucose under static and flow conditions.

Results

Compared to non-DKD controls, DKD patient plasma exhibited higher levels of platelet activation (PF4), neutrophil extracellular traps (NET) formation (H3Cit, NE) and endothelial dysfunction (sVCAM1) markers. Although these markers did not correlate with HbA1c in DKD patients, they did positively correlate with one another and negatively linked with eGFR, indicating a primary relationship with kidney function in DKD. In DKD plasma, upon flow cytometry analysis, we observed increase in netting neutrophils and platelet-neutrophil conjugates correlated with kidney function parameter. In-vitro, platelets aggravated NET-mediated barrier disruption, inflammation (pNF-κB p65), and endothelial dysfunction (p-eNOS, KLF4) when GENC were exposed to the supernatant of platelet-neutrophil co-culture. Flow experiments revealed that when GENC was co-cultured with platelets and neutrophils under high glucose conditions in the channel sides, there was an increase in platelet activation and it was seen co-localizing with NETs. Mechanistic studies in mice revealed that targeting platelet activation (aspirin) or platelet-neutrophil conjugates (P-selectin ligand on neutrophils) prevents albuminuria and reduces NETs in diabetic glomeruli. Plasma levels of PF4, H3Cit, NE and sVCAM-1 improved. Levels of pNF-κB p65, p-eNOS and KLF4 was restored in glomerular lysates of treatment mice as compared to non-treated diabetic controls. These findings suggest inhibition of platelet activation or neutrophil-platelet interaction can cause disease reversal.

Conclusion

Hyperglycemia promotes platelet activation, platelet-neutrophil interactions and NET formation enhancing inflammation and glomerular endothelial dysfunction and aggravating experimental DKD. Inhibition of platelets or platelet-neutrophil

interactions improves kidney function and injury, suggesting that targeting platelets, platelet-neutrophil interactions or NETs are promising therapeutic strategies for treatment of DKD.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-04-02

Reversing Risk: Impact of Lifestyle-Induced Weight Loss on the Cardiovascular Related Proteome

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Introduction

Lifestyle-induced weight loss (LIWL) improves the clinical trajectory of common cardiometabolic diseases such as atherosclerosis and type 2 diabetes, which may even be reversible in some cases. However, long-term weight maintenance remains challenging, and weight regain is common. Intriguingly, prior studies suggest that initial weight loss may confer lasting metabolic benefits even if weight is regained.1,2 We hypothesized that these benefits could be mediated by persistent changes in the cardiovascular proteome.

Methods

In a single-center, randomized controlled trial, 74 middle-aged men with metabolic syndrome underwent a 6-month LIWL program and participated in annual follow-up assessments for up to 5 years. The intervention included caloric restriction, a low-glycemic diet, and moderate physical activity. A total of 39 participants attended all scheduled follow-up visits and were included in the present analysis. Proteomic profiling of plasma samples was performed at baseline (T1), post-intervention (6 months, T2), and long-term follow-up (5 years, T3) using the Olink® Cardiovascular II panel. Protein expression (NPX values) was correlated with clinical markers such as BMI, lipids, blood pressure, HbA1c, CRP, and IL-6.

Results

The LIWL intervention led to a substantial BMI reduction from 33.02 kg/m^2 to 28.77 kg/m^2 within six months. Proteomic analysis revealed significant changes in 26 cardiovascular-related proteins between T1 and T2, many of which are involved in inflammation, lipid metabolism, and vascular function. Notably, 5-year follow-up data showed that most participants had regained their initial weight (BMI 32.45 kg/m^2). Nevertheless, only 6 of the 26 initially altered proteins reverted significantly between T2 and T3. The remaining 20 proteins remained significantly altered compared to baseline, suggesting a persistent proteomic shift. Several of the stable proteins are known to be involved in vascular inflammation, further supporting their potential role in long-term modulation of cardiovascular risk.

Discussion

Despite substantial weight regain, the majority of the initial LIWL-induced proteomic changes were maintained five years post-intervention. These findings suggest that early weight loss may induce lasting molecular adaptations in pathways relevant to cardiovascular risk, particularly inflammation. This durable proteomic signature highlights a potential mechanism by which even transient lifestyle changes may exert long-term health benefits beyond weight loss itself.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-09

Dynamic Changes in Cardiac Innate Immune Cell Populations During LPS-Induced Systemic Inflammation

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Introduction:

Endotoxin-induced cardiomyopathy is a complex pathological condition driven by systemic inflammation caused by endotoxins, such as lipopolysaccharides (LPS), typically associated with gram-negative bacterial infections. This condition results in impaired cardiac function and poses significant challenges for patient outcomes in septic shock. While the interplay among bone marrow-derived macrophages (BMDM), tissue-resident macrophages, cardiomyocytes, and neutrophils has been extensively studied in myocardial infarction, the cellular mechanisms underlying endotoxin-induced cardiomyopathy remain poorly understood. Elucidating these mechanisms is essential for developing novel intervention strategies to mitigate endotoxin-induced cardiac dysfunction

Materials and Methods:

LPS (5mg/kg body weight) or phosphate-buffered saline (PBS) was administered intraperitoneally into B57/Bl6 J. After removal the left ventricle was digested and neutrophils, monocytes , BMDMs and tissue resident macrophage populations were isolated and quantified (in % relative to live, CD45+ cells, presented as Mean ± SEM) by flow cytometry 6h, 24h and 48h post PBS or LPS-injection. Statistical significance was assessed using the Kruskal-Wallis test followed by Dunn's post-hoc-test for multiple-comparisons.

Results:

Neutrophils increased from 0.49% in PBS-treated mice to around 3.00% ($p < 0.05$) at 6h post LPS- treatment and remained high throughout the 48h time point. Monocytes gradually increased up to 3.2 fold at 48h post LPS treatment compared to PBS-control mice (0.36% vs. 1.17%). Surprisingly, the percentage of total macrophages remained constant in LPS- treated mice. Whereas the CCR2-, MHCII+ subfraction of tissue resident macrophages was almost completely diminished at 48h post LPS-injection, the fraction of CCR2-, MHCII- macrophages, which are known to support the resolution of inflammation, increased during the same period by 2.5 fold (82.9% vs. 33.9% in PBS-treated mice, $p < 0.01$). Regarding BMDM, the CCR2+, MHCII+ subfraction strongly decreased overtime (0.24% at 48h post LPS injections vs. 12.8% in PBS-treated mice, $p < 0.01$), whereas CCR2+MHCII- macrophages increased (0.46% 48h post LPS injection vs. 1.34% in control treated mice, $p < 0.05$).

Conclusion:

During LPS-induced systemic inflammation cardiac innate immune cell populations undergo dynamic changes, with significant recruitment of neutrophils and monocytes. Shifts in macrophage subpopulations, including the depletion of CCR2-, MHCII+ tissue-resident macrophages and an increase in resolution-supportive CCR2-, MHCII- macrophages will be further characterized by functional and multi-omic-based analyses.

DGKL: 02. Molekulare Labormedizin und Grundlagenwissenschaft und Weiteres

P-07-10

Metabolic Profiling of Type I Interferonopathies to Develop Targeted Decision-Making Tools

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Introduction

Type I interferonopathies are a group of rare genetic disorders characterized by chronic overactivation of the type 1 interferon signaling pathway due to impaired nucleic acid metabolism or sensing, leading to persistent inflammation and tissue damage leading to increased morbidity and mortality early in live.

This project aims to improve our understanding of the link between chronic activation of type I interferon signaling in patients with interferonopathies and its impact on cellular metabolism, laying the foundation for advancing precision medicine through the development of innovative treatment strategies and advanced diagnostic tools.

Materials and Methods

In a cross-sectional study plasma samples from patients with different subtypes of Aicardi-Goutières syndrome (AGS) and age- and sex-matched healthy individuals were analyzed using liquid chromatography coupled with mass spectrometry (LC-MS/MS) to quantify metabolites across various metabolic pathways. The resulting data were analyzed using MetaboAnalyst 6.0, performing principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (oPLS-DA) to explore metabolic differences between groups. The biomarker analysis tool was employed to calculate the area under the curve (AUC) of receiver operating characteristic (ROC) curves for individual metabolites and metabolite ratios, aiming to identify potential biomarkers distinguishing AGS from healthy individuals. Additionally, random forest analyses were applied to construct predictive models capable of accurately classifying metabolomic profiles into healthy and AGS groups.

Results

Distinct metabolic profiles were observed for each AGS subtype, with key metabolites such as spermin (polyamine-pathway), cis-aconitate (citric acid cycle, xanthurenic acid, kynurenine, (both kynurenine pathway) and citrulline (urea cycle) among the top-regulated biomarkers. ROC curve analysis demonstrated strong diagnostic performance, with metabolites such as the citrulline/spermin ratio (AUC: 0.94) or xanthurenic acid (AUC: 0.92) showing high predictive accuracy for identifying patients with AGS Type 7.

Conclusion

The preliminary results emphasize subtype-specific and shared metabolic alterations, offering potential biomarkers for AGS diagnosis and classification. Moreover, these results will be compared with findings from primary fibroblasts to identify potential correlations. Through these planned experiments, we aim to identify new biomarkers and potential therapeutic targets, ultimately striving to improve patients' quality of life and clinical outcomes.

DGKL: 03. Hämostaseologie

P-03-01

The effects of high shear stress on platelets in sepsis patients

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Introduction

Platelets are small, nucleus-free cells that originate from megakaryocytes. In recent years, it has been demonstrated that platelets play a crucial role in immunomodulation in diseases such as acute respiratory distress syndrome and sepsis [1].

Sepsis is currently defined as a life-threatening condition involving organ dysfunction, which results from an abnormal immune response. It is preceded by an infection of bacterial, viral or fungal origin. Serious complications can include disseminated intravascular coagulation or septic shock which can increase the probabilty of mortality [2]. Intensive care measures such as extracorporeal membrane oxygenation (ECMO) are intended to support patients in their recovery.

Methods

This study aims to investigate the impact of high shear stress resulting from intensive care interventions such as ECMO and ventricular assist devices (VADs and Impellas) on platelet functionality and surface receptors in sepsis patients.

The study was approved by the ethics committee of the Heart and Diabetes Centre North Rhine-Westphalia (HDZ NRW) in Bad Oeynhausen (reg. no. 2019-556_2). To be considered suitable for inclusion in the study, patients (aged ≥18 years) had to show an increase of at least 2 points in their SOFA score from baseline, and elevated infection parameters such as PCT had to be present. Additionally, an infection had to be suspected, ideally with positive microbiology results. For this study, 29 sepsis patients were enrolled and compared with an analysis of 31 randomly selected blood donors.

Results

Platelet function was analyzed using light transmission aggrengometry with ten different agonists. In comparison with the healthy control group, functionality was markedly impaired. These restrictions were primarily related to activation by collagen, PAF, arachidonic acid and TRAP-6 and were exacerbated by ECMO and cardiac support systems. Additionally, IPF analysis showed significantly higher abundances in the sepsis cohort and a higher trend in the sub-cohort with ICU measures.

Flow cytometric analysis revealed significant fluctuations in signal intensity within the sepsis cohort, irrespective of the high shear forces induced by ICU measures. This primarily impacted the expression of different platelet receptors. The analysis revealed a slight decrease in CD36 expression and a slight increase in CD42a and CD42b expression in the sepsis group. Notably, there was considerable variation in MFI for CD36 in particular. A significant loss of signal was observed in patients admitted to the ICU between days 7 and 14 suggesting a fundamental biological mechanism.

Conclusion

This study showed that high shear stress influences various platelet receptors and negatively affects platelet functionality. The next step is to verify the results using a larger sample size. Following verification, signalling pathway analyses will be conducted to investigate the alteration in CD36 signal intensity, for example.

DGKL: 03. Hämostaseologie

P-03-02

Recombinant von Willebrand-factor concentrate promotes better platelet adhesion under flow conditions in patients with left ventricular assist devices

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Introduction

Left ventricular assist devices (LVADs) are an established treatment for advanced heart failure and are used as bridge-to-transplant, recovery, or destination therapy. The Heartmate 3® (HM3) is the latest device. Gastrointestinal bleeding (GIB) is a common serious complication associated with LVAD use. It is caused by factors such as reduced pulsatility, arteriovenous malformations, acquired von Willebrand syndrome (aVWS), and anticoagulation. Treating GIB is challenging; VWF/FVIII therapy is possible but rarely used. Our study examined whether LVAD patient platelets adhere to and activate properly on VWF-coated surfaces and among different VWF concentrates.

Methods

We studied 14 patients with HM3 LVAD and 20 healthy donors. Platelet adhesion to VWF was tested using a microfluidic flow chamber. The VWF concentrates examined were Haemate P®, Wilate®, Willfact® (plasma-derived, pdVWF) and recombinant Veyvondi® (rVWF). The chambers were coated overnight and PPACK-anticoagulated blood was pumped at 2000 s^{-1} through the chamber. Adhered platelets were stained and quantified by immunofluorescence, and platelet activation was assessed by flow cytometry.

Results

Our results show that platelets from LVAD patients adhered significantly better to the Veyvondi®-coated surface after one min compared to Haemate P® ($p < 0.05$), Wilate® ($p < 0.01$) and Willfact® ($p < 0.05$). After two min, significantly more platelets adhered to Veyvondi® than to Wilate® ($p < 0.001$) and Willfact® ($p < 0.05$) with no difference to Haemate P®. In healthy controls, no significant differences between the preparations were observed at either time point. Compared to LVAD patients, healthy donor platelets adhered significantly better after 1 min to Haemate P® ($p < 0.01$) and Willfact® ($p < 0.05$), with no differences after 2 min. Flow cytometry analysis revealed significant differences in activation markers (CD62P, CD63, PAC-1), but not in adhesion receptors.

Conclusion

We demonstrated that rVWF leads to better in vitro platelet adhesion than pdVWF. GPIbα (CD42b) is not reduced in HM 3 patients, indicating sufficient VWF binding capacity. Overall, rVWF may be a promising option for bleeding in LVAD patients.

DGKL: 03. Hämostaseologie

P-03-03

Antiphospholipid Antibody Testing in a Maximum Care Hospital - Method-Dependent Differences and Impact on Current Classification

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Introduction: Antiphospholipid antibody (aPL) testing is critical for the classification of antiphospholipid syndrome. The 2023 ACR/EULAR classification criteria recommend the use of enzyme-linked immunosorbent assays (ELISAs) and specific thresholds for aPL positivity. Since non-ELISA methods are increasingly used, we compared and evaluated ELISA and non-ELISA aPL assays in a real-world maximum care hospital setting.

Methods: Between January 2021 and June 2024, anticardiolipin (aCL; IgG and IgM) and anti-beta2 glycoprotein I (aß2GPI; IgG and IgM) antibodies were measured using ELISA (n = 5115) and a chemiluminescence-based automated immunoassay (CLIA) (n = 3820). Results of parallel testing were compared, and associations with clinical and laboratory characteristics were evaluated.

Results: A total of 946 samples were tested using ELISA and CLIA in parallel. A total of 136 (14%) specimens were positive for at least one aPL, and 55 (6%) specimens were from patients diagnosed with APS. Among the latter, 47 (85%) and 41 (75%) patients were positive when ELISA- or CLIA-based aPL assays were used, respectively. After applying the >40 units threshold of the new classification criteria, the number of aPL-positive specimens was significantly lower. In the entire cohort, the agreement between ELISA and CLIA aPL assays was acceptable only for aß2GPI IgG; the results from the two methods did not agree for aCL IgG/IgM and aß2GPI IgM. In APS patients, the agreement between ELISA and CLIA aPL assays was acceptable for aß2GPI IgG and IgM but poor for aCL IgG and IgM. Antibody levels in APS patients were significantly higher using CLIA compared to ELISA.

Conclusion: The method-dependent discrepancies between ELISA- and CLIA-based aPL assays regarding the quantitative and qualitative results are substantial. Both methods are suitable for APS classification, but the choice of aPL assay may influence the classification, and therefore, aPL results should be interpreted carefully in the clinical context.

DGKL: 03. Hämostaseologie

P-03-04

Antiplatelet and anticoagulant (APAC) heparin proteoglycan mimetic restricts thrombo-inflammation and maintains cardiac function following myocardial injury

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Introduction

Myocardial injury (IRI) is hallmarked by thromboinflammation, both thrombotic and inflammatory effects contribute to organ damage. Therapies which not only target coagulation but simultaneously convey cytoprotective effects by dampening inflammatory mechanisms are expected to provide benefits. We speculate that a novel compound APAC, a heparin proteoglycan mimetic, conveying both antiplatelet and anticoagulant (APAC) functions may ameliorate thromboinflammation triggered by myocardial IRI.

Methods

Wild type or Nlrp3A350V mutant mice were either pretreated or post-treated with APAC (0.5mg/kg, intravenously) or PBS (control). Myocardial IRI was induced via LAD ligation (30 min ischemia followed by 24 h of reperfusion).

Results

APAC pre- or post-treatment efficiently reduced infarct size, inhibited coagulation and platelet activation. snRNAseq data revealed that APAC decreased immune cells accumulation (macrophages and natural killer cells) within the hearts and alters peripheral immune cell populations. Congruently APAC markedly reduced Ly6g+ monocytes population and restricted the expression of Nlrp3 inflammasome markers (Nlrp3 and IL-1β) in the blood. Importantly APAC treatment post myocardial IRI likewise provide cardioprotection. The constitutively active Nlrp3A350V mutation abolished the effect of APAC, indicating that NLRP3 suppression is important for APAC-mediated cardioprotection. APAC also alleviated myocardial fibrosis and improved the LV ejection fraction, output, and stroke volume at 28 days after IRI, indicating beneficial cardiac effects beyond the effects on acute tissue injury.

Conclusions

Both APAC pretreatment and posttreatment restricted myocardial IRI, as reflected by the infarct size and NLRP3 inflammasome activation. With multifunctional antithrombotic, anti-inflammatory, and vascular injury-targeting properties, APAC may convey therapeutic benefits in myocardial IRI

DGKL: 03. Hämostaseologie

P-03-05

A loss-of-function point mutation in thrombomodulin gene promotes accelerated aging in mice

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Background: With generally improved health, aging has become the primary risk factor for morbidity and mortality worldwide. Aging is a complex and heterogeneous process, as cellular damage accumulates over time, causing progressive deterioration of organ functions. Vascular aging emerges as a central driver of organ function decline during aging, where endothelial dysfunction initiates and amplifies vascular pathology in different organs. Thrombomodulin (TM), an endothelial glycoprotein, maintains vascular homeostasis by binding thrombin and activating protein C (PC), thereby promoting anti-inflammatory and barrier-protective functions. TM dysfunction, as induced by mutations, aging-related downregulation, or shedding through different proteases, is strongly correlated with organ dysfunction, but the mechanisms are not fully understood. Our aim is to identify the role of TM dysfunction in aging and to elucidate the involved mechanisms.

Methods: We compared wild type (WT) mice to TMPro/Pro mice, that have a point mutation in the thrombin binding domain of TM receptor, resulting in markedly reduced activated PC (aPC) generation, for a total duration of 12 months. Physical function, blood pressure and kidney function were investigated in these mice in addition to histological analyses of the heart and kidneys. An unbiased approach (RNA sequencing) was used to gain mechanistic insights for heart and kidney phenotypes.

Results: TMPro/Pro mice showed reduced physical function, increased body weight and kidney dysfunction (reflected by albuminuria) compared to age matched WT mice. Histological analyses of the heart and kidneys revealed hypertrophy accompanied by fibrosis, inflammation and senescence in TMPro/Pro mice. RNA-seq analysis revealed a large number of differentially expressed genes (DEGs) in hearts and kidneys of TMPro/Pro mice compared to WT mice. Functional annotations of the DEGs revealed downregulation of pathways related to mitochondrial function, ATP generation, and mitochondrial biogenesis and cristae formation in TMPro/Pro mice, reflecting mitochondrial dysfunction and reduced energy supply in both organs. The downregulation of genes involved in cardiolipin (CL; specialized lipid moieties in the mitochondrial cristae) biosynthesis, modification and clearance of oxidised CL pinpoints to abnormalities that may drive the mitochondrial dysfunction phenotype resulting in increased ROS production and organ damage.

Conclusion: Loss of TM function drives abnormalities in CL that contribute to mitochondrial dysfunction. Mitochondrial dysfunction in turn drives abnormalities in energy production in the heart and kidneys of aged TMPro/Pro mice eventually promoting accelerated aging phenotype. These data suggest that endothelial dysfunction with loss of TM-function promotes mitochondrial dysfunction and aging.

DGKL: 03. Hämostaseologie

P-03-06

Towards Accurate Coagulation Diagnostics in Pediatric Patients: Age-Dependent Reference Intervals from a Population-Based Cohort

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Introduction

Reference intervals for coagulation parameters are typically established using adult cohorts, excluding special patient groups such as infants and children. Hemostasis differs significantly during childhood due to maturation of the coagulation system. Therefore, age-specific reference intervals are essential for accurate diagnosis and treatment in pediatric patients. Previous studies using routine clinical samples face limitations like non-standardized pre-analytics and incomplete phenotyping.

Methods

This study is based on the population-based longitudinal LIFE Child cohort study, in which pediatric samples are drawn under standardized conditions during scheduled visits. Citrated plasma samples are collected from individuals aged 3 months to 18 years, aiming to include over 2000 samples in total, with at least 100 healthy subjects per age group. Each study visit is complemented by a structured questionnaire assessing the patient's coagulation history. For neonates and infants under 6 months, surplus clinical routine samples are also analyzed (≥30 per subgroup). Samples are centrifuged and stored at -80°C immediately until analysis. Tests for aPTT, Prothrombin time, fibrinogen, thrombin time, antithrombin, D-dimer, coagulation factors (FVII, FVIII, FIX, FX, FXI, FXII, FXIII), von Willebrand factor activity, Protein C and S, and others are determined on the COBAS t711 coagulation instrument (Roche Diagnostics). Interim evaluations after 6 months assess

data quality and sample size. The results are stratified into 22 age-specific groups: neonates are subdivided into 0–14 days and 15–30 days; infants into 1–3 months, 3–6 months, and 6–12 months. From one year of age up to 18 years, data are analyzed in one-year age intervals. The primary objective of this study is to calculate age-specific non-parametrically reference intervals in order to define 2.5th and 97.5th percentiles with 95% confidence intervals. Sex-specific analyses are planned for later stages.

Results

By July 2025, approximately 35 samples per age group have been analyzed, representing about 35% of the target cohort size. Preliminary analyses revealed clear age-dependent trends in several coagulation parameters, supporting the necessity of age-specific reference intervals. Further, preliminary reference intervals were estimated to determine 2.5th and 97.5th percentiles.

Conclusion

This interim analysis highlights the need for reliable, age-specific pediatric reference intervals for coagulation parameters, based on standardized sample collection and analysis procedures. The interim findings suggest that the final analysis, expected in 2027, will yield robust reference intervals suitable for clinical application.

DGKL: 03. Hämostaseologie

P-03-07

Distinct Platelet and Thrombo-Inflammatory Signatures in Early- vs. Late-Onset Preeclampsia

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Distinct Platelet and Thrombo-Inflammatory Signatures in Early- vs. Late-Onset Preeclampsia

Authors

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Introduction:

Preeclampsia (PE) is a thrombo-inflammatory vascular disorder of pregnancy with limited therapeutic interventions. Based on gestational age at onset, PE is classified into early-onset (EOPE) and late-onset (LOPE) subtypes. While platelet activation and inflammation are recognized contributors to PE pathophysiology, their differential regulation in EOPE versus LOPE remains unclear.

Objective:

To delineate mechanistic differences in platelet activation, inflammatory signaling, and endothelial dysfunction between EOPE and LOPE.

Methods:

Whole blood and plasma samples from EOPE, LOPE, and gestational age-matched normotensive pregnancies were analyzed. Platelet activation markers (CD41, CD62P, αIIbβ3) were assessed via flow cytometry. Circulating levels of IL-1β and sVCAM-1 were quantified as indicators of inflammation and endothelial dysfunction. Transcriptomic analyses included whole blood and placental bulk RNA sequencing, and single-nucleus RNA sequencing (snRNA-seq) of placental tissue.

Results:

Both EOPE and LOPE showed elevated platelet activation relative to controls, with LOPE demonstrating significantly higher levels. IL-1β and sVCAM-1 were increased in both subtypes, but their correlation with platelet activation was exclusive to LOPE. Reanalysis of whole blood bulk RNAseq data revealed distinct expression signatures: EOPE was associated with altered metabolic, immune, and aging pathways, while LOPE showed enrichment of inflammatory and platelet activation pathways. Placental bulk RNAseq indicated dysregulation of angiogenesis, hypoxia, and inflammatory pathways in EOPE; in contrast, LOPE placentae exhibited differential regulation predominantly in KRAS signaling. snRNA-seq further revealed pronounced transcriptomic shifts in trophoblasts and immune cells in EOPE, whereas LOPE showed limited transcriptomic changes in both the cell types. Additionally, both the types of PE, showed dynamic changes in the endothelial cell such as inflammatory and coagulation pathway being enriched in the EOPE, whereas LOPE showed apoptosis, ER stress and mitochondrial damage.

Conclusion:

The data indicate that platelet activation and thrombo-inflammatory responses are mechanistically distinct in EOPE and LOPE. LOPE appears to be driven primarily by maternal factors, whereas EOPE originates from placental pathology with secondary maternal involvement. These insights underscore the need for tailored diagnostic and therapeutic strategies for PE subtypes.

DGKL: 03. Hämostaseologie

P-03-08

sFLT1 overexpression in the placenta promotes placental thrombo-inflammation

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Introduction: Preeclampsia (PE) is a thrombo-inflammatory gestational vascular complication characterized by hypertension, proteinuria, and elevated levels of anti-angiogenic factors, including soluble fms-like tyrosine kinase-1 (sFLT1). PE causes long-term consequences on maternal and child health. The only known treatment is placental delivery. Constitutive in-vivo overexpression of human (hsFLT1) resulted in PE-like phenotype in mice with placental insufficiency and fetal death. PE patients are known to have elevated platelet activation and placental thrombo-inflammation. The expression of sFlt1 and its role in platelet activation and thrombo-inflammation remain ambiguous. Our objective is to evaluate whether sFlt1 overexpression regulates placental thrombo-inflammation.

Methods: Doxycycline-induced systemic hsFLT1 overexpression from 10.5 days post conception (dpc) was employed in hsFLT1/rtTA-transgenic mice. PE heterozygous (hsFLT1+/+; rtTA+/-) and PE wt (hsFLT1+/+; rtTA-/-) foetuses were obtained by breeding hsFLT1/rtTA double transgenic mice. PE homozygous (hsFLT1+/+; rtTA+/+) genotype was lethal. Consequently,

placentas were classified as either hsFLT1-maternal (PE wt, exclusive maternal hsFLT1 overexpression) or hsFLT1-placental (PE het, maternal and feto-placental expression of hsFLT1). Placentae were analyzed using immunoblotting, immunostaining and bulk RNAseq.

Results: Placenta with either hsFLT1-placental and hsFLT1-maternal overexpression showed elevated thrombo-inflammation characterized by increased expression of cleaved IL-1β, cleaved caspase-1, and PAD4 and reduced expression of thrombomodulin. Compared to controls, both hsFLT1 groups showed increased platelet activation (CD62P) and Neutrophil Extracellular Traps (NETs: H3Cit, MPO) within the placenta. Bulk-RNAseq analysis showed enrichment in NETs pathway in maternal hsFlt-1 overexpression, while those of metabolic and diabetes related pathways in hsFLT1-placental overexpression.

Conclusions: These findings indicate that while placental sFlt1 overexpression promotes platelet activation and placental thrombo-inflammation, circulating maternal hsFlt1 expression was sufficient to cause these effects. In hsFLT1-maternal placenta DEGs related to NETs pathway are enriched, suggesting neutrophil-driven thrombo-inflammation. The mechanisms by which placental or maternal sFLT1 overexpression regulates thrombo-inflammation remains under investigation.

DGKL: 03. Hämostaseologie

P-03-09

Semi-automated Multimere Analysis of the Von Willebrand Factor

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The Von Willebrand syndrome (vWS) is a common inherited bleeding disorder that requires a structured and multi-level diagnostic approach. Multimer analysis of von Willebrand factor (vWF) enables the qualitative assessment and classification of von Willebrand syndrome (vWS) subtypes. Complex manual multimer analysis using agarose gel electrophoresis is still widely performed; however, it is technically demanding and lacks standardization.

In this implementation project, we establish a workflow to support the more consistent and practical use of multimer analysis in routine laboratory diagnostics. We apply a commercially available assay kit for semi-automated multimer analysis, which has been approved for in vitro diagnostic use but lacks reference ranges for Germany.

We have collected data from both healthy individuals and patients with confirmed vWS subtypes. Current work involves optimizing gel image evaluation and validating the method according to the CLSI guidelines. For a subpopulation, we have also measured the vWF–collagen binding activity and related it to the results of the multimere analysis.

We present the current state of the implementation, including methodological aspects and observations.

DGKL: 03. Hämostaseologie

P-03-10

Impact of Pneumatic Tube vs Bicycle Courier Transport on Platelet Aggregation: Influence of Sex and Diabetes

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Introduction

Pneumatic tube (PT) transport can impact platelet function, often altering platelet aggregation. Consequently, manual transport is frequently recommended to mitigate preanalytical effects on platelet function tests. However, comparative data between PT and bicycle courier (BC) transport remain limited.

Methods

In this study, two S-Monovette® Citrate syringes of whole blood were collected from 96 participants (43 female, 53 male, median age 63 years). Samples were transported simultaneously by PT or BC to the central laboratory of University Hospital Heidelberg. Platelet function was assessed via light transmission aggregometry (LTA) using five agonists: ADP, arachidonic acid, ristocetin, collagen, and epinephrine.

Results

BC transport showed subtle reductions in platelet aggregation compared to PT for most agonists: ADP (2.5 %, $p = 0.02$), arachidonic acid (1.0 %, $p = 0.006$), ristocetin (2.0 %, $p = 0.003$), and collagen (3.0 %, $p = 0.002$). These differences were more pronounced in women and individuals with diabetes, particularly for collagen-induced aggregation (3.5 %, $p = 0.02$). Epinephrine-induced aggregation was unaffected by transport mode ($p = 0.58$). Women exhibited higher aggregation values overall (arachidonic acid: $p = 0.02$ vs males). Among participants, antiplatelet drugs inhibited aggregation mediated by ADP, arachidonic acid, and epinephrine but did not affect collagen or ristocetin responses.

Conclusion

PT transport caused minor increases in platelet aggregation compared to BC transport, which may not be clinically relevant for most patients but could affect specific subgroups. Increased platelet mechanosensitivity in women and individuals with diabetes may contribute to their elevated cardiovascular risk and merits further study.

DGKL: 04. Hämatologie

P-06-01

Interprofessionelle Zusammenarbeit in der Laboratoriumsmedizin – ein innovativer Mikroskopierkurs für Medizinstudenten und MTL-Schüler

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Zielsetzung: Die zunehmende Komplexität medizinischer Diagnostik erfordert eine enge Zusammenarbeit verschiedener Berufsgruppen. In der Laboratoriumsmedizin müssen Ärzte mit Medizinischen-Technologen für Laboratoriumsanalytik (MTL) eng kooperieren, um eine präzise Diagnosestellung zur optimalen Patientenversorgung zu gewährleisten. Vor diesem Hintergrund wurde ein interprofessionell angelegter Pilot-Kurs mit dem Ziel entwickelt, Medizinstudenten und MTL-Schülern fachliche sowie kommunikative Kompetenzen zu vermitteln, um die interprofessionelle Zusammenarbeit zu fördern.

Methoden: Das didaktische Konzept des zweitägigen, interprofessionellen Kurses (16UE à 45min) basiert auf dem Prinzip des problemorientierten Lernens und kombiniert theoretische Grundlagen mit praktischen Übungen unter Anleitung eines interprofessionellen Dozenten-Teams. Inhaltlich wird der Weg des labormedizinischen Befundes von der Probenentnahme bis zur Befundübermittlung erarbeitet. Im Fokus stehen hierbei Analyse und Management kritischer Schnittstellen in der interdisziplinären Patientenversorgung sowie das Lernen voneinander, miteinander und übereinander. Anhand von praxisbezogenen Fällen der Hämatologie und Zytologie werden Fach- und Methodenkompetenzen zur Mikroskopie, Zytomorphologie und Interpretation von Laborparametern gemeinsam erarbeitet und in den Gesamtkontext des Krankheitsbildes eingeordnet. Hierbei wird ein multimedialer Ansatz verfolgt sowie der interprofessionelle Austausch in Kleingruppen oder Kreisgespräch gefördert. Das Unterrichtsformat wurde mittels online-Fragebogen evaluiert und getrennt nach den Teilnehmergruppen ausgewertet.

Ergebnisse: Das neu entwickelte Lehrkonzept wurde in zwei zeitlich getrennten Durchläufen (07/2023 & 07/2024) evaluiert, der dritte Durchlauf ist für 07/2025 anberaumt. Bis dato nahmen 12 Medizinstudenten im Praktischen Jahr und 10 MTL-Schüler an der Evaluation teil – bei 100% Rücklaufrate. Beide Gruppen gaben an, durch den Kurs die Notwendigkeit und verschiedenen Aspekte der interprofessionellen Zusammenarbeit sehr gut nachvollziehen zu können, sowie über ihre Aufgaben im Team nachgedacht zu haben. Der interprofessionelle Austausch wurde in beiden Gruppen als sehr positiv hervorgehoben. Die Kommunikation in den Kleingruppen war rege und wertschätzend. Die gesamte Veranstaltung wurde von den PJ-Studenten mit 1,1 und MTL-Schülern mit 1,8 bewertet (Skala 1 bis 5) und als besonders wertvoll für die spätere berufliche Tätigkeit angesehen - beide Teilnehmergruppen wünschten sich mehr gemeinsame Unterrichtsformate.

Diskussion und Schlussfolgerung: Im Rahmen der Pilot-Kurse konnten sowohl diagnostische Lehrinhalte vermittelt als auch die Reflexion über die eigene Rolle im interprofessionellen Team angeregt werden. Die Implementierung interprofessioneller Lehrformate kann einen wichtigen Schritt darstellen, um die Kommunikation und Qualität der Zusammenarbeit im Berufsleben zu verbessern.

DGKL: 04. Hämatologie

P-06-02

Evaluation of the hematology digital imaging system Scopio X100HT in a university hospital setting

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Introduction:

In the field of peripheral blood count testing, hematology analyzers are used for automated classification and quantification of peripheral cells, pathological results are verified by manual microscopy as gold stand-ard in cell morphology. To overcome diverse limitations of manual microscopy, digital imaging platforms were introduced for many years now, however, routine application of current instruments is limited based on the limited diagnostic quality in detecting abnormal cells. Scopio X100HT instrument is a newly devel-oped digital imaging platform, introducing full-field computational imaging. The aim of the present study was to evaluate this new technology in a university hospital setting.

Methods:

In a central study element, 400 adult samples - 300 randomly selected routine samples and 100 with pre-defined pathology criteria - were analyzed with Scopio X100HT, quantitative and flagging preclassified and user verified results were compared to Sysmex XN hematology analyzer and manual microscopy as gold standard. Furthermore, a workflow study including 20

routine samples per day over a one week period and comparing turn around times between digital imaging analysis and manual microscopy was performed. Evaluations were performed in accordance with CLSI standards.

Results:

Method comparison evaluations revealed good correlations for normal peripheral blood cells ranging from 0.82 to 0.99. Correlations ranging from 0.94 to 0.97 were found for blasts as standard abnormal cell type. The workflow study revealed an average reduction of hands on time of approximately 26%, when compar-ing user verified digital imaging analysis and manual microscopy.

Conclusion:

The first results of our validation study demonstrate a good performance of Scopio X100HT digital imaging analyzer and indicate potential for routine application in a university hospital setting. The first results of our workflow study, revealing reduced hands on times, support the idea that Scopio X100HT is suitable to increase efficiency in peripheral blood count testing.

DGKL: 04. Hämatologie

P-06-03

Examples for the use of QIP-MS (EXENT®) in gammopathy disease management

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Introduction

In gammopathy disease management (e.g., MGUS or multiple myeloma (MM)), the initial diagnosis often begins with a suspicious serum electrophoresis displaying an M-gradient, followed by immunofixation electrophoresis (IFE), quantification of the free light chains, and bone marrow puncture, which is then followed by sequencing or flow cytometry [1]. In the course of the therapy, this M-gradient should fade away, and the IFE should become inconspicuous. If complete remission is reached, one might look for MRD (minimal residual disease). In some cases, IFE remains positive for IgG-kappa despite a clinically successful therapy [2].

In such cases, the question arises whether this is a truly pathogenic clone (tumor product of an MM) or if this IFE signal is directed against a therapeutic monoclonal antibody (tmAB) [3].

Methods

Serum analysis for gammopathy patient follow-up. For patients from an oncology department, serum samples were analyzed by electrophoresis and IFE. For questionable results – MRD negative in the bone marrow and IFE positive – an attempt for clarification with QIP-MS (EXENT®) was tried.

In the presented examples, these sera are tested for IgG, IgA, and IgM using Optilite with reagents from The Binding Site, allowing for quantitative analysis. In addition, light chains kappa and lambda are analyzed with QIP-MS (EXENT®, The Binding Site).

Results

In both presented cases, we could clearly show the presence of a tmAB (Daratumumab). In one case, two clones were visible via QIP-MS. One displays the tmAB, and the other is (proofed by QIP-MS) the originally pathogenic tumor product.

Conclusion

We demonstrate the utility of the new QIP-MS technology for distinguishing tmAB from pathogenic tumor products in the management of myeloma disease. This capability will become increasingly important as the number of therapies using tmAB continues to increase exponentially. For this reason, the number of small gradients or slight bands visible by serum electrophoresis or IFE will also increase, and it is necessary to distinguish these therapy-related signals from true pathologies with unambiguous certainty.

For optimised patient care, we believe it is better not only to resolve unclear cases retrospectively with reserve samples (these will not always be available), but also to introduce EXENT® analysis as part of the characterisation of a gammopathy in the long term.

DGKL: 04. Hämatologie

P-06-04

The Intensive Care Infection Score (ICIS) as a prognostic biomarker for sepsis and short-term mortality in patients with liver cirrhosis

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Introduction Infections and sepsis are common complications in patients with liver cirrhosis. They frequently require intensive care and are associated with high short-term mortality. The Intensive Care Infection Score (ICIS) was developed to predict sepsis but has not been specifically validated in liver cirrhosis. Therefore, the aim of this study was to prospectively validate ICIS in a cohort of patients with liver cirrhosis and liver-associated sepsis. Methods Patients with confirmed liver cirrhosis from the hepatology unit of the UKB with a decompensation event requiring hospitalization were included. ICIS was measured at baseline (time of admission) and then daily for up to 10 days after admission. The primary endpoint was sepsis-associated mortality. ICIS was measured on XN9000™ (Sysmex) at the central laboratory within a maximum of 30 minutes after blood draw. Results 208 patients were included. 116 patients (56%) were admitted to a general ward (Group A), 37 patients (18%) to the ICU (Group B), and 55 patients (26%) served as control patients. 153 (74%) had at least one type of decompensation, ascites (71%) and infections (43%) being most common. Patients from group B were more decompensated and showed more infections. Within 90 days, 40% of patients in Group A developed sepsis or died, compared to 65% in Group B. Median ICIS at baseline was 2 (0–8) in Group A and 2 (0–12) in Group B ($p < 0.001$). Over the 10-day observation period, ICIS remained consistently higher in Group B compared to Group A, whereas C-reactive protein (CRP) levels lost discriminatory power between the groups after baseline. ICIS, CRP and MELD score were identified as independent predictors of sepsis or mortality. Cut-off values defining a high-risk group were ICIS ≥ 3 ($p = 0.027$) and CRP > 22 mg/L ($p < 0.001$). Group B was further stratified into two subgroups: those who died during their ICU stay (Group B1) and those who survived within 90 days after ICU admission (Group B2). Median CRP levels did not differ significantly at baseline or at any further time point. In contrast, median ICIS was significantly higher in B2 vs. B1 (4 [0–12] vs. 2 [0–7], $p = 0.004$), and this difference remained significant. Both ICIS and MELD were identified as independent predictors of outcome. An ICIS ≥ 5 was determined as the cutoff for identifying high-risk patients ($p = 0.027$). Conclusion Decompensation and hospital admission in patients with liver cirrhosis are associated with high short-term mortality. In non-septic patients, an ICIS ≥ 3 may serve as an early biomarker indicating the need for intensified monitoring and care. In septic patients, an ICIS cutoff of ≥ 5 aligns with findings from non-cirrhotic populations and demonstrated superior predictive performance compared to CRP during the ICU stay. ICIS may thus represent a more sensitive marker to guide therapeutic decisions in critically ill liver cirrhosis patients. Further studies are needed to validate these cutoffs.

DGKL: 05. Onkologie

P-04-13

Comprehensive analytical comparison of two serum-free light chain laboratory assays in a pan-hematologic patient cohort

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Introduction:

Methodological comparison and routine application of laboratory assays for detecting serum-free light chains (FLC) is an important clinical issue in diagnosis and monitoring of plasma cell related disorders, including multiple myeloma. Various laboratory assays serving as screening and diagnostic tools, with each using distinct methodologies and antibody types to measure kappa (κ) and lambda (λ) FLC are available. Their notable quantitative result differences can impact clinical outcomes, making their comparative diagnostical performance an essential area in hematooncological patient care. Furthermore, incorrect measurements may falsely indicate a disease exacerbation or improvement and can seriously impact subsequent clinical decisions.

Methods:

Seven hundred ninety-three serum specimens from three hundred thirty-six hematooncological patients (female: 44%, male: 56%, age: 19-89 years) who routinely received κ and λ FLC measurements are selected for the study. The cohort is grouped into three sub-groups: myeloma patients with one timepoint κ and λ FLC measurement (proportion: ~ 18%, number specimens: n=61), myeloma patients with longitudinal κ and λ FLC measurements (~ 35%, n=404), and non-myeloma hematooncological patients (~ 47%, n=328). Patient samples are analyzed side-by-side by two different immunological FLC assays, Freelite® (The Binding Site) and N Latex (Siemens) applying cobas® 6000 (Roche) and BN II (Siemens) full automated laboratory systems, respectively.

Results:

We will systematically perform the concordance analysis comparing laboratory results for selected routine parameters (κ, λ and κ/λ-ratio) assessed by two different immunological FLC assays. The intra-day and inter-day imprecision for both assays (Freelite®, N Latex) and corresponding laboratory systems (cobas® 6000, BN II) is determined by measuring quality control samples. Clinical disease annotations are selected retrospectively and are considered in combination with laboratory results.

Conclusion:

The clinical validity of well-established laboratory FLC assays is still challenging in routine hematooncological diagnostics. While different immunological FLC assays may demonstrate a high analytical correlation, they could also show unequal results in clinical practice, indicating a notable discrepancy in patient-specific outcomes.

DGKL: 05. Onkologie

P-04-01

Diagnosemuster bei Asthma und COPD: Haupt- und Nebendiagnosen sowie Komorbiditäten im Rahmen einer Real-World-Analyse (CALM-QE).

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Einleitung / Zielsetzung

Asthma bronchiale und chronisch obstruktive Lungenerkrankung (COPD) zählen zu den häufigsten chronischen Atemwegserkrankungen mit hohem Versorgungsbedarf. Beide Erkrankungen treten nicht nur als Hauptdiagnosen, sondern auch als relevante Nebendiagnosen im stationären Setting auf – häufig gemeinsam mit kardiovaskulären, metabolischen oder infektiologischen Komorbiditäten. Ziel dieser retrospektiven Real-World-Analyse im Rahmen des CALM-QE-Projekts war es, Diagnosemuster bei Patient:innen mit Asthma und COPD systematisch zu erfassen. Im Fokus standen sowohl die Häufigkeit als Haupt- und Nebendiagnose als auch die Analyse assoziierter Begleiterkrankungen.

Methoden

Im Rahmen des CALM-QE-Verbundprojekts wurden strukturierte Routinedaten aus der stationären Versorgung am Universitätsklinikum Marburg retrospektiv analysiert. Eingeschlossen wurden vollstationäre Fälle mit kodierten ICD-10-Diagnosen J44.x (COPD) und J45.x (Asthma) im Zeitraum von Januar 2014 - Dezember 2024. Analysiert wurden Häufigkeit, Positionierung als Haupt- oder Nebendiagnose sowie diagnostische Kontexte (z. B. führende Hauptdiagnosen bei Nebendiagnose COPD/Asthma). Darüber hinaus erfolgte eine Erhebung typischer Nebendiagnosen zur Bestimmung relevanter Komorbiditäten.

Ergebnisse

Die Datenauswertung ist zum Zeitpunkt der Abstract-Einreichung noch nicht abgeschlossen. Es wird erwartet, dass COPD vermehrt als Nebendiagnose bei internistischen Hauptdiagnosen dokumentiert ist und Asthma häufiger als Hauptdiagnose erscheint. Typische Komorbiditäten könnten arterielle Hypertonie, Diabetes mellitus und Herzinsuffizienz sein. Diese Hypothesen sollen durch die retrospektive Analyse im Rahmen von CALM-QE geprüft werden.

Diskussion / Schlussfolgerung

Die Analyse von Haupt- und Nebendiagnosen bei Asthma und COPD stellt einen ersten wichtigen Schritt zur Beschreibung realweltlicher Versorgungsmuster dar. Die gewonnenen Erkenntnisse sollen zukünftig die Basis für weiterführende Analysen im CALM-QE-Projekt bilden und dabei helfen, klinisch relevante Subgruppen und Risikoprofile besser zu identifizieren.

DGKL: 05. Onkologie

P-04-03

Automating High Molecular Weight DNA Extraction in Clinical Genetics: Evaluation of the chemagic™ 360 Instrument in a Hospital Lab for Routine Genetic Testing

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High-quality nucleic acid extraction is fundamental to reliable molecular testing in clinical research laboratories. With the increasing use of long read sequencing and a growing demand for genetic testing, laboratories are faced with the challenge of obtaining high quality High Molecular Weight (HMW) DNA in a reliable manner at scalable throughputs.

In 2015, the European Georges Pompidou Hospital integrated the chemagic 360 instrument from Revvity into their routine molecular testing workflow to address the need for high-throughput, high-quality, and automation-compatible DNA extraction from whole blood.

Over eight years of continuous use, the system's performance was evaluated across more than 30,000 extractions, focusing on yield, purity, integrity, and robustness. DNA extracted routinely demonstrated A260/A280 ratios between 1.8–2.0, minimal fragmentation, and was consistently double-stranded, meeting quality thresholds for complex applications including CRISPR-Cas9–based enrichment, nanopore sequencing, and methylation analysis. Here, data from reproducibility testing, cross-contamination controls, and downstream assay performance (e.g., NGS, MLPA and Sanger Sequencing) will be showcased.

The system's gentle separation technology using proprietary M-PVA Magnetic Beads with optimized buffer dispensing enabled fast isolation of high integrity DNA with significantly reduced failures, removing the requirement for Nanodrop quantification. The output files from the chemagic 360 system were also easily integrated into the Laboratory Information System (LIS), creating a traceable workflow fulfilling regulatory requirements.

These data support the chemagic 360 instrument as a robust and scalable platform for clinical research labs requiring high-quality HMW DNA extraction, enabling expanded analysis capabilities, and preparing for future transitions to long-read testing.

DGKL: 05. Onkologie

P-04-04

MARCKS-independent effects of MARCKS N-terminal sequence-derived inhibitor peptides

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Introduction

Myristoylated alanine-rich C kinase substrate (MARCKS) is a ubiquitously expressed, unstructured protein with a myristoylated N-terminal domain and a lysine-rich effector domain (ED). Via the myristoyl group and the ED, unphosphorylated MARCKS associates with the membrane, while ED phosphorylation induces its translocation to the cytosol. MARCKS is

involved in numerous molecular mechanisms and cellular processes including cytoskeletal rearrangements, signal transduction/gene expression, adhesion/migration, and reactive oxygen species (ROS) production. Since alterations of MARCKS levels and function are associated with various diseases, MARCKS is a promising target for pharmacological intervention. To control MARCKS' function, inhibitor peptides have been developed, e.g., the myristoylated N-terminal sequence (MANS, reflecting the first 24 amino acids (aa) of MARCKS) and its shorter derivative BIO-11006, comprising aa 1-10.

Methods/Results

In this study, we applied MANS and BIO-11006 to test whether MARCKS inhibition via these peptides is able to mimic the suppressive effect on monocytic ROS production exerted by MARCKS deficiency recently demonstrated by our group. In monocytic THP-1 and PLB-985 cells as well as primary human monocytes, MANS significantly decreased total ROS production induced by various stimuli (opsonized E. coli, S. aureus, zymosan, and PMA), while BIO-11006 predominantly affected PMA-induced ROS levels. TNF preincubation enhanced monocytic ROS production, but was not able to compensate for MANS treatment or MARCKS deficiency. Unexpectedly, an inhibition of ROS formation by both peptides could also be observed in MARCKS KO cells, indicating a target-independent effect. Comparable negative effects of MANS in both WT and KO cells could also be observed when transmigration was assessed.

Conclusion

Our data indicate that MARCKS inhibitor peptides MANS and (to a lesser extent) BIO 11006 are able to inhibit MARCKS-associated cellular processes by MARCKS-independent mechanisms.

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P-04-05

Sonderfall in der Zöliakie-Diagnostik: Nachweis von Endomysium- bzw. Transglutaminase-IgA-Ak trotz selektivem IgA-Mangel (sIgAD)

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Einleitung: Die Zöliakie, eine Gluten-abhängige Autoimmunerkrankung, manifestiert sich in erster Linie an der Darmschleimhaut. Anders als bei den meisten anderen Autoimmunerkrankungen sind deshalb Autoantikörper vom IgA-, nicht IgG-Typ diagnostisch relevant, im Speziellen IgA-Antikörper gegen die Gewebstransglutaminase (TG-IgA), bzw. bei Untersuchung im IFT Endomysium-IgA-Ak. Bei nachweisbarem Gesamt-IgA sollen Ak der IgG-Klasse nicht untersucht werden, da sie im Vergleich zu den IgA-Ak eine zu geringe diagnostische Sensitivität besitzen und zudem auch häufiger unspezifisch reagieren.

Der selektive IgA-Mangel (sIgAD) ist der häufigste angeborene Immundefekt, definiert als Serum-IgA < 0.06 g/l bei unauffälligen IgG- und IgM-Konzentrationen und fehlendem T-Zell-Defekt. Ein sIgAD wird erst ab einem Alter von 4 Jahren diagnostiziert, da eine verzögerte IgA-Ausreifung nicht selten ist. Das sekretorische IgA (sIgA) wird bei der Diagnose nicht berücksichtigt

Aufgrund einer HLA-Assoziation treten Zöliakie und sIgAD häufiger gemeinsam auf. Wenn aufgrund eines sIgAD kein IgA gebildet werden kann, switcht der Körper bei der Autoantikörper-Bildung ersatzweise auf den IgG-Typ, so dass im Falle eines sIgAD zusätzlich TG-IgG- bzw. Endomysium-IgG-Ak untersucht werden müssen.

Fallbeschreibung: In einigen Fällen konnten wir im Rahmen der Zöliakie-Diagnostik erhöhte TG- bzw. Endomysium-IgA-Ak nachweisen, obwohl im weiteren Verlauf ein sIgAD diagnostiziert wurde. In allen Fällen ließ sich sekretorisches IgA nachweisen, welches bei der Definition der sIgAD nicht berücksichtigt wird. Z.T. konnte während der floriden Phase der

Zöliakie transient auch IgA im Serum nachgewiesen werden, was jedoch unter glutenfreier Diät parallel zu den TG-IgA-Ak wieder unter die Nachweisgrenze abfiel. Bisher haben wir dieses Phänomen nur bei Kindern beobachtet. In einem Fall ließ sich in der Langzeitbeobachtung bis zum Erwachsenenalter eine spontane Normalisierung des Serum-IgAs beobachten.

Diskussion: Der Nachweis von sIgA bei diesen Patienten zeigt, dass ihnen prinzipiell die Bildung von IgA-Ak möglich ist. Es erklärt, warum bei diesen Patienten trotz des Serum-IgA-Mangels TG- bzw. Endomysium-IgA-Ak nachweisbar war. Ob der Nachweis von sIgA der Diagnose eines sIgAD grundsätzlich widerspricht oder womöglich eine Subgruppe des sIgAD darstellt oder aber als ein Hinweis auf eine stark verzögerte Ausreifung der IgA-Bildung zu werten ist, bleibt in zukünftigen Studien zu klären. In mindestens einem unserer Fälle wurde die Diagnose eines sIgAD jedoch zu früh gestellt.

Fazit: Die Fälle unterstützen die Zöliakie-Leitlinien-Empfehlung, grundsätzlich mit Gesamt-IgA und IgA-Ak-Diagnostik zu starten und eine IgG-Diagnostik bei Bedarf nur ergänzend durchzuführen.

Eine IgA-Ausreifung kann sehr stark verzögert sein, die Diagnose eines sIgAD sollte womöglich erst im Erwachsenenalter gestellt werden.

Es sollte die Einbeziehung des sekretorischen IgAs zur Diagnostik des sIgAD erwogen werden

DGKL: 05. Onkologie

P-04-06

Sustainability Challenges and Opportunities of SARS-CoV-2 Antigen Rapid Detection Tests in the COVID-19 Pandemic – a life cycle assessment

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Introduction

The widespread use of SARS-CoV-2 antigen rapid detection tests (RDTs) has been instrumental in managing the COVID-19 pandemic by enabling large-scale point-of-care testing. However, their unprecedented global deployment has resulted in significant resource consumption for production, transport, and large volumes of waste. Despite their importance, the ecological sustainability of RDTs - critical for future use in detecting other respiratory pathogens - remains unexplored. This study evaluates the carbon footprint of RDTs through a life cycle assessment (LCA), analysing production, transport scenarios, and the overall CO_2 impact during the COVID-19 pandemic in Germany.

Methods

The study applies the processes production and transport of an LCA framework based on ISO 14040/44 standards. RDT components, including packaging, were separated by presumable material, weighed, and analysed to calculate CO_2-equivalent (CO_2-eq) emissions. A MEDsan® SARS-CoV-2 Antigen Rapid Test (MEDsan GmbH, Hamburg, Germany) was used to measure component weights. CO_2-eq values for raw materials were sourced from the German Federal Office of Economics and Export Control,(1) while transport emissions were based on reference data from the UK Department for Energy Security and Net Zero.(2)

Results

A single RDT including packaging amounts 16.0g CO_2-eq, with the largest contributor being the test cassette plastic. Transport emissions vary considerably depending on the production location and shipping method: production in Germany results in an additional 0.5g CO_2-eq for a 500km transport, while production in China leads to 191.0g CO_2-eq when shipped by air, or to 2.8g CO_2-eq when transported sea freight.

During the COVID-19 pandemic, 756 million RDTs were billed by health insurance companies in Germany.(3) These RDTs generated a total of 12,116.0 tons CO_2-eq. Depending on the production and transport scenarios, emissions would increase by an additional 342.1 tons CO_2-eq for German production to 144,361.6 tons CO_2-eq for Chinese production with air transport.

At the University Hospital Würzburg 1,925,000 RDTs were used during the COVID-19 pandemic, corresponding to 30.9 tons CO_2-eq for the RDTs themselves. Since almost exclusively German-produced RDTs were ordered, an additional 0.9 tons CO_2-eq were generated due to production. If only RDTs produced in China had been sourced, this would have resulted in 5.4 (sea) / 367.6 (air) additional tons. Thus, 4.5 (sea) / 366.7 tons of CO_2-eq were saved.

Conclusion

While RDTs have been crucial in combating COVID-19 pandemic, their long-term sustainability is essential for future public health crises. By prioritizing performance, affordability, and environmental responsibility, RDTs can become a model for a sustainable medical innovation. Integrating RDT LCAs and waste management into pandemic preparedness plans will minimize environmental impact, positioning RDTs as a cornerstone of eco-friendly healthcare.

DGKL: 05. Onkologie

P-04-07

Amino Acid Trajectories in the Early Phase Following Intracranial Hemorrhage: An Observational Study Using Nuclear Magnetic Resonance Spectroscopy

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Introduction

Critically ill neurological patients often exhibit complex metabolic alterations during the early phase of intensive care. While energy metabolism and macronutrient regulation have been widely studied, little is known about how individual amino acid concentrations behave in the first days after a sudden-onset critical illness such as acute intracranial hemorrhage. This study aimed to characterize the dynamic changes in circulating free amino acids during the first 72 hours of critical care.

Methods

A prospective, monocentric observational study was conducted in which 24 patients with severe acute neurological or neurosurgical conditions (subarachnoid hemorrhage [SAH] grades 4–5, intracerebral or subdural hemorrhage, or traumatic brain injury with GCS < 9) were included. Over the first three days of intensive care, 2 ml of arterial blood samples were collected every eight hours. After centrifugation, the concentrations of alanine, glutamine, glycine, histidine, leucine, and valine were quantified using a 600 MHz NMR and the Plasma/Serum B.I.Quant-PS methods from Bruker BioSpin GmbH. We applied a repeated measures analysis of variance to evaluate possible alterations in amino acid concentrations.

Results

No significant changes were observed for five of the six analyzed amino acids (alanine, glutamine, histidine, leucine, and valine) during the study period. Only glycine, which also acts as an inhibitory neurotransmitter, showed a transient but statistically significant increase approximately 36 hours after admission ($F(3.7;84.8) = 2.6$; $p = 0.05$). This isolated pattern may indicate a specific neuronal or glial response following acute cerebral injury.

Conclusion

The data suggest a remarkable stability in free amino acid levels during the early stages after intracranial hemorrhage, with glycine being the only exception, showing a transient increase. Larger-scale studies (e.g. such as Schefold et al. 2019)

including broader and stratified patient populations are needed to elucidate metabolic regulatory mechanisms in neurotraumatized patients.

DGKL: 05. Onkologie

P-04-08

Isoelectric Focusing as an Alternative to the Hydrashift Assay: Differentiation of Therapeutic Monoclonal Antibodies and Paraproteins

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Introduction

An increasing number of diseases are treated with therapeutic monoclonal antibodies (tmAbs). These antibodies can be misinterpreted as paraproteins in serum immunofixation electrophoresis (IF). It has been demonstrated that anti-tmAb antibodies can be used to circumvent this challenge. A few years ago, Sebia introduced the Hydrashift assay, an anti-tmAb antibody-based commercially available product for the elimination of IF-interference by daratumumab (DmAb) or isatuximab (ImAb). However, usage of this assay is limited to these two antibodies and is only available for the Sebia platform. Furthermore, we observed a few cases with questionable shifting of tmAbs by the Hydrashift assay. Therefore, we aimed to evaluate isoelectric focusing (IEF) as a common and widely applicable alternative for clinical laboratories.

Methods

We collected samples of commonly used tmAbs in the university hospital of Marburg and analysed these antibodies with IEF to assess antibody-specific charge variant patterns. Subsequently, the neutralization capacity of the DmAb and ImAb Hydrashift assay were assessed. Clinically relevant concentrations of DmAb and ImAb were spiked into paraprotein-positive samples and analysed by IEF and the Hydrashift assay. Potential unshifted tmAb and identification of tmAb and paraproteins was evaluated in these and routinely collected patient samples.

Results

Up to 0.5 g/L DmAb and 1.5 g/L ImAb were shifted reliably by the Hydrashift assay. IEF and the Hydrashift assay were equally reliable for the identification of antibody interferences and paraprotein detection. In addition, IEF identified two pseudomonoclonal bands and other tmAbs besides DmAb and ImAb.

Conclusion

We did not observed insufficient tmAb neutralisation within the clinically relevant range of antibody concentration. In addition, we demonstrated that IEF is a simple and flexible alternative to the Hydrashift assay that can be used for the identification of tmAb-interference during paraprotein detection.

DGKL: 05. Onkologie

P-04-09

Application of a fast and reliable cardiolipin focused LC-MS/MS method to murine heart tissue and microglia cell lysates

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Introduction. Cardiolipins (CL) are a phospholipid (PL) group with four fatty acyl substituents, enriched in mitochondria. The multitude of possible fatty acyl chain combinations can lead to significant isobaric overlap, complicating identification and quantitation. Additionally, CL have a 100- to 1000- fold lower abundance compared to other PL classes. Their separation from other PL classes is therefore a requirement for method development. As these two challenges have not been overcome in a fast enough method yet, large cohort studies to take advantage of the specificity of CL as a biomarker candidate are to date missing from the literature. In this work, we present a rapid HILIC method capable of robust and reliable CL separation and its successful application to two different sample types, murine heart tissue and ultracentrifugation fractions (UCF) of microglia cell lysates.

Methods. A standard mixture of 7 CL standards (50 ng/ml each) and the SPLASH Lipidomix (diluted 1:180, all: Avanti Polar Lipids, USA) in eluent B (97/3 v/v ACN/ aqueous ammonia acetate (AA) buffer (pH 5.8, 15 mM)) was used for method development. 1 mg aliquots of murine heart tissue C57BL/6 mice were extracted with n-hexane/iso-propanol (IPA) 60/40 v/v [1]. 50 ul aliquots of ultracentrifugation factions (UCF) from SIM-A9 microglia cells were prepared by protein precipitation with IPA [2]. A Nexera HPLC system (Shimadzu, JP) carried out the 8.5 minute long gradient program on a zwitterionic SeQuant ZIC HILIC (50 x 2.1 mm, 3.5 µm, Merck, DE) with eluents A (97/3 v/v aq. AA buffer (pH 5.8, 15 mM)/ ACN) and B. MS/MS acquisition was achieved on a QTRAP 6500 mass spectrometer (SCIEX, CA).

Results. Rapid head group separation of 10 PL classes including CL was developed, where CL were baseline separated from all tested PL groups except phosphatidic acid, serins and inositols. CL species had a reproducible retention time of 2.5 ± 0.01 minutes, with the other PL classes eluting between 0.9 minutes (glycerols) and 3.4 minutes (lyso-cholines). Intraday repeatability of the MRM transition peak areas for CLs ranged from 1% (CL (14:1)4) to 6% (various CL) and from 1% (inositol) to 26% (sphingomyelin) for the other PLs. Application of the method to murine heart tissue revealed the anticipated CL (18:2)4 as the main CL. PL classes showed distinctive fraction-dependent patterns in ultracentrifugally separated cell lysates. Comparing heart tissue to the UCF, enrichment of CL in either sample type was species dependent. Serines and inositols were enriched in the UCF, whereas ethanolamines and cholines had comparable intensities between the sample types.

Conclusion. A rapid and robust HILIC separation of PL classes was presented and applied. In the next steps, further sample matrices such as plasma are to be tested, as well as additional validation data is to be measured. To conclude, the rapidness of the HILIC separation is the key to unlocking large cohort studies of CL in the future.

DGKL: 05. Onkologie

P-04-11

IgE Sensitization Patterns Among Adult Asthmatic Patients in rural and urban Kilimanjaro region, Tanzania

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With rising asthma prevalence alongside urbanization in sub-Saharan Africa (SSA), understanding allergen sensitization patterns is increasingly important. This study investigated IgE sensitization profiles in 76 Tanzanian adults from urban Moshi and rural Siha districts at the Kilimanjaro region, including 38 asthmatic patients (21 urban, 17 rural) and 38 non-asthmatic controls (21 urban, 17 rural).

Sensitization patterns were assessed using the ALEX^2 multiplex assay, which measures IgE levels to 300 allergen extracts and molecular components. This microarray covers aeroallergens (mites, pollens, animal dander, moulds) and food allergens (cereals, milk/egg, fruits, nuts, seafood, meat, vegetables, spices).

Urban asthmatics exhibited the highest rates of polysensitization, particularly to house dust mites Der p 23 (12/21, 57%) and Der p 1 / Der f 1 (10/21, 47.6%)—followed by Blomia tropicalis (5/21, 23.8%) and cat dander Fel d 1 (6/21, 28.5%). Urban asthmatics showed the highest sensitization to tropomyosin, arginine kinase, NPC2 and mite group 5 & 21 families among all four groups. Sensitization levels were significantly lower in both urban and rural controls, and differences in sensitization to tropomyosin were statistically significant across all group comparisons ($p < 0.0001$).

Rural asthmatics showed lower rates but were more frequently sensitized than controls, especially to meat allergen Alpha-Gal 3/17 (17.6%) and horse allergen Equ c 4 2/17 (11.7%). Urban asthmatics also showed notable sensitization to seafood allergens (e.g., Pen m 1, Mac r 1, Hom g) in 5/21 (23.8%) and to meat proteins (e.g., Loc m, Ten m, Ach d) in 7/21 (33.3%). Sensitization to tree and weed pollens was generally low across all groups.

These findings highlight distinct IgE sensitization patterns influenced by the environmental exposure and disease status. Urban life is associated with a higher frequency and diversity of sensitization, emphasizing the need for context-specific allergy diagnostic. Future steps will explore links between sensitization patterns, asthma severity, and environmental exposures in urban and rural SSA settings.

DGKL: 05. Onkologie

P-04-10

Etablierung von ICP-MS in der Routinediagnostik zur Bestimmung von Zink, Kupfer und Selen in den Matrices Urin und Plasma/Serum

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Einleitung: Die induktiv gekoppelte Plasma-Massenspektrometrie (ICP-MS) hat sich aufgrund ihrer Sensitivität, technischen Vielseitigkeit und Genauigkeit als eine bevorzugte Methode zur Quantifizierung von Spurenelementen etabliert. Im Vergleich zur klassischen Flammenabsorptionstechnik (AAS) bietet ICP-MS zahlreiche Vorteile, insbesondere in der Analyse von niedrigen Konzentrationen einer breiten Elementpalette, einschließlich schwerer Metalle und toxischer Substanze, sowie der Möglichkeit von Multielementanalysen während einer Einzelmessung.

Methoden: ICP-MS wurde als in-house Methode zur Detektion von Zink, Kupfer und Selen in den Matrices Plasma und Serum validiert. Zusätzlich erfolgte eine Validierung von Kupfer in Urin-Matrix. Die technische Umsetzung erfolgte mittels des Geräts iCAP-TQ (Fa. ThermoFisher) in den Messmodi M-SQ-KED, M-TQ-O2. Als interner Probenstandard wurde Germanium gewählt. Die Methodenkalibration erfolgte mehrstufig einschließlich der Messung von Qualitätskontrollen (QK).

Ergebnisse: Es wurden 100 Single-Donor-Proben und 40 Ringversuchsproben, 13 QK-Intervalle und 10 hochreine Reagenzien zur Methodenetablierung eingesetzt. Die QK-Präzision lag zwischen 0,038 - 4,442% (innerhalb der Messserie) und 0,004 - 3,277% (zwischen einzelnen Messserien). Die Richtigkeit innerhalb einer Probenmessung lag bei 0,338 - 2,751% und zwischen einzelnen Probenmessungen 0,750 - 3,157%. Dabei wurde verschiedene Probengefäße/-matrices sowie die Einflüsse verschiedener Probenzusätze (Detergens, Aminosäuren, Chelatoren) berücksichtigt. Eine besondere Herausforderung stellte die Entwicklung eines Vorgehens zum Ausschluss von potentiellen Matrixeffekten dar. In der Routine konnten seit Etablierung im Durchschnitt monatlich 834 Serum/Plasma-Proben und 51 Urin-Proben gemessen werden.

Diskussion und Schlussfolgerung: Die hohe Empfindlichkeit von ICP-MS ist vorteilhaft in der Bestimmung von Spurenelementen in biologischen Proben, wo die Konzentrationen der zu analysierenden Elemente oft im Bereich von

Nanogramm bis Pikogramm pro Liter liegen. Neben der Nutzung von speziellen Messmodi zur Eliminierung von Interferenzen mittels einer Kollisionszelle, ist die gleichzeitige Multielementanalyse ein weiterer wesentlicher Vorteil der ICP-MS. Die Etablierung der ICP-MS erfordert eine Vielzahl an technischen Voraussetzungen, als auch qualifiziertes Personal, kann aber die Turnaround-Time gegenüber den herkömmlichen Methoden wie die AAS deutlich reduzieren und wirtschaftlich betrieben werden. Die methodische Weiterentwicklung und Verfeinerung dieser Technologie wird künftig zu noch präziserer und effizienterer Diagnostik von Spurenelementen und einer damit verbundenen verbesserten Patientenversorgung führen.

DGKL: 05. Onkologie

P-04-12

Effects of hypoxia training on stress parameters

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Introduction: Physical fitness influences the processing of stress, particularly on a psychological level. It is now technically possible to conduct hypoxia training at sea level in special rooms through the addition of nitrogen, which enhances the training effect. So, this study investigates the influence of hypoxia training on various stress parameters.

Participants and methods: For this purpose, 20 subjects were divided into a control group (n = 6) and a hypoxia group (n = 14). Both groups completed an approximately five-week training programme, the control group under normoxic conditions, the hypoxia group in corresponding rooms in simulated high-altitude air. As parameters cortisol levels in saliva were measured during initial, intermediate and final measurements, and questionnaires on subjective stress perception were completed.

Results: In the hypoxia group, data showed a tendency towards improvements in the stress index and a reduction in the subjective assessment of stress. In the control group, on the other hand, there were no significant changes in measured values, only in the subjective stress assessment. The training showed no clear alterations of cortisol levels in saliva.

Conclusion: Cortisol levels in both groups (normoxia and hypoxia) were already decreased at the beginning of the training programme in comparison to a normal range, indicating a good physical fitness. Overall, it can be suggested that hypoxia training could have potential positive effects on stress parameters.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-01

Comparison of artificial intelligence algorithms on numerical databases to develop screening strategies from routine hospital results.

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Introduction

Machine learning (ML) has established its utility for disease prognosis and prediction by enabling the analysis of large medical data sets to uncover subtle patterns that support rapid diagnosis or disease exclusion.

Methods

In this study, we developed and tested different ML-algorithms based on routine laboratory data for the diagnosis of cancer-associated venous thromboembolism (VTE). The study cohort includes 1,223 patients admitted between 2010 and 2023 encompassing a wide range of malignant tumor types. Key parameters were selected using statistical and visual analyses to ensure relevance and to minimize bias. Data preparation involved multiple steps including handling missing values, applying transformations to correct asymmetries, normalizing scales and balancing the dataset with techniques such as ADASYN and SMOTEENN. We evaluated various ML models, including logistic regression (LR), support vector machines (SVM), random forest (RF), XGBoost (XGB) and a neural network (NN) utilizing Python libraries.

Results

XGB and ensemble model combinations demonstrated the best performance, with AUC scores of 0.96 and 0.95, respectively. Followed by RF (AUC of 0.93) and NN (AUC of 0.90).

Conclusion

These results highlight the potential of tree-based models, particularly XGB and RF to analyze laboratory data and classification. In this way, it is feasible to increase the prevalence of uncommon conditions to be investigated leading to more successful screening procedures.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-02

Development and Validation of Big Data-driven Digital Strategies for Precision Laboratory Diagnostics in Neurological Setting

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Introduction:

Precision laboratory diagnostics call for integration and joint assessment of large-scale heterogeneous datasets - clinical, radiological and laboratory - to improve diagnostic and therapeutic accuracy by developing tailored diagnostic algorithms and predictive scores. Applying machine learning and advanced statistical methods, laboratory clinicians have an excellent opportunity to analyze the laboratory data in various clinical settings. In general, digital approaches for precision laboratory diagnostics have the potential to optimize patient stratification, identify disease-specific signatures, predict disease progression and improve therapy workflows. Due to their clinical urgency as well as diagnostic complexity, neurological disorders are ideal models for development and validation of big data-driven digital diagnostic strategies.

Methods:

Our study aims to provide a holistic digital laboratory procedure for clinical routine diagnostics. We focused on neurological disease spectra and analyzed clinical and laboratory data using several machine learning approaches. Gradient-boosted trees, random forests and regularized logistic regression models were trained to predict each relevant disease category based on the input features.

Results:

We retrospectively screened all inpatient encounters at a tertiary center from 2013 to 2023, identifying >300 000 cases with neurological ICD-10 codes. Encounters were grouped into eight overarching disease categories (29 entities). For each patient we extracted age, sex and >100 routine laboratory parameters. Depending on the specimen matrix used and the number of laboratory parameters tested, the AUROC values (area under the receiver operating characteristic) for each superordinate clinical category were calculated ranging from 0.77 to 0.92.

Conclusion:

Our big data-driven machine learning approach has potential to efficiently analyze large amounts of laboratory data, recognize neurological disease patterns and support their clinical diagnoses. By integrating our classifier models into routine laboratory information system, combining them with radiological records and prospective clinical validation, we propose that a personalized, faster and more reliable cost-effective patient care is possible.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-03

Machine learning-based thalassemia prediction – Model development and over one year experience from routine diagnostics

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Introduction

Thalassemias are among the most common inherited blood disorders worldwide, and early identification of carriers is important for genetic counseling of couples at risk. The present study describes the development of a machine learning-based prediction model and shows results from its application in clinical routine diagnostics.

Methods

Hemoglobinopathy test results from 18,848 individuals were retrospectively (2013-2023) extracted from the database of Fürst Medical Laboratory, Norway. An eXtreme Gradient Boosting (XGB) model was trained to identify positive thalassemia cases using 11 parameters, including gender, age, erythrocyte indices and clinical chemistry results. After validation, hyper-parameter tuning and evaluation of the results by hematology and AI experts, the algorithm was put into production to evaluate all incoming samples to the clinical routine laboratory Fürst. Positive prediction of thalassemia resulted in an active recommendation to the requesting physician for further investigation, negative thalassemia screening results were not communicated. Validation of the algorithm was also performed on an external dataset (1,178 samples) compiled at MLL Munich Leukemia Laboratory, Germany.

Results

On the total training set from Fürst, the algorithm showed the following performance metrics in predicting the presence of thalassemia: Sensitivity 0.94, specificity 0.91, accuracy 0.91, and F1-score 0.90.

Two months after start in production (03/2024) at Fürst, prediction performance was re-evaluated on data unknown to the model comprising 2,048 samples (10/2023-05/2024): Sensitivity 0.88, specificity 0.81, accuracy 0.80, F1-score 0.79.

Implementing feedback from requesting physicians, the prediction threshold of the model was adjusted from 0.5 to 0.7 to increase specificity and precision. In the following seven months (06/2024-12/2024), the model evaluated more than 700,000 samples and predicted thalassemia in 6,657 cases. Of these samples, further thalassemia testing was requested and performed in 783 cases, and a diagnosis of thalassemia confirmed in 84% (n=657). In the same period, the laboratory received 1,574 samples for hemoglobinopathy testing with no previous recommendation for thalassemia testing (i.e. no or a negative previous prediction from the algorithm). Of these samples, only 36% (n=566) were tested positive for thalassemia.

External validation of the trained model on the MLL dataset resulted in a sensitivity of 0.88, a specificity of 0.67, an accuracy of 0.80, and an F1-score of 0.83, respectively.

Conclusions

Machine learning based prediction of thalassemia is a feasible and effective approach for identifying thalassemia patients in high-throughput routine medical laboratories.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-04

Research on the Application of cfMeDIP-Seq in Psychotic Disorders

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Research on the Application of cfMeDIP-Seq in Psychotic Disorders

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Cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq) is an emerging technology that enables genome-wide methylation profiling of low-input cfDNA samples without the need for bisulfite conversion. This method offers several advantages, including high sensitivity, cost-effectiveness, and significantly reduced DNA degradation. While cfMeDIP-seq has already shown great potential in oncology—particularly in early cancer detection—its application in psychotic disorders remains unprecedented.

In this study, we apply cfMeDIP-seq for the first time to analyze cfDNA from the cerebrospinal fluid (CSF) of patients with schizophrenia and Alzheimer's disease. Traditional methylation studies in psychiatric disorders have primarily relied on blood samples or post-mortem brain tissues, which fail to accurately reflect the dynamic epigenetic landscape of the central nervous system in living patients. In contrast, cfDNA from CSF offers a minimally invasive and more specific window into the pathological processes occurring in the central nervous system. We aim to identify disease-associated differentially methylated regions (DMRs). In a first step, we will focus on known risk genes associated with schizophrenia and Alzheimer's

disease while also conducting hypothesis-free genome-wide screening to discover novel epigenetic biomarkers. Based on the results, we will train machine learning models for disease prediction and classification.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-05

Using Large Language Models for Therapeutic Drug Monitoring Reporting – a Proof-of-Concept

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Therapeutic Drug Monitoring (TDM) is an indispensable tool in the adjustment of patients to medication, the intake of which should be monitored regularly. TDM is becoming increasingly popular, especially in the field of psychopharmacotherapy. In addition, it becomes helpful if the desired therapeutic effect is not achieved with standard dosages and possible drug interactions with the often-extensive co-medication needs to be further clarified. Writing TDM reports often takes a lot of time, as there are currently no tools that can bundle information from the numerous information silos, like laboratory information system, interaction databases and drug information.

We developed an LLM-based tool which produces a structured TDM report based on input patient information. In order to provide trustworthy and traceable medical reporting, this tool is additionally equipped with a broad repository of substance-substance interactions and detailed drug-related data. In doing so, it follows the paradigm of Retrievel Augmented Generation (RAG). The large language models GPT4.1 and Gemini 2.5 serve as the basis for text generation. We evaluate these first proof-of-concept TDM reports using the CLEAR evaluation framework, which focuses on completeness, absence of misinformation, evidence, appropriateness, and relevance, compare the results of both models, and discuss the merits and limitations of the current tool.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-06

EU AI Act: Was bedeutet KI-Kompetenz für das medizinische Labor?

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Seit August 2024 ist der EU AI Act in Kraft getreten. Die verschiedenen Teile und Artikel werden in den nächsten Jahren Gültigkeit erlangen. Seit dem 02.02.2025 sind nun auch die Betreiber von KI-Lösungen dazu verpflichtet, ihre Mitarbeiter:innen nach Artikel 4 EU AI Act in Hinblick zur Erlangung einer „KI-Kompetenz“ zu schulen. Die im April 2021 gegründete AG Digitale Kompetenz der Sektion Junges Labor, die im Mai 2025 den Weg in eine eigene Sektion gefunden hat, hat sich in den letzten vier Jahren mit den verschiedenen Möglichkeiten und Grenzen von KI-Algorithmen auseinandergesetzt. In diesem Poster soll aus den Erkenntnissen und dem Wissensschatz der AG eine grundlegende Struktur einer Schulung nach Artikel 4 EU AI Act zur Erlangung einer grundlegenden KI-Kompetenz speziell für medizinische Labore entwickelt werden. Neben dem grundsätzlichen Verständnis von Daten und deren Auswertungen (Data Literacy) werden Grundlagen zu den verschiedenen Bereichen der KI wie dem „Machine Learning“ oder dem „Deep Learning“ , sowie notwendige Kenntnisse zu den Foundation-Modellen (z.B. Large Language Models (LLMs)) integriert (AI Literacy).

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-07

Direct Prediction of Metabolite Concentrations from NMR Spectra Using Machine Learning: A Case Study

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Introduction

The quantification of metabolites in human blood plasma using NMR spectroscopy typically involves extensive preprocessing steps, including baseline correction, signal annotation, and peak integration. This process is challenged by overlapping signals and individual-specific background contributions from proteins and lipids. We explore whether machine learning (ML) models can directly predict metabolite concentrations from raw NMR spectra, bypassing conventional preprocessing.

Methods

NMR spectra and conventional quantified metabolite concentrations were collected from Study of Health in Pomeranian SHIP Trend-1. Spectra were normalized to an internal standard to reduce variability due to measurement conditions. Two spectral preprocessing strategies were compared within SHIP data: (1) unannotated spectra, using the full raw signal, and (2) annotated spectra, limited to expert-defined regions containing known quantifier signals. Gradient-boosted decision trees were trained to predict conventional quantified metabolite concentrations either from the full spectrum or from relevant parts of the spectrum identified through expert knowledge. Model performance was assessed using Passing-Bablok regression, Bland-Altman analyses and the symmetric Mean Absolute Percentage Error (sMAPE) between predicted and reference concentrations. Established feature importance measures (SHAP) were used to gain insights into the parts of the spectrum exploited by the model.

Results and Discussion

Models trained on raw NMR spectra achieved high agreement with conventional quantified values. Passing-Bablok slopes ranged from 0.75 to 1.00 and Spearman correlations exceeded 0.8 for alanine, glycine, glutamine, creatinine, and others. Median sMAPE remained below 0.2 across these metabolites, indicating high predictive accuracy. For these targets, spectral regions corresponding to their established quantifier signals showed the highest feature importance, confirming their dominant role in model predictions.

Conclusion

Direct prediction of metabolite concentrations from unprocessed spectra is feasible and reliable for selected metabolites. Annotation of metabolite single region improves cross-cohort consistency and model robustness.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-08

Foreground and Colony Detection on Agar Plates Using a Convolutional Neural Network

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Introduction

Accurate automated detection of microbial colonies on agar plates is a crucial step for enabling high-throughput microbiological analysis. In our research, we focused on developing a robust pipeline for foreground and colony detection using digital images of agar plates. Our dataset comprised images of mono-cultures from 20 different bacterial species as well as plates exhibiting mixed bacterial growth, reflecting the diversity and complexity encountered in clinical and research microbiology.

Methods

We first implemented a semi-automated labeling approach to identify colonies and foreground regions, leveraging brightness differences in the RGB color channels of the plate images. This initial segmentation was performed programmatically in Python with results being double-checked by a physician. Recognizing that automated colorimetric methods can occasionally mislabel complex or ambiguous cases—such as overlapping colonies or atypical backgrounds—we manually corrected labeling for approximately 2% of the images to ensure high-quality ground truth annotations.

To facilitate deep learning, all images and their corresponding masks were subdivided into standardized patches of 96×96 pixels. The final dataset was comprised of 20000 patches. These patches served as input for training a U-Net convolutional neural network, a model architecture well-suited for biomedical image segmentation tasks. The dataset was split into training and validation sets to rigorously evaluate model performance.

Results

The trained U-Net model demonstrated excellent performance in foreground and colony segmentation, achieving a segmentation accuracy of 99.2% on the validation set. The combination of automated color-based labeling, selective manual correction, and patch-based deep learning proved effective in handling the diverse visual characteristics of microbial colonies and agar backgrounds.

Conclusion

Our study demonstrates that integrating colorimetric analysis with deep learning, supported by selective manual annotation, enables highly accurate and scalable detection of microbial colonies on agar plates. By using Python and TensorFlow, and by training on images representing a wide range of bacterial species and growth conditions, we established a workflow that achieves 99.2% segmentation accuracy. This approach offers a reliable foundation for automated microbiological analysis and can be adapted to other colony detection and segmentation tasks in laboratory diagnostics and research.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-09

CLAM2030 and TDM-Supportes Adherence to Angiotensin-Converting-Enzyme-Inhibitors in Children with Alport-Syndrome

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Background:

Therapeutic drug monitoring (TDM) is a cornerstone of personalized medicine. Beyond its traditional role in dose adjustment, TDM can also serve as an objective measure of medication adherence—particularly relevant for non-TDM-targeted drugs such as antihypertensives. In children with Alport syndrome (AS), a genetic disorder leading to progressive kidney

failure and reduced lifespan, angiotensin-converting enzyme inhibitors (ACEi) like ramipril have shown efficacy in delaying disease progression. However, nonadherence may undermine therapeutic benefits.

Aim of the study:

This study aimed to develop and validate a fully automated LC-MS/MS method integrated into the CLAM2030-LCMS8060NX (Shimadzu Corporation) platform for adherence assessment of ramipril in urine samples from pediatric AS patients participating in the EARLY PRO-TECT trial. The fully automated open LC-MS/MS platform enables adding methods for new compounds facilitating 24/7 use in clinical laboratories.

Methods:

A total of 106 pseudonymized urine samples from treated and untreated patients were analyzed at two different time points. Nine children (16%) were not receiving ACEi therapy at baseline. The samples were processed automatically using the CLAM-2030 system (Shimadzu Corporation). Analytical performance was evaluated based on parameters including peak area, area ratio, ion ratio, retention time, and internal standard response. Acceptance criteria were established individually for each analyte and internal standard to ensure system integrity, accurate identification, quantification, and reliable result transmission.

Results:

The LC-MS/MS method demonstrated high sensitivity and specificity for ramipril detection in urine. An adherence rate of 96% and 95% was observed across both sampling points. The assay exhibited excellent linearity over the calibration range and acceptable intra- and inter-day imprecision. Long-term stability studies confirmed the robustness of calibration curves. The fully automated platform supports continuous operation suitable for clinical routine use.

Conclusions:

This novel LC-MS/MS method provides an objective tool for monitoring adherence to ramipril in pediatric patients with Alport syndrome. Its high accuracy, reproducibility, and automation make it suitable for 24/7 application in clinical and toxicological diagnostics via the CLAM2030-LCMS8060NX platform.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-10

A Cross-Platform Approach to Optimizing Adjusted Calcium

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Introduction: Calcium (Ca) plays a central role in coagulation, intracellular signalling and bone turnover. Ca disorders are common in patients but can easily be overlooked due to the absence of classic symptoms. Total calcium (tCa), free calcium (fCa) and adjusted calcium (aCa) are available. While fCa is readily measurable, it is hindered by the need for laborious blood sampling, preanalytical vulnerability and costs. tCa is routinely ordered in blood sampling, but interpretation is challenging due to dysalbuminemia. aCa is a well-supported calculation recommended by guidelines for primary hyperparathyroidism, chronic kidney disease and multiple myeloma. It is endorsed by the European Society of Endocrinology (ESE) and the U.S. National Institutes of Health. To prevent misdiagnosis, every lab must tediously develop their own equation using their own machinery and single-site data. We aim to develop an aCa standard.

Methods: Retrospective data analyses were performed on 8.4 million results (1.2 million patients) from the University Hospital Hamburg-Eppendorf (2014–2024) and the University Medicine Leipzig (2014–2019). 3.9 million whole blood (fCa) samples were measured on the ABL (Radiometer). Plasma and serum measurements (3.1 million tCa, 1.4 million albumin) were analyzed by: Siemens Atellica (At, 2020–2025), Siemens Vista (Vi, 2014–2019), and Roche Cobas (Cob, 2014–2019). The impact on aCa equations were investigated for: reference range (RefineR), decision limits (Cohen's κ), patient selection and measurement time. The resulting aCa equations were ranked according to the average Cohen's κ.

Results: The best equations for At (κ = 0.53) and Vi (κ = 0.44) have similar features (0s-30m IFU). The best equation for Cob (κ = 0.52) differs (0s-3h refineR). Considering measurement times improves aCa compared to no Consideration. Reworked reference ranges for tCa by refineR do not always improve the aCa: At (4/10), Vi (2/10), and Cob (10/10). RefineR improves Cohen's κ for fCa by 0.05 for hypocalcemia. Literature-proposed aCa cutoffs for severe hypercalcemia are higher (ESE: 3.5 mmol/L, At: 3.15 mmol/L, Vi: 3.11 mmol/L, and Cob: 3.14 mmol/L) and lower for severe hypocalcemia (ESE: 1.9 mmol/L, At: 1.91 mmol/L, Vi: 1.9 mmol/L, and Cob: 1.93 mmol/L). Patient classification for severe calcemia by aCa is comparable (At: 1.12%, Vi: 0.97%, Cob: 1.07%). Patient classification for aCa regarding to tCa reference range was in concordance between Siemens (At: 13.55%, Vi: 14.93%, Cob: 43.53%).

Conclusion: The interpretation of aCa exceeds tCa. Transferability of aCa for the interpretation of severe calcemia is feasible across platforms, especially Siemens, though reference-range classification remains inconsistent. We plan to develop separate hypo- and hypercalcemia aCa equations and launch an open-source web app to standardize aCa calculation in clinical laboratories.

DGKL: 06. Präzisionsmedizin und KI in der Labormedizin

P-08-11

Fettgewebeinsulinresistenz als früher Indikator für die Entwicklung einer metabolischen Dysfunktion-assoziierten steatotischen Lebererkrankung (MASLD)

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Einleitung:

Adipositas und Diabetes sind mit der metabolisch-dysfunktionsassoziierten steatotischen Lebererkrankung (MASLD) assoziiert. Unklar ist, ob die Beeinträchtigung der metabolischen Funktion des Fettgewebes die Progression der MASLD unabhängig von der Fettmasse begünstigt.

Methoden:

Menschen mit Adipositas und bevorstehender bariatrischer Operation (n=115, 26/89 Männer/Frauen, BMI 45,6±5,8 kg/m^2, Alter 45,6±11,4 Jahre) wurden nach den Ergebnissen eines oralen Glukosetoleranztestes in glukosetolerante (GT n=40), prädiabetische (PD n=43) und diabetische Gruppen (T2D n=32) eingeteilt. Laboruntersuchungen, Elastographie und Histologie von intraoperativ entnommenen Leberproben (n=67) wurden durchgeführt. Der Adipo-IR als etablierter Biomarker der Fettgewebsinsulinresistenz wurde als Produkt des Nüchterninsulins und der freien Fettsäuren [pmol/L x mmol/L] bestimmt. Marker für MASLD und Leberfibrose (Fatty Liver Index [FLI], Fib-4 Score, NAFLD-Score, Controlled Attenuation Parameter [CAP], Lebersteifigkeit) wurden analysiert. Die Leberproben wurden anhand des SAF-Scores auf Steatose, Aktivität und Fibrose untersucht. Die Datenanalyse erfolgte mittels Spearman-Korrelation, Wilcoxon-Test, Kruskal-Wallis-Test und Mann-Whitney-U-Test.

Ergebnisse:

Adipo-IR korrelierte mit FLI (R=0,456; P < 0,0001) und CAP (R=0,323; P=0,002), jedoch nicht mit Fib-4 (R=0,142; P=0,147), NAFLD-Score (R=0,116; P=0,236) und Lebersteifigkeit (R=0,18; P=0,094). CAP korreliert mit FLI (R=0,370, P=0,0004) und histologischen Steatose-Score (Score 0 vs 1: P=0,354, Score 0 vs. 2+3: P=0.0004, Score 1 vs. 2+3: P=0,056). Adipo-IR war bei höherem Steatose-Score erhöht (Score 0: 54,8 [31,3/96,01]; Score 1: 79,9 [64,0/100,3] und Score 2+3: 113,7 [82,2/141,7] pmol/L x mmol/L; Score 0 vs. 1: P=0,135; Score 0 vs. 2+3: P=0,0004; Score 1 vs. 2+3: P=0,133), aber nicht bei erhöhtem Aktivitäts- (gesamt P=0,377) oder Fibrose-Score (gesamt P=0,624). Der Adipo-IR sank 6 Monate nach bariatrischer Operation um 59 % (P< 0,0001), mit stärkerer Reduktion bei höheren Steatose-Scores (P=0,0004), und gleichbleibender bei höheren Aktivitäts- (P=0,654) und Fibrose-Scores (P=0,583). Ähnliche Korrelation von Adipo-IR mit den MASLD Markern zeigten GT (FLI: R=0,361; P=0,022; CAP: R=0,357; P=0,053 und Steatose-Score: P=0,012), und PD (FLI: R=0,055; P=0,0002; CAP: R=0,372; P=0,028 und Steatose-Score: P=0,023), nicht jedoch T2D (FLI: R=0,320; P=0,127; CAP: R=0,204; P=0,350 und Steatose-Score: P=0,266).

Zusammenfassung:

Insulinresistenz im Fettgewebe korreliert mit Markern der Lebersteatose, jedoch nicht mit Fibrose-Markern. In der T2D-Subgruppe wurde keine signifikante Korrelation gefunden, während Adipo-IR als Frühmarker für Steatose bei Adipositas und Prädiabetes dient. Reduktion der Lipolysehemmung und Stimulation der Lipogenese im Fettgewebe durch Insulin könnte demnach den Substratfluss vom Fettgewebe zur Leber und damit die Entwicklung der Steatose begünstigen.

DGKL: 07. Immunologie, Liquor

P-10-01

Liquordiagnostik der Alzheimer-Krankheit: Statistische Analyse des Amyloid-Beta-Quotienten in einem großen Datensatz aus der labormedizinischen Routinediagnostik

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Einführung. Bereits viele Jahre vor der klinischen Manifestation der Alzheimer-Krankheit bilden sich im Gehirn der Erkrankten Plaques aus fehlgefalteten Amyloid-Beta-(ABeta) Peptiden und Neurofibrillen aus dem Tau-Protein sowie phosphoryliertem Tau-Protein („Phospho-Tau“), wodurch Nervenzellen irreversibel geschädigt werden. Mit Blick auf einen zukünftigen Einsatz von therapeutischen monoklonalen Antikörpern (wie Lecanemab), welche die Bildung von Amyloid-Beta-Plaques hemmen können, gewinnt die Früherkennung der Alzheimer-Krankheit an Bedeutung.

Zielsetzung. Labormedizinisch erfolgt die Diagnose der Alzheimer-Krankheit durch die Untersuchung der Biomarker Amyloid-Beta (1-42)(bezogen auf ABeta1-40), Tau-Protein und phosphoryliertem Tau-Protein im Liquor cerebrospinalis anhand von Entscheidungsgrenzen, welche durch die Untersuchung von Patientenkollektiven mit der klinischen Diagnose einer Alzheimer-Erkrankung in Abgrenzung zu anderen Demenzformen definiert wurden. Im Unterschied zu diesem Endpunkt-basierten Ansatz ist die statistische Untersuchung von labormedizinischen Routinedaten eine ergänzende Methode zur Identifizierung von Entscheidungsgrenzen, der in unserer Studie verfolgt wird.

Methoden. In diesem Beitrag legen wir labormedizinische Routinedaten von > 12.000 Liquor-Untersuchungen der Marker ABeta1-42, ABeta1-40, des ABeta-Quotienten ABeta1-42/ABeta1-40 (ABetaQ) sowie von Gesamt-Tau-Protein und Phospho-Tau (181) zugrunde, die mittels ELISA-Technik (Euroimmun) über den Zeitraum von 2020 bis 2024 in unserem Labor gemessen wurden. Zur Datenanalyse setzen wir das Programm R ein, um an die beobachteten Daten statistisch bewertbare lognormale Verteilungen anzupassen, deren Anpassungsgüte durch Q-Q-Plots charakterisiert wird.

Ergebnisse. Unsere Analysen zeigen altersabhängige bimodale Häufigkeitsverteilungen der ABetaQ-Werte und unsere Kurvenanpassungen sprechen dafür, dass in unserer Patientengruppe bei ca. 60% der Fälle eine Alzheimer-Krankheit anzunehmen ist. Basierend auf den ermittelten Wahrscheinlichkeitsdichten errechnen wir ROC-Kurven zur Detektion der Alzheimer-Krankheit durch den Marker ABetaQ unter Berücksichtigung des Geschlechts und Alters.

Diskussion und Schlussfolgerungen. Unsere Ergebnisse legen nahe, dass für den ABeta- Quotienten jenseits etablierter Endpunkt-orientierter Entscheidungsgrenzen ein Grenzbereich in die diagnostische Bewertung einbezogen werden sollte, um eine möglichst frühzeitige Diagnose der Alzheimer-Krankheit zu ermöglichen. Zur Validierung dieses Grenzbereichs wird in Zukunft die Einbeziehung weiterer diagnostischer Modalitäten wie des Amyloid-PETs und die Korrelation mit klinischen Verlaufsdaten von Bedeutung sein.

DGKL: 07. Immunologie, Liquor

P-10-02

NMDA-Rezeptor-Antikörper Autoimmunenzephalitis mit stark erhöhtem CXCL13 im Liquor nach einer Infektion mit Parvovirus B19

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Einführung

Die NMDA-Rezeptor-Antikörper Autoimmunenzephalitis wurde zunächst als Krankheitsbild junger Frauen beschrieben mit einem typischen Verlauf, bei dem sich an eine unspezifische Prodromalphase schizophreniforme Symptome und später oft neurologische Symptome wie epileptische Anfälle, Bewegungsstörungen und autonome Störungen anschließen. Bei älteren Patientinnen wurde in ca. 60% der Fälle eine paraneoplastische Genese aufgrund eines Ovarialteratoms berichtet. Post-infektiöse Fälle z.B. im Anschluss an eine Herpes simplex Enzephalitis wurden ebenfalls mitgeteilt.

Ergebnisse und Diskussion

Wir berichten über einen 63-jährigen Patienten, der nach einer prodromalen Phase mit über 1 1/2 Wochen zunehmenden Kopfschmerzen generalisierte tonisch-klonische Anfälle entwickelte, an welche sich eine zunehmende Vigilanzminderung durch einen nonkonvulsiven Status epilepticus anschloss.

Ursächlich für diese Symptomatik war eine Autoimmunenzephalitis mit einer Pleozytose, einer mittelschweren Liquor-zirkulationsstörung zunächst ohne quantitativ fassbare intrathekale Antikörpersynthese im Reiber-Diagramm, aber mit positiven Anti-NMDA-Rezeptor-Autoantikörpern im Liquor und schwach positiven liquorspezifischen oligoklonalen IgG-Banden. Zusätzlich fand sich ein stark erhöhter CXCL13-Wert im Liquor bei einem fehlenden klinischen und serologischen Anhalt für eine Neuroborreliose.

Im weiteren Verlauf war eine intrathekale IgG-/IgA-/IgM-Synthese im Reiber-Diagramm quantitativ nachweisbar mit positiven liquorspezifischen oligoklonalen Banden und einer MRZ-Reaktion. Für eine paraneoplastische Genese ergab sich aufgrund der Zusatzdiagnostik mittels MRT des Schädels, CT von Thorax und Abdomen, Hoden-Sonographie und FDG PET-CT kein Anhalt.

Umfangreiche serologische Untersuchungen ergaben bei stark erhöhten Werten spezifischer IgM- und IgG-Antikörper den Hinweis auf eine vor kurzem durchgemachte Virusinfektion mit Parvovirus B19. Die wiederholte PCR-Diagnostik auf diesen Erreger im Liquor war negativ.

Unter einer eskalierten antiepileptischen Therapie aufgrund eines superrefraktären Status epilepticus mit Levetiracetam, Valproat, Lacosamid, Perampanel und Phenobarbital sowie einer Therapie mit Glucocorticoiden i.v., einer Plasmapherese und einer B-Zell-Depletion mit Rituximab kam es trotz einer zwischenzeitlichen Beatmungspflichtigkeit infolge einer Aspiration insgesamt zu einer weitgehenden Besserung des klinischen Bildes. Hierbei zeigte sich eine Korrelation der Krankheitsaktivität mit dem CXCL13-Wert im Liquor.

Schlussfolgerung

Dieser Fall zeigt, dass bei stark erhöhten CXCL13-Werten im Liquor und einer für eine Neuroborreliose atypischen Enzephalitis die Differentialdiagnose einer NMDA-Rezeptor-Antikörper Autoimmunenzephalitis in Betracht gezogen werden sollte. Hierbei kann eine vorangegangene Infektion mit Parvovirus B19 ursächlich sein.

DGKL: 07. Immunologie, Liquor

P-10-03

Automated detection of DFS-70 autoantibodies (AC-2) by multiplex fluorescence immunoassay and retrospective comparison to microscopic detection

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Abstract:

DFS70- antibodies are a subtype of ANA characterised by dense fine speckled staining of interphase nuclei and metaphase plate in IIF. They have been reported in a variety of non-SARD-inflammatory conditions such as atopic dermatitis, bronchial asthma or alopecia areata (typically at low frequencies and high titers), prostate cancer, as well as in apparently healthy individuals [1]. It is therefore not surprising that DFS70 tests are not routinely used to detect or confirm the presence of autoimmune diseases. Instead, DFS70 antibodies have been proposed as an exclusion criterion for SARD and are only tested in suspected samples showing the DFS pattern on Hep-2 cells, to prevent incorrect interpretation of positive IIF results. The correct identification of the DFS pattern requires a certain level of experience due to its similarity to AC-1 and AC-29 patterns, and/or the simultaneous presence of other ANAs. This diagnostic approach creates a gap between the suspicion of DFS70 positivity based on IIF-HEp2 and the actual prevalence of DFS70-positive cases. We aimed to validate a novel PMAT-Aptiva immunoassay for DFS70 antibody detection and to determine the number of DFS70-positive samples that remain undetected in a routine laboratory setting at a university hospital.

Methods: We compared the positive rate of DFS70 antibodies in over 8000 consecutive samples for whom ANA testing was ordered over the same 6-month period of two successive years. In the first period, only DFS-pattern suspected samples were analysed for the presence of DFS-70 antibodies. In the second period, DFS70 antibodies were determined in each IIF-positive sample, regardless of staining pattern(s) on Hep-2 cells, using particle-based multi-analyte technology (PMAT), allowing simultaneous detection of DFS70 along with ten classic ANA antibodies.

Results: The number of DFS70-positive samples has doubled since the implementation of PMAT/Aptiva in routine ANA testing. The rate of DFS70 positive results rose by more than 900% at a dilution of 1:80 and by 90% at a titer of 1:160, whilst the rate of DFS70 positive samples with moderate and high ANA titre (≥ 1:320) has not changed significantly. 17% of DFS70-

positive sera were positive for at least one other classic ANA antibody. Anti-RO52, anti-Ro60 and anti-Rib P were the most commonly detected antibodies and systemic lupus erythematosus was the most frequent diagnosis.

Conclusion: IIF alone is not a reliable method for detecting anti-DFS70 antibodies, especially for samples with weak positive IIF staining.

DGKL: 07. Immunologie, Liquor

P-10-04

Plant sterol enrichment alters membrane microdomains composition and reduces cellular inflammatory response in microglial cell lines

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Introduction: Plant sterols (PS), structurally similar to cholesterol, are exclusively derived from plants and have demonstrated anti-inflammatory properties in brain [1]. Cholesterol rich cell membrane microdomains facilitate specific cellular signalling and trafficking by organising free sterols, phospholipids and proteins. This work investigates the impact of PS on inflammatory responses in microglial cells.

Methods: SIM-A9 cells were cultured in a PS-enriched medium in various concentrations (6.25 µM, 12.5 µM, 25 µM each campesterol and sitosterol) prior to lipopolysaccharide (LPS) stimulation. After detergent-free extraction of membrane microdomains, the sterol, poly unsaturated fatty acid (PUFA) and eicosanoid composition was analysed by targeted LC- MS/MS [2]. The oxidative stress response was assessed using Western blot and NO-assays. Non-targeted proteomic analysis of membrane microdomains utilizing an HPLC-HRAM MS/MS with a data-dependent acquisition (DDA) approach. Whole transcriptome RNA sequencing was performed using the Illumina NovaSeq 6000 platform, generating high-throughput paired-end reads for downstream transcriptomics analysis. A differential gene expression analysis was conducted between control and PS treated cells, both with and without LPS activation.

Results: Microglia cultured with ascending PS concentrations, showed a dose-dependent accumulation of PS within microdomains, resulting in an alteration in the abundance of proteins involved in cholesterol transport and homeostasis. PUFA profiling revealed an increased proportion of PUFAs in PS-treated cells, accompanied by a decreased NO-release in response to LPS activation, indicating anti-inflammatory effects.

The analysis of RNA-sequencing data demonstrated significant downregulation of inflammatory genes in cells treated with PS prior to LPS activation. A downregulation of COX-2 expression was not observed in the RNA-sequencing data or in the Western Blot.

Conclusion: PS exhibit anti-inflammatory effects demonstrated by reduced NO-release in microglia. However, it is not a consequence of the downregulation of COX-2 but rather caused by alterations in its metabolism. Proposing a shift in PUFA metabolism, resulting in alterations of eicosanoid profiles and oxidative stress signalling pathways.

DGKL: 07. Immunologie, Liquor

P-10-05

Circulating Endocannabinoids Reflect Cognitive Status but Not Cognitive Decline in Older Adults

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Introduction: Endocannabinoids (eCBs) are lipid-derived signaling molecules involved in neuromodulation, inflammation, and synaptic plasticity—key processes in cognitive function and aging. While preclinical evidence suggests a role in neurodegeneration and cognitive decline, human data remain limited. This study investigated cross-sectional and longitudinal associations between circulating eCB levels and cognitive performance in older adults from the population-based LIFE Adult cohort.

Methods: A total of 463 participants (mean age 71) with baseline cognitive assessments (SISCO score), clinical lab data, and serum eCB measurements were included. A subset (n = 225) had follow-up cognitive data. The eCBs N-Arachidonoylethanolamide (AEA), N-Oleoylethanolamide (OEA), N-Palmitoylethanolamide (PEA), 1-Arachidonoylglycerol (1-AG), and 2-Arachidonoylglycerol (2-AG) were quantified via LC-MS/MS. Cognitive impairment was defined by SISCO cut-offs (≥53: unimpaired, ≤45: impaired). Analyses included group comparisons (Mann-Whitney U), Spearman correlations, and multiple linear regressions adjusted for age, sex, BMI, HbA1c, cholesterol, smoking, and physical activity. Cognitive change (ΔSISCO) was assessed relative to baseline eCB concentrations.

Results: At baseline, impaired individuals had significantly higher AEA and OEA levels, while 1 AG was lower compared to unimpaired participants. AEA showed the strongest negative correlation with cognitive performance ($r = –0.23$, $p < 0.001$). In multivariable models, AEA remained a robust negative predictor ($\beta = –2.13$, $p < 0.001$), whereas PEA ($\beta = +1.55$, $p = 0.002$) and 1-AG ($\beta = +0.91$, $p = 0.002$) were positively associated with cognitive scores. OEA showed no significant adjusted associations. Longitudinally, cognitive decline was more pronounced in initially unimpaired individuals, likely due to ceiling effects and regression to the mean. No eCB significantly predicted ΔSISCO. AEA showed a weak trend toward cognitive resilience ($\beta = +0.92$, $p = 0.064$); other analytes showed negligible or no associations.

Conclusion: Serum levels of selected endocannabinoids – particularly AEA, PEA, and 1-AG – are cross-sectionally associated with cognitive status in older adults. Elevated AEA may reflect neuroinflammatory stress, whereas higher PEA and 1-AG may indicate neuroprotective activity. However, their lack of predictive value for cognitive decline limits their utility as prognostic biomarkers. eCBs appear to reflect current neurobiological states rather than future cognitive trajectories. Future studies should extend follow-up durations, incorporate repeated eCB measurements, and include neuroimaging to unravel the mechanistic role of eCBs in cognitive aging and neurodegenerative disease progression.

DGKL: 07. Immunologie, Liquor

P-10-06

Association of ABO Blood Type with Humoral Immune Responses to SARS-CoV-2 Vaccination and Infection

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Introduction

During the COVID-19 pandemic, studies suggested a potential link between blood type and susceptibility to SARS-CoV-2 infection. Evidence on the association between blood type and humoral immunity induced by COVID-19 vaccination or SARS-CoV-2 infection has been inconsistent, with no definitive conclusions. This study examines the relationship between ABO blood type and vaccination-induced humoral immunity against SARS-CoV-2 among healthcare workers (HCWs).

Methods

As a substudy of the CoVacSer study - a prospective, longitudinal cohort study conducted among HCWs from September 2021 to December 2023 - 429 HCWs fulfilling the study inclusion criteria were assessed for ABO and Rhesus blood type. Participants provided blood serum samples and completed questionnaires 14 days, 3, 6, and 12 months after their most recent COVID-19 vaccination or SARS-CoV-2 infection. Follow-up schedules restarted after each new COVID-19 immunisation or infection event. Anti-SARS-CoV-2-Spike IgG levels were measured using the SERION ELISA agile SARS-CoV-2 IgG assay (SERION Diagnostics, Würzburg, Germany), ABO and Rhesus blood typing was conducted via column agglutination technology.

Results

In the overall cohort, 42.7% of participants had blood group O, 40.3% had blood group A, 6.1% had blood group AB, and 11.0% had blood group B. Stratification by Rhesus factor revealed that 84.8% were Rhesus positive and 15.2% Rhesus negative. Anti-SARS-CoV-2-Spike IgG antibody levels increased significantly following both the third and fourth COVID-19 vaccinations, independent of blood group. No significant differences were observed in the changes of IgG levels before and after vaccination across ABO or Rhesus blood type. Similarly, no significant differences were detected in the occurrence of post-vaccination side effects, self-reported work ability, or health-related quality of life between blood groups. The risk of breakthrough SARS-CoV-2 infection did not differ significantly between ABO or Rhesus blood groups.

Conclusion

The study found no evidence that ABO or Rhesus blood group status significantly affects vaccine-induced antibody response, post-vaccination side effects, work ability, quality of life, or susceptibility to SARS-CoV-2 infection in vaccinated healthcare workers. Thus, blood group status does not appear to be a reliable predictor of vaccine response or infection risk and should not influence COVID-19 vaccination strategies, clinical risk assessment, or occupational health policies.

DGKL: 07. Immunologie, Liquor

P-10-07

Interim Analysis NfL Lab: Assessment of Real-World Implementation and Execution of Routine Neurofilament Light Chain Diagnosis for Multiple Sclerosis in German Laboratories

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Introduction:

Neurofilament light chain (NfL) is a biomarker to detect neuronal damage. Neurologists see added value in determining NfL to monitor multiple sclerosis (MS) disease activity. Improved monitoring and clinical decision-making of MS patients potentially helps to minimize disease progression (1-4). Recently, new NfL assays in blood (serum, sNfL) were developed for clinical application (6,9,11-12).

This data collection aims to understand the sNfL diagnostic landscape and test usage.

Methods:

The data collection is conducted in up to 10 diagnostic laboratories providing sNfL testing.

Baseline and end of study data are collected once. Structured tables with anonymized data on sNfL measurements, patient demographics, clinical diagnoses and submitter specifications are provided quarterly by each laboratory over a period of 12 months.

Results:

2474 sNfL samples were documented from 5 diagnostic laboratories, with 0.6 % (SD 0.9) deemed unsuitable. Samples were sent from medical practices (32.5 %), hospitals (29.8 %), and laboratories (36.8 %). 28.1 % were sent by neurologists.

Diagnoses were mostly unknown (69.7 %), 16.7 % were diagnosed with MS. 73.4 % of patients were between 20 – 59 years old, 24.3 % falling in the 40 – 49 years age group.

The overall median sNfL value was 11 pg/ml, [95 % percentile 59.2 pg/ml]. sNfL distribution was 4.6 % (0-5 pg/ml), 42 % (6-10 pg/ml), 36.3 % (11-20 pg/ml) and 17 % ≥ 21 pg/ml.

Median NfL in the groups 20 - 59 years range from 7.6 pg/ml [20.4 pg/ml], 8 pg/ml [23 pg/ml], 9.8 pg/ml [22.3 pg/ml] to 11.8 pg/ml [34.0 pg/ml] respectively, in groups ≥ 60 years levels were higher (max. 44 pg/ml).

Conclusion:

We show that sNfL testing is established in clinical routine with therapeutic relevance in neurology for monitoring of MS activity but also possible utility in other indications (1-4,6,8).

The low percentage of unsuitable samples indicates that sNfL is an easy, stable laboratory parameter, confirming published data on preanalytical stability (5-7).

Overall median sNfL level was comparable to published threshold values for MS disease activity (14-16). Available data along with our observation show that sNfL levels generally increase with age, but there is high variation on an individual level (13). As sNfL is influenced by various confounders, such as BMI and comorbidities, a patient-individual approach, along with frequent, longitudinal sNfL measurement might be needed to accurately monitor MS activity (10).

While sNfL is a reliable, therapeutically relevant and easy-to-use biomarker, more data is needed to determine the optimal frequency of sNfL measurements for monitoring MS activity. Additionally, the threshold sNfL level that necessitates a change or escalation in therapy needs further investigating.

DGKL: 07. Immunologie, Liquor

P-10-08

Ferritin in cerebrospinal fluid – reference interval for measurement on the Siemens Atellica IM Analyzer

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Introduction: Circulating ferritin concentrations reflect the iron status of the organism and are crucial in the diagnosis of iron-related diseases. In cerebrospinal fluid (CsF), the ferritin concentration is low under physiological conditions. It rises after hemorrhagic events in the subarachnoid space, therefore the laboratory-based measurement supports the detection of intracranial bleedings [1]. CsF ferritin was further suggested to reflect iron accumulation and oxidative stress in the central nervous system and higher concentrations have been reported to be associated with severity of neurodegenerative diseases, as Alzheimer's [2]. Additionally, increased CsF ferritin levels may point to inflammative or neoplastic conditions [3, 4]. In the Institute of Clinical Chemistry and Laboratory Medicine of the University Medicine Greifswald, the Atellica® IM analyzer (Siemens Healthineers, Erlangen, Germany) is used in patient care for the measurement of ferritin in plasma or serum samples. This method was used to measure CsF ferritin from surplus patient material to establish a reference interval.

Methods: Surplus material from 128 inpatients with intact blood-brain barrier and no signs of bleeding or inflammation was collected and CsF ferritin concentrations were measured using the Atellica® IM analyzer. The distribution of the measurement results was visualized and the central 95% reference interval determined by calculating the 2.5th and 97.5th percentiles. Another 79 surplus patient samples were used for duplicate measurements on the Atellica® IM analyzer and the older Dimension Vista® 1500 system (Siemens Healthineers, Erlangen, Germany). The results of these measurements were compared using the rank correlation coefficient Kendall's Tau and Passing-Bablok regression.

Results: In the 128 individuals of the reference population, the CsF ferritin concentration ranged between 1.7 µg/l and 9.7 µg/l. The median concentration was 6.1 µg/l (1st-3rd quartiles: 5.0 - 7.5 µg/l) and the central 95% reference interval was 3.0 - 9.2 µg/l. The correlation between both analyzers was high (Kendall's Tau 0.95), but the Atellica® IM analyzer produced lower results than the Dimension Vista®1500 (Passing-Bablok regression: slope=0.78, intercept=2.50).

Conclusion: CsF ferritin concentrations correlated well between the new Atellica® IM analyzer and the older Dimension Vista® 1500 system, with lower values obtained by the new method. This is also reflected in the reference interval, which was 2.0 - 12.6 µg/l for the Dimension Vista® 1500 and is 3.0 - 9.2 µg/l for the Atellica® IM analyzer system. Our normative data may aid in the diagnosis of several severe diseases, including intracranial bleedings.

DGKL: 07. Immunologie, Liquor

P-10-09

Verifizierung und Methodenvergleich eines ELISA zur Bestimmung der freien Leichtketten

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Die Bestimmung der freien Leichtketten (FLC) ist ein essentieller Bestandteil beim Screening, Prognosebestimmung und Monitoring bei Plasmazell-Neoplasien. Neben den schon lange etablierten nephelometrischen und turbidimetrischen Messverfahren ist auch ein ELISA verfügbar.

in der Methodenverifizierung zeigte sich eine gute Präzision und eine akzeptable klinische Konkordanz. Der korrelierte gut mit der nephelometrischen Bestimmung. Bei der indirekten Referenzintervallermittlung wurde ein etwas breiteres Referenzintervall ermittelt.

Der FLC-ELISA ist eine robuste und praktikable Alternative zur turbidimetrischen oder nephelometrischen Bestimmung der freien Leichtketten.

DGKL: 07. Immunologie, Liquor

P-10-10

Correlation of Serum Glial Fibrillary Acidic Protein and Neurofilament Light Chain Levels During Induction of Immunomodulatory Therapy in Multiple Sclerosis

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Introduction

Neuroinflammation in multiple sclerosis (MS) impairs quality of life and can lead to permanent neurological damage. Immunotherapy, particularly depletion of B cells that are involved in the disease mechanism, can alter the overall activity level of MS by reducing the ongoing inflammatory processes in the central nervous system (CNS). Real-time monitoring of disease activity and severity can be achieved by regular clinical and imaging controls. However, especially subclinical and progressive disease activity is difficult to assess by these modalities. Different studies have demonstrated that longitudinal measurement of serum parameters for neuroaxonal damage hold potential to asses and even predict disease activity.

Methods

We use the novel Roche Elecsys assays for measurement of glial fibrillary acidic protein (GFAP) and neurofilament light chain (NFL) in serum samples. Samples from MS patients under different disease-modifying therapies (DMT) are tested longitudinally for serum concentrations of GFAP and NFL. We also investigate the correlation of MS disease activity scores (expanded disability status scale, EDSS; inflammatory MRI lesion load in T2-weightened images, T2L; clinical apparent relapses) with the serum GFAP and NFL levels at different visits after treatment initiation. Immunoassays that can detect low levels of serum GFAP and NFL are run on the Roche Cobas Pro integrated solutions c703 module according to manufactures protocols.

Results

During acquisition, we have noted a high stability of results in both daily quality control values and a highly reproducible median of all measured values across different cohorts of patients including MS patients under different DMT, indicating a high level of reliability in the detection of serum GFAP and serum NFL levels applying this methodological approach. Across all measurements, GFAP and NFL levels showed a clear clustering of most values around a median of 50 pg/ml for GFAP and of 1 pg/ml for NFLC (n = 347 samples with parallel measurements). In a direct correlation analysis, some samples showed elevation of both and some samples showed an elevation of primarily NFL, which was already associated to relapsing disease activity (overall significant correlation, albeit with low R^2, slope 95% CI 6-18 pg/ml for serum NFL on x-axis). The effect of

B cell depletion appeared as trend towards lower NFL values and lower MS disease severity as indicated by EDSS. Next, more data will be analyzed and effect sizes will be calculated.

Conclusion

These data help to monitor MS disease activity under different disease-modifying therapies. These findings also demonstrate the possible impact of measuring CNS-specific target cell markers with the novel serum analytical methods to rapidly detect patients with ongoing astrocyte or neuron damage-inducing neuroinflammation.

DGKL: 07. Immunologie, Liquor

P-10-11

Auswertung der Anzahl der oligoklonalen Banden bei Multipler Sklerose: Erkenntnisse aus einer großen Kohortenanalyse

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Zielsetzung: Liquor spezifische oligoklonale Banden (OKB) dienen als wichtiger diagnostischer Marker bei der Multiplen Sklerose. Rezentere Arbeiten beginnen zu untersuchen, inwieweit die Anzahl der OKB als möglicher Marker für die Wirksamkeit einer auf das zentrale Nervensystem ausgerichteten Behandlung dienen kann. In diesem Zuge wurden die Anzahl der OKB bereits beginnend als potenzieller Endpunkt in Therapieversuchen z.B. mit chimären Antigenrezeptor T Zellen bei MS definiert. Die klinischen Implikationen der Liquor spezifischen OKB sind jedoch nur begrenzt erforscht. Ziel dieser Studie war es, die Korrelation zwischen der Anzahl der Liquor spezifischen OKB und demographischen, sowie klinischen und paraklinischen Parametern zu Untersuchen. Zusätzlich sollte der Zusammenhang zu weiteren Liquor Markern wie z.B. der intrathekalen IgG Synthese und Kappa freien Leichtketten (KFLC) untersucht werden

Methoden: Es wurden die Daten aller Patienten ausgewertet, welche zwischen 2010 und 2017 in die Klinik für Neurologie der MHH aufgenommen wurden und bei denen die Erstdiagnose MS oder klinisch isoliertes Syndrom (CIS) nach den aktualisierten McDonald Kriterien von 2017 gestellt wurde. Ein Follow Up konnte bei 293 Patienten erhoben werden, mit einer durchschnittlichen Dauer von 9 Monaten (IQR 2-27) für MS Patienten und 8 Monaten (IQR 2-20) für CIS Patienten. Die Anzahl der OKB wurde durch isoelektrische Fokussierung von Liquor/Serumprobenpaaren auf einem Polyacrylamidgel und anschließender Silberfärbung bestimmt.

Ergebnisse: Es konnte eine Kohorte von 454 Patienten rekrutiert werden, hiervon waren 304 (67%) Frauen, der Altersmedian lag bei 33 Jahren. Bei 314 (69%) Patienten wurde die Diagnose einer MS gestellt und bei 140 (31%) die eines CIS. 99% der MS Patienten waren OKB Positiv (OKB Typ 2 oder 3) quantitativ wurden im Median bei diesen Patienten 19 Liquor spezifische OKB ausgezählt. Es wurde kein signifikanter Zusammenhang zwischen der Anzahl der OKB und dem Alter, dem Geschlecht, der klinischen Präsentation einschließlich der Höhe des Expanded Disability Status Scale (EDSS) oder der Anzahl der T2 Läsionen in der Magnetresonanztomographie bei Erstdiagnose einer MS festgestellt. Des Weiteren fand sich kein Zusammenhang zwischen der Anzahl der Liquor spezifischen OKB bei Erstdiagnose und dem Auftreten eines Schubes oder der Progression des EDSS im Follow Up. Es wurden jedoch starke Korrelationen zwischen der OKB Zahl und der intrathekalen Synthese von IgG und den KFLC beobachtet.

Diskussion und Schlussfolgerung: Diese Ergebnisse deuten darauf hin, dass die Anzahl der Liquor spezifischen OKB die klinische und paraklinische Krankheitsaktivität zum Zeitpunkt der Diagnose nicht ausreichend widerspiegelt und somit nicht als zuverlässiger prognostischer Marker zu sehen ist. Ob ein Rückgang der OKB Anzahl unter bestimmten Therapien eine Verringerung der humoralen Immunantwort im ZNS anzeigen kann, erfordert weitere prospektive Untersuchungen.

DGKL: 07. Immunologie, Liquor

P-10-12

Laboratory Validation of Neurofilament light chain measurement in cerebrospinal fluid on the Atellica® Immunoassay Analyzer

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Introduction: The measurement of Neurofilament light chain (NfL) in human serum is validated using a highly sensitive automated immunoassay. Neurofilament light chain (NfL) is a neuron specific structural protein that can be found in blood and in cerebrospinal fluid (CSF) in an even higher concentration as a biomarker for axonal injury or degeneration. Neurofilaments separate from the axon into the interstitial space and eventually into CSF after damage. The NfL as a marker for neuroaxonal damage has been reported in multiple sclerosis, traumatic brain injury, Amyotrophic lateral sclerosis, Parkinsons`s disease and Alzheimer`s disease, for example. The aim of our study was to validate NfL assay for liquor samples using the serum application.

Methods: The evaluation of the linearity, extended measuring interval, stability and recovery of liquor samples were evaluated using the fully automated Atellica® IM Neurofilament Light Chain (NfL) Serum-Assay running on the Atellica® Immunoassay (IM), in accordance with CLSI guidelines. Tests were performed using manually prediluted cerebrospinal fluid (1:50) with the IM NfL Atellica® diluent. To assess sample recovery, a basic spike study using the pure solvent with known NfL concentration on undiluted liquor was performed. The detection capability (Limit of Blank, Limit of Detection and Limit of Quantification) was not determined separately; it was accepted based on the serum data provided in the package insert.

Results: NfL CFS measurements showed linearity from 150 - 15000 pg/ml and up to 75000 pg/ml with an extended measuring range using an additional 1:5 automated dilution. The assay showed a mean recovery of 90–110%. Stability of the samples up to 5 days refrigerated 4-8°C was also analyzed.

Conclusion: Using the Atellica® IM NfL- Serum Assay for NfL measurement in cerebrospinal fluid demonstrated acceptable analytical performances. It has the capacity to substitute the labor-intensive manual ELISA measurement of Neurofilament light chain in cerebrospinal fluid.

DGKL: 08. Endokrinologie, Biobanking

P-09-01

Adjusting total Calcium for Albumin – Does a locally derived equation improve performance?

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Introduction: While measuring free (ionized) calcium (fCa) remains the diagnostic standard for the detection of disturbed calcium homeostasis), the measurement of total Calcium (tCa) is also commonly applied in the screening for these disorders. As tCa levels are significantly influenced by albumin homeostasis, it is common practice to calculate a total calcium that is adjusted for albumin (aCa). The literature yields several equations for this purpose, but it is also possible to derive local equations for aCa, based on real world data from the clinical routine. It is expected that a locally derived equation improves the performance of aCa in the prediction of hypo- and hypercalcemia, as it is better suited for local (and current) methods of

measurement of tCa and albumin and is baesd on a much larger sample size than existing, decades-old equations. This study aims to investigate whether deriving a local equation for aCa does indeed improve this performance relevantly.

Methods: Based on observed concurrent measurements for fCa, tCa, and albumin in real world data, a new equation for aCa was derived, using a linear least squares regression. The resulting equation was compared to that of Payne et al. with regards to sensitivity and specificity in the detection of hypo- and hypocalcemia, comparing different cutoffs for both fCa and aCa.

Results: Correlations between aCa and fCa were comparable between both equations (Payne: R = 0.61; local: R = 0.7; $p < 0.001$ for both). Sequential calculation of ROC curves for different fCa cutoffs show that both equations perform better for severe dyscalcemia, the prediction of hypercalcemia being superior to that of hypocalcemia. The local equation was able to achieve slightly better AUC values overall. The prediction of mild hypocalcemia was mediocre for both equations (AUC for fCa < 1.1 mmol/l: 0.67 (Payne); 0.76 (local). Sequential calculation of Cohen's kappa with different aCa cutoffs showed mediocre agreement of both equations with fCa in the case of severe hypocalcemia (fCa < 0.9 mmol/l; maximum Cohen's kappa: 0.53 (Payne); 0.58 (local)) and good agreement for severe hypercalcemia (fCa > 1.6 mmol/l; maximum Cohen's kappa: 0.79 (Payne); 0.8 (local)). Again, the local equation performed slightly better. ROC analyses for the determined respective optimal cutoffs showed that hypocalcemia tends to be overestimated by aCa, while hypercalcemia tends to be underestimated by both equations.

Conclusion: The performance of aCa in the prediction of dyscalcemia can be improved by deriving a local equation. However, the overall performance of both compared equations in the prediction of hypocalcemia was mediocre, due to a large percentage of false positives at the optimal aCa cutoff. Especially for hypocalcemia, aCa should rather be used as a screening tool, with cutoffs optimized for sensitivity, while the diagnosis of disorders of calcium homeostasis remains a domain of the measurement of fCa.

DGKL: 08. Endokrinologie, Biobanking

P-09-02

GDF-15 mitigates fibrosis development in patients with liver injury – data from a community-based cohort study

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Background & Aims: Liver diseases are a major global health issue. Growth Differentiation Factor 15 (GDF-15), a stress-induced cytokine, is elevated in various pathological conditions and may protect against liver fibrosis progression, through neuro-metabolic-immunologic mechanisms. In obesity and diabetes, GDF-15 is thought to regulate energy and lipid homeostasis modulating hepatic steatosis. This study investigates the potential protective role of GDF-15 in hepatic steatosis and fibrosis, accounting for prior liver injury, alcohol intake, insulin resistance and obesity.

Methods: We conducted a retrospective cohort study of 626 participants from the LIFE Adult study. Associations between GDF-15, alcohol intake, FIB-4-score and metabolic risk factors with hepatic steatosis and fibrosis over a 6-year-follow-up period were examined, using linear regression models.

Results: In subjects with elevated baseline FIB-4, the interaction between GDF-15 and FIB-4 was positively associated with liver stiffness at follow-up (ß = 0.47, SE = 0.23, t = 2.02, p = 0.045). Interactions between GDF-15 and higher alcohol intake (3rd and 4th quantiles) were negatively associated with liver stiffness (ß = -1.68, SE = 0.55, t = -3.08, p = 0.002 and ß = -1.43, SE = 0.68, t

= -2.09, p = 0.038), suggesting a protective effect. In obese subjects, GDF-15 was associated with higher steatosis levels at follow-up (ß = 37.14, SE = 13.15, t = 2.82, p = 0.006). In subjects with higher HOMA-IR (3rd and 4th quantile) increased steatosis at follow up was observed (ß = 31.15, SE = 14.33, t = 2.17, p = 0.032 and ß = 38.15, SE = 16.48, t = 2.32, p = 0.023), but HOMA-IR interactions with GDF-15 were inversely associated (ß = - 38.98, SE = 14.29, t = -2.73, p = 0.008 and ß = - 38.54, SE = 16.11, t = - 2.39, p = 0.019), indicating protective modulation.

Conclusions: GDF-15 may modulate liver steatosis and fibrosis, particularly in metabolically or lifestyle-compromised individuals, highlighting its potential as a future therapeutic target and the need for further research on the neuro-metabolic-immunologic axis.

DGKL: 08. Endokrinologie, Biobanking

P-09-03

Three Years of Proficiency Testing in Liquid Biobanking: A Robust Approach to Identifying Process and Sample Quality Variations

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Introduction:

Ensuring the integrity of biospecimens is a cornerstone of high-quality biobanking. Proficiency testing (PT) serves as a critical quality assurance mechanism, enabling biobanks to evaluate and improve their procedures. Over the past three years, our PT program for liquid biobanking has aimed to uncover process inconsistencies and variations in sample quality across both national and international biobanks.

Methods:

The PT program assesses multiple operational aspects, including sample entry control, processing times, aliquoting precision, and sample homogeneity. In its third iteration, the program introduced a nuclear magnetic resonance (NMR) spectroscopy–based contamination test to detect potential disinfectant residues in biological samples. Additionally, newly implemented progress checks provided a longitudinal view of participating biobanks' adherence to standardized protocols.

Results:

This latest round of PT involved 24 national biobanks and revealed significant variability in sample handling and processing practices. While progress checks indicated gradual improvements in standardization, they also identified persistent gaps in specific procedural areas. The implementation of NMR analysis proved effective in detecting contamination risks, further reinforcing the program's diagnostic capacity.

Conclusions:

The third cycle of PT represents a key advancement in the standardization of liquid biobanking. The integration of contamination detection and longitudinal performance monitoring has significantly enhanced the program's ability to identify and address critical quality issues. The growing participation of national biobanks reflects the program's increasing value in promoting consistency, reliability, and high standards across the biobanking community.

DGKL: 08. Endokrinologie, Biobanking

P-09-04

Steroid Panel LC-MS by Tecan: Exploring Some Essential Issue For Sexual Steroids Determination.

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INTRODUCTION:

Tecan offers the kit “Steroid Panel LC-MS” for the simultaneous determination of 17 steroids and dexamethasone. Interesting features of this kit are the limited volume of serum required (250 µL), the automated and simple sample preparation, and the high sensitivity in particular for sexual steroids testosterone and estradiol with the possibility to also determine estrone. In our laboratory at Andrology, Female Endocrinology and Gender Transition Unit of Azienda Universitaria Ospedaliera Careggi (Florence, Italy). The essential requirements for sexual hormones in which we are interested are high sensitivity and accuracy for testosterone at low concentrations (females and children) and for estradiol at low concentrations (men and postmenopausal women) together with the possibility to quantify estrone (adults). Before adopting this new method, it is imperative to ascertain that the crucial analytical concerns mentioned above can be satisfactorily addressed.

METHODS:

The Steroid Panel LC-MS kit, including the quantitative analysis of 17 steroids and dexamethasone, was used. Calibration range and retention time for all the included steroids are reported. After sample preparation via SPE, 20 µL of the extract was injected into the system. Focus was set on the comparison of estradiol and testosterone on their agreement on external standard material from UK-NEQAS.

RESULTS:

The analytical performance was in line with the internal standard material. We were able to show an accuracy between 80 and 110% for the measurement of standard material from UK-NEQAS, as well as interassay CV% below 8% for the measurement of Estradiol samples.

CONCLUSION:

The performance of the Steroid Panel LC-MS met our first goals, especially regarding accuracy for the two main sexual steroids. For validation purposes, precision and accuracy will be evaluated on all the other steroids. Moreover, LoD, LoQ and linearity will be assessed and a study in order to verify the comparability with the existing LDT will be performed in the future on a large number of real samples.

DGKL: 08. Endokrinologie, Biobanking

P-09-05

Effect of 11-Year Biobanking on Oxylipins as Quality Biomarkers for In Vitro Degradation Processes in Human Blood

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Introduction:

Biobanks are essential infrastructures for biomedical research, providing not only high-quality biospecimens but also comprehensive metadata on sample origin, processing, and donor characteristics. These resources enable robust retrospective and large-scale analyses. However, sample integrity may be compromised by pre-analytical factors during processing and storage. Oxylipins, a class of bioactive lipid mediators involved in inflammation and other physiological processes, are particularly sensitive to enzymatic and oxidative degradation and have emerged as candidate biomarkers for assessing sample stability. Long-term data on their behaviour under routine biobanking conditions remain limited. This study investigates whether selected oxylipins can indicate in vitro degradation in blood samples stored for up to 11 years, focusing on storage temperature and tube type.

Methods:

This longitudinal study, initiated in April 2014, examined the long-term stability of oxylipins and long-chain fatty acids (LCFAs) quantified by targeted LC-MS/MS under highly standardised and strictly controlled conditions. Blood samples were collected, aliquoted, and stored according to predefined protocols. Key variables included three tube types, two storage temperatures (minus 80 °C and below minus 150 °C), and two fill volumes. Analyses were conducted at 3 and 6 months, annually for 5 years, and again after 11 years. In addition to oxylipins, the broader study design included clinical chemistry markers, steroids, and coagulation parameters.

Results:

Storage duration was the main factor influencing oxylipin degradation, reflected by both increases and decreases in analyte concentrations over time. These shifts suggest a combination of enzymatic activity and non-enzymatic oxidation during storage. Most analytes remained within acceptable change limits for up to four years. Beyond that, degradation became evident, with many oxylipins exceeding defined thresholds by year 11. No statistically significant differences were observed between samples stored at minus 80 °C and those stored in the gas phase of liquid nitrogen below minus 150 °C, indicating that minus 80 °C is sufficient for long-term preservation. Fill volume had no effect. However, tube type significantly affected analyte stability: of nine oxylipins with significant differences, eight were more stable in straw tubes than in conventional storage tubes.

Conclusion:

Storage duration is the principal determinant of oxylipin degradation. Although relevant degradation was observed after four years, storage at minus 80 °C maintained acceptable analyte profiles and represents a practical long-term storage option. The absence of significant differences compared to ultra-low temperatures supports this conclusion. Straw tubes conferred superior stability and are recommended. Oxylipins serve as sensitive quality biomarkers and support evidence-based improvements in long-term biobanking.

DGKL: 08. Endokrinologie, Biobanking

P-09-06

Method Comparison and Reference Range Verification of the new anti-Thyroid peroxidase assay on the Atellica Immunoanalyser

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Introduction: As one of the most common anti-thyroid autoantibodies, the anti-thyroid peroxidase (TPO) measurements have a considerable contribution to the diagnosis of autoimmune thyroiditis, together with the clinical assessment. The anti-TPO assays are known for the method variability, due to antibody heterogeneity inherent in the patients, that cannot be influenced by standardisation efforts. The new aTPOII-assay (Siemens Healthineers) has a different TPO antigen and detecting antibody source. It is also traceable to another international standard compared to the old aTPO-assay (Siemens Healthineers). The aims of this study were to verify the new assay through a method comparison (n=562) with the old aTPO-assay from the same manufacturer and to verify the Reference range (n=62) of the new aTPOII-assay on Atellica Immunoanalyzer (Atellica IM).

Methods: The assay comparison was determined using a 2×2 contingency table in accordance with the CLSI EP12-A2 document. A total of 562 patient serum samples were tested using the Atellica IM aTPO and Atellica IM aTPO II assays, and the positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) were calculated. The reference range verification on 62 euthyroid patient samples was performed in accordance with the recommendations set out in CLSI Document EP28-A3c. Additionally, we evaluated the within- and between-day precision and accuracy using commercially available quality control materials.

Results: Within- and between-day coefficients of variation were 9,1% at concentration of 27,1 kU/l and 5,9 % at concentration of 76,8 kU/l for and in accordance with manufacturer claims.

In the analytical accuracy analysis, the aTPOII-asssay revealed a trueness of 5,9% and 3,4 % for anti-TPO concentrations of 27,1 kU/I and 76,8 kU/I respectively.

The PPA between methods was at 79,1 %. The NPA was at 100% and the OPA was at 98,8 %.

The manufacturer’s reference interval (below 13.8 kU/L) was verified and confirmed in our population.

Conclusion: The wide heterogeneity of both exogenous antibodies and patient autoantibodies can lead to poor correlation and inconsistent patient results between anti-TPO assays. These two methods cannot be used interchangeably. Therefore, the new aTPOII-assay may substitute the old aTPO-assay only considering the following: patients must be monitored starting again with the new aTPOII-assay (not in comparison with the previous results of the Patient from the old aTPO-assay).

DGKL: 08. Endokrinologie, Biobanking

P-09-07

Prädiabetes und Knochenmikrostruktur: Geschlechtsspezifische Zusammenhänge mit MASLD-Markern

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Zielsetzung:

Diabetes mellitus Typ 2 (T2D) und Osteoporose erhöhen das Frakturrisiko und beeinträchtigen Lebensqualität und Mortalität. Der T-Wert, gemessen mittels Dual-X-Ray Absorptiometrie ist der Standard zur Frakturrisikobeurteilung, überschätzt jedoch bei gestörtem Glukosestoffwechsel die Knochendichte und liefert keine Informationen über die Knochenmikrostruktur. Ziel dieser Studie war es, den trabekulären Knochenscore (TBS) als Marker für die Knochenmikrostruktur in Bezug zu HbA1c und Markern der metabolisch-dysfunktionsassoziierten steatotischen Lebererkrankung (MASLD: Fib-4 Score und AST to Platelet Ratio Index [APRI]) zu untersuchen.

Methoden:

Zwischen Oktober 2021 und Dezember 2023 wurden bei 190 Personen (49/141 Männer/Frauen, Alter 65 [22/82] Jahre, HbA1c 5,5 [4,1/8,0] %) TBS (TBS iNsight v3.1.2) und T-Wert an Lendenwirbelkörpern 1 bis 4 gemessen und retrospektiv analysiert (Hologic Horizon W, Hologic Deutschland GmbH, Deutschland). Subgruppen wurden basierend auf HbA1c (< 5,7% vs. ≥ 5,7%; n=128/62) und Fib-4 (< 1,3 vs. ≥ 1,3; n=95/95) gebildet. Statistische Analysen erfolgten mittels Mann-Whitney Test, 2way ANOVA, Fisher-LSD Test und Spearman-Korrelation.

Ergebnisse:

Bei HbA1c ≥ 5,7% war der TBS um 3% reduziert (P=0,0259), der T-Wert blieb unverändert. Männer mit HbA1c ≥ 5,7% wiesen einen 70% höheren T-Wert auf (P=0,0111). Bei Fib-4 < 1,3 waren TBS (5%; P=0,0351) und T-Wert (40%; P=0,0251) bei Männern im Vergleich zu Frauen erhöht, während Männern mit Fib-4 ≥ 1,3 einen um 6% niedrigeren TBS zeigten (P=0,0164). TBS korrelierte bei Männern mit Fib-4 (R=-0,3767; P=0,0076) und APRI (R=-0,3868; P=0,0060) und bei Frauen mit Alter (R=-0,3232; P < 0,0001) und HbA1c (R=-0,2141; P=0,0108). T-Wert korreliert nur bei Männern mit HbA1c (R=0,3936, P=0,0051). Unabhängig von Subgruppen hatten Frauen tendenziell niedrigere Werte für TBS (3%; P=0,1305) und T-Wert (40%; P=0,0295). In Regressionsanalysen waren Alter (P < 0,001) bei Frauen und APRI bei Männern (P=0,041) signifikante Prädiktoren für TBS. Für T-Wert waren HbA1c (P=0,044) und APRI (P=0,027) insgesamt und HbA1c für Männer (P=0,045) signifikant.

Diskussion und Schlussfolgerung:

TBS als Marker für die Knochenmikrostruktur ist bei hohen HbA1c-Werten reduziert und korreliert bei Männern mit Markern für MASLD. Im Gegensatz dazu zeigen Männern mit erhöhtem HbA1c höhere T-Werte, was die Diskrepanz zwischen Knochendichte und Mikrostruktur unterstreicht. Diese Ergebnisse verdeutlichen die Auswirkung von Prädiabetes auf den Knochenstoffwechsel und betonen die Notwendigkeit einer geschlechtsspezifischen Berücksichtigung in der Diagnostik und Therapie metabolischer Erkrankungen.

DGKL: 08. Endokrinologie, Biobanking

P-09-08

Frühe mikrovaskuläre Veränderungen bei Prädiabetes und Typ-2-Diabetes: Bedeutung des EASIX-Scores und der sublingualen Mikroskopie

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Zielsetzung: Endotheliale Dysfunktion spielt eine zentrale Rolle bei der Entstehung vaskulärer Komplikationen bei Typ-2-Diabetes (T2DM). Der „Endothelial Activation and Stress Index“ (EASIX) hat sich als Marker für Endothelschäden in verschiedenen klinischen Szenarien erwiesen. Die sublinguale Mikroskopie ermöglicht zudem eine direkte Analyse der Mikrozirkulation. Ziel der Studie ist es, die endotheliale Dysfunktion bei T2DM näher zu charakterisieren und ihren Zusammenhang mit diabetesbedingten (mikro-)vaskulären Veränderungen und Komplikationen zu untersuchen.

Methodik: Es wurden Personen mit Typ-2-Diabetes (T2DM, n=59), Prädiabetes (PRED, n=20) und gesunde Kontrollprobanden (CON, n=17) untersucht. Der EASIX-Score wurde anhand von LDH, Kreatinin und Thrombozytenzahl berechnet. Metabolische Parameter und sogenannte mikrovaskuläre Komplikationen (Nephropathie, Neuropathie, Retinopathie) wurden

ebenfalls evaluiert. Ergänzend erfolgte eine sublinguale Mikroskopie (Glycocheck®) zur Analyse der Kapillardichte, der Flussgeschwindigkeit und der Perfused Boundary Region (PBR 4-25).

Ergebnisse: Nach Adjustierung für Alter, Geschlecht und Kreatinin zeigte sich kein signifikanter Unterschied im EASIX-Score zwischen den Gruppen (p=0,258). Innerhalb der T2DM-Gruppe korrelierte der EASIX-Score mit dem HOMA-IR (R=0,355, p=0,010), unabhängig von Alter, Geschlecht und BMI. Zudem war ein höherer EASIX mit einer erhöhten Prävalenz mikrovaskulärer Komplikationen bei T2DM assoziiert (R=0,268, p=0,046).

Die sublinguale Mikroskopie zeigte in den T2DM- und PRED-Gruppen eine reduzierte Dichte kleinster Kapillaren (4–9 µm) im Vergleich zur Kontrollgruppe, unabhängig vom Alter (CON=29,08±5,98; PRED=20,09 [95%-KI: 18,17–28,79]; T2DM=21,74±6,97; p=0,012). Zwischen den Gruppen bestand kein signifikanter Unterschied in der PBR4-25 (p=0,545). Innerhalb der T2DM-Gruppe zeigte sich jedoch, dass eine erhöhte PBR4-25 mit einer niedrigeren GFR nach CKD-EPI (R=-0,412, p=0,013) und einem höheren Kreatininspiegel (R=0,430, p=0,009) assoziiert war, unabhängig von Alter, Geschlecht und BMI.

Schlussfolgerung: Die Ergebnisse zeigen eine reduzierte Kapillardichte bei Patienten mit T2DM und Prädiabetes. Die bereits bei Prädiabetes verminderte Dichte kleinster Kapillaren weist auf frühe vaskuläre Schäden hin. Zusätzlich deutet der erhöhte EASIX-Score auf Endothelschäden im Zusammenhang mit Insulinresistenz und mikrovaskulären Komplikationen hin. Die Ergebnisse zeigen, dass morphologische Veränderungen der Mikrozirkulation und der EASIX-Score unterschiedliche, aber komplementäre Aspekte der Pathogenese widerspiegeln.

DVTA: 02. Aus Qualitätssicherung und Labormanagement

P-05-01

Benchmarking für Laborexzellenz: Identifikation innovativer Verfahren in klinischen Laboren

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Die dynamischen Entwicklungen im Gesundheitswesen mit sich rasch ändernden Anforderungen beeinflussen auch die Organisation und die Zielsetzungen großer Laborbereiche in Kliniken. Neben der technologischen Ausstattung und dem operativen Betrieb rücken dabei zunehmend strategische Fragestellungen und organisatorische Zielsetzungen der zugeordneten Bereiche in den Fokus.

Die Autoren beleuchten aktuelle Herausforderungen großer Kliniklabore und zeigen Ansatzpunkte für Benchmarks auf, die wegweisend für die Ausrichtung auf zukünftige Zielsetzungen im Hinblick auf Effizienz, Standardisierung, Digitalisierung und stetige Optimierung der Abläufe sind.

Im Rahmen einer Benchmark-Analyse wurden die Abläufe in südbayerischen Kliniklaboren systematisch auf Basis einer objektivierten Statusbestimmung untersucht. Der Fokus lag dabei auf einer prozessorientierten Betrachtung der Laboratorien. Die Datenerhebung erfolgte durch direkte Beobachtung und gezielte Nachfragen, gestützt durch einen strukturierten Fragebogen zur Erfassung relevanter Prozesskennzahlen. Die Ergebnisse dienen als Modell für die Etablierung von Optimierungsansätze in großen Laborbereichen.

DVTA: 02. Aus Qualitätssicherung und Labormanagement

P-05-02

Stabilitätsvergleich von Kalium im Lithiumheparinatplasma und Serum

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Das Ziel dieser Arbeit ist es zu ermitteln, welchen Einfluss das Untersuchungsmaterial auf die Ermittlung des Kaliumspiegels in einer Probe hat. Am 14.04.2023 aktualisierte die Bundesärztekammer die Richtlinien für den Parameter Kalium. Ab April 2026 ist nur noch Lithiumheparinatplasma für die Messung zugelassen, da laut der Bundesärztekammer im Serum falsch hohe Werte auftreten. Kalium ist von hoher medizinischer Bedeutung, daher wird eine Vergleichsmessung durchgeführt. Vor der Messung werden die Proben bei Raumtemperatur für 2, 4, 6 und 24 Stunden gelagert und die Abweichungen mittels t- und p-Test ausgewertet. Die Ergebnisse zeigten signifikante Abweichungen zwischen Plasma und Serum. Im Lithiumheparinatblut lagen die Werte deutlich unter denen im nativen Blut. Im zeitlichen Verlauf war ein Anstieg des Kaliums im Plasma mit einer zunehmenden Streuung der Messergebnisse erkennbar, während im Serum ein geringerer Anstieg und eine minimale Streuung der Werte beobachtet wurde. Im Lithiumheparinatplasma konnte eine Stabilität von mindestens sechs Stunden und im Serum von mindestens 24 Stunden ermittelt werden. Die Aussage der Bundesärztekammer ließ sich nicht bestätigen. Beide Materialien eignen sich für die Kaliumbestimmung. Die Verwendung unterschiedlicher Referenzbereiche wird empfohlen. Aufgrund der Stabilität sollte Serum ab einer Lagerdauer von sechs Stunden bevorzugt werden. Weitere Forschungen sind notwendig, um engere Stabilitätszeiträume zu definieren.

DVTA: 02. Aus Qualitätssicherung und Labormanagement

P-05-03

Correlation analysis and verification of routine laboratory parameter PIVKA-II considered for the novel GAAD algorithm-associated diagnostics

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Introduction:

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related mortality worldwide. Early risk stratification is critical for optimizing clinical management and enabling individualized therapeutic strategies. The GAAD score — which includes alpha-fetoprotein (AFP), des-γ-carboxy prothrombin (DCP), also known as protein induced by vitamin K absence or antagonist-II (PIVKA-II), as well as patient sex and age — has recently emerged as a non-invasive biomarker-based algorithmic tool for prognostic assessment in patients with chronic liver diseases at increased risk for developing HCC. Despite its clinical promise, the routine integration of the GAAD score in laboratory diagnostics has not yet been widely adopted. Standardized and automated calculation and reporting of the GAAD score could enhance its practical utility into clinical workflows.

Aims and Methods:

This study aims at verification of the routine laboratory parameter PIVKA-II and evaluation of GAAD score-associated laboratory parameters for score implementation into routine clinical laboratory diagnostics. The laboratory information system was adapted to enable automated calculation of the score based on routinely measured parameters. A retrospective laboratory data analysis of samples obtained from HCC patients will be conducted to assess the technical feasibility, accuracy and clinical relevance of GAAD score reporting. The score integration into clinical routine was supported by interdisciplinary collaboration between laboratory medicine and clinical departments.

Results:

Patient samples underwent comparative measurements using both laboratory methods AFP (measurement at an accredited site) and PIVKA-II (comparative measurement at an accredited site vs. an already established laboratory site). The automated implementation of the GAAD score and the requirement of technical adjustments to the existing laboratory infrastructure will be assessed. A systematic evaluation of the reliability of score calculation and its implementation in the final laboratory report will be performed. Retrospective correlation of the GAAD score and routine laboratory parameter AFP will help identify incongruent cases. The turnaround time for score availability will be assessed to ensure clinical usability.

Conclusion:

AFP and PIVKA-II are well-established biomarkers used in the surveillance of patients with chronic liver diseases. Their measurement within routine laboratory diagnostics is feasible and provides meaningful clinical information for HCC risk stratification.

However, an additional algorithmic GAAD score calculation could further improve the clinical utility of these parameters. Standardized reporting of the score may contribute to more efficient decision-making in interdisciplinary oncological care and supports the integration of biomarker-based tools into routine clinical practice.

DVTA: 02. Aus Qualitätssicherung und Labormanagement

P-05-04

Verkürzung der TAT für die Bestimmung der Anti Xa Inhibitoren bei vollautomatisierter Probenabarbeitung 24/7

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Zielsetzung

Die Turnaround Time (TAT) spielt in jedem Labor eine große Rolle. Besonders in Notfallsituationen, wie beispielsweise einem Schlaganfall, zählt jede Minute, bis die Laborergebnisse dem Arzt vorliegen und die richtige Therapieentscheidung getroffen werden kann. Wichtige Messgrößen aus dem Bereich der Gerinnung sind die aktivierte partielle Thromboplastinzeit (aPTT), der International Normalized Ratio (INR) Wert und die Anti Xa Inhibitoren (Rivaroxaban, Apixaban, Edoxaban, unfraktioniertes Heparin (UFH), niedermolekulares Heparin (NMH), Fondaparinux, Orgaran).

Bei einer vollautomatisierten Probenbearbeitung unterschiedlicher Materialien sind unterschiedliche Zentrifugationsanforderungen in einem Workflow zu vereinen. Bei Untersuchungsmaterialien wie Serum und Lithiumheparin ist eine Zentrifugation von fünf Minuten bei 3.280 x g ausreichend. Für die Gerinnungsanalysen wird von der Clinical & Laboratory Standards Institute (CLSI) Guideline (H21-A4, Vol.23 No.35) ein thrombozytenarmes Citratplasma gefordert [1].

Um diese Bedingungen zu erfüllen und eine parallele Bearbeitung aller Materialien auf einer Automation unter Berücksichtigung der kurzen Zentrifugationszeit von fünf Minuten zu realisieren, wurde eine doppelte Zentrifugation für Citratröhrchen eingeführt. Bereits im Jahr 2014 konnte gezeigt werden, dass es keine signifikanten Unterschiede bei der aPTT und INR Messung nach einfacher und doppelter Zentrifugation gibt [2]. Ziel der vorliegenden Arbeit war es, die TAT der direkten oralen Antikoagulanzien (DOAKs) ebenfalls hinsichtlich einer Verkürzung der Zentrifugationszeit zu optimieren. Aktuell liegt die durchschnittliche TAT am Beispiel der einfach zentrifugierten INR bei 36 min (Median: 34 min, 75% Perzentil: 40 min, 90% Perzentil: 50 min)

Methoden

Als Probenmaterial wurde Citratplasma von 51 Proben mit bekannter DOAK-Konzentration verwendet. Dieses wurde nach einmaliger Zentrifugation (fünf Minuten bei 4.000 x g) am CS5100 (Siemens Healthineers) mittels des chromogenen Assays von Hyphen (BIOPHEN™ Heparin LRT) bestimmt. Anschließend wurden die Proben erneut fünf Minuten bei 4.000 x g zentrifugiert und die DOAK-Konzentration nochmals bestimmt.

Ergebnisse

Es zeigten sich keine signifikanten Unterschiede in den Messergebnissen zwischen erster und zweiter Zentrifugation. Für alle untersuchten DOAKs (Rivaroxaban, Apixaban, Edoxaban) und Heparine/Heparinanaloga (UFH, NMH, Fondaparinux, Orgaran) betrug die Steigung der Regressionsgeraden 1,0 und der Korrelationskoeffizient 0,99.

Diskussion und Schlussfolgerung

Die einfache, und damit um fünf Minuten reduzierte, Zentrifugation von Citratplasma liefert dieselben Messwerte bei der Bestimmung der Anti Xa Inhibitoren wie die zweifache Zentrifugation. Dies erlaubt, die TAT für diese wichtigen Messgrößen zu verkürzen. Die schnellere Verfügbarkeit der Ergebnisse wäre für eine schnellere Therapie bei sehr zeitkritischen Notfällen wie dem Schlaganfall möglich.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-05

Heart and Liver: hepatocyte secreted coagulation factor XI regulates the collagen fibrogenesis of human cardiac fibroblasts in an in vitro model for cardiac fibrosis

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Introduction:

Liver disease is known as a risk factor for heart failure, but the underlying mechanism of this interaction escaped elucidation until recently. In 2022, Cao et al. published research showing that coagulation factor XI (FXI), secreted by the liver, might mediate signalling along the liver-heart axis. In a cohort of genetically diverse inbred mouse strains, FXI expression was inversely correlated with diastolic dysfunction. Cao et al. concluded that FXI protects against diastolic dysfunction and therefore heart failure. While the mechanism was clearly revealed in mice, a human cohort did not show significant results in this regard. In this study, we established an in vitro cell-culture model using human primary cells to elucidate whether this effect is relevant to human cardiac pathobiology.

Methods:

Human primary cardiac fibroblasts (HCFs) were isolated from hearts derived from patients undergoing heart transplantation. The isolated cells were analysed using immunofluorescence and flow cytometry to verify homogeneous fibroblast cultures (>98%). Human hepatocellular carcinoma cells (HepG2) were used as liver cells. CRISPR/Cas9-mediated knockout of FXI was performed in HepG2 and validated using Sanger sequencing, qPCR and Western blot. HepG2 wildtype (wt) and HepG2 FXI-/- were used to produce conditioned cell culture media. HCFs were cultured in conditioned media. To imitate profibrotic conditions, cells were induced with transforming growth factor β1 (TGFβ1). Changes in matrix component secretion and profibrotic gene expression were assessed using qPCR and Western blot.

Results:

Using CRISPR/Cas9, we successfully induced mutations within the F11 gene of HepG2. After selection and single-clone isolation, a clone with a homozygous nonsense F11-mutation was generated. Treating HCFs with conditioned media of wt HepG2s, the inductive effects of TGFβ on matrix component expression, such as collagen type-I and fibronectin, were significantly increased compared to fibroblasts cultivated in non-conditioned media. Using conditioned media derived from FXI-/- HepG2s, no such effect was observed.

Conclusion:

Our findings imply that, at least in vitro, hepatocytes secrete proteins that upregulates matrix biosynthesis in cardiac fibroblasts under profibrotic conditions. A knockout of F11 in hepatocytes reverses this effect, indicating that FXI may mediate it. Our in vitro study does show opposite effects compared to the cohort mice study of Cao et al. The effect Cao et al. found in mice may be due to secondary interactions of F11 with other tissues and organs leading to a cardio-protective effect mediated by not yet identified compounds. Further, Cao et al. were unable to detect any significant effects in a human cohort, indicating a possible species specificity of the observed effects. Nevertheless, the results obtained by our group highlight the important role of hepatocyte related metabolites in systemic- and local tissue remodelling.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-06

Xylosyltransferase-II deficiency alters macrophage polarization and osteoclast differentiation in bone remodelling

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Introduction: The extracellular matrix (ECM) is a non-cellular, 3D-structure and occurs ubiquitously in all tissues, including bone. It contains proteoglycans (PG), whose glycosylation is initiated by xylosyltransferase (XT), which has two human isoforms, XT-I and XT-II, encoded by XYLT1 and XYLT2. Mutations in XYLT2 cause the rare skeletal disorder spondylo-ocular syndrome, mainly characterised by severe osteoporosis and skeletal abnormalities.

Bone remodeling is primarily mediated by osteoclasts, which differentiate from macrophages (MΦ), derived from monocytes. MΦ are divided into three subtypes: M0 (inactive), M1 (pro-inflammatory) and M2 (anti-inflammatory). MΦ are essential for successful bone regeneration, and an absence of M1-like MΦ in particular leads to reduced formation of osteoclasts, as well as reduced activity of the NF-κB signalling pathway.

Methods: Monocytes were seeded at a density of 1×10^5 cells/cm^2 for macrophage differentiation and 2×10^5 cells/cm^2 for osteoclastogenesis. Macrophages were cultured for 8 d, with polarization induced on day 6. Osteoclasts were cultivated for 14 d. A XYLT2-siRNA-knockdown was performed 5 d after seeding and in osteoclasts this procedure was performed every 72 h

afterwards. Macrophages were analyzed via quantitative real-time PCR, flow cytometry and a phagocytic activity assay. Osteoclasts were analyzed by quantitative real-time PCR, Tartrate-resistant acid phosphatase (TRAP) staining, immunofluorescence and a pit assay.

Results: After XYLT2 knockdown in macrophages, a significant reduction in M1 markers was observed in M1-like MΦ. Conversely, a significant increase in the expression of M2 markers was detected in M1-like MΦ, while these markers increased in M2-like MΦ after XYLT2 knockdown. Furthermore, the NF-κB signalling pathway was downregulated in all MΦ phenotypes. Altered cellular functionality was detected using a phagocytosis assay: M1-like MΦ exhibited significantly increased phagocytic activity, while M2-like MΦ exhibited decreased activity.

Reduced osteoclast formation was observed following a serial XYLT2 knockdown during monocyte-MΦ-osteoclast differentiation. This was demonstrated by significantly reduced gene expression and immunofluorescence of various osteoclast markers and by TRAP staining. Functionality was analyzed using a pit assay.

Conclusion: The reduction of M1 markers and NF-κB components in M1-like MΦ after XYLT2 knockdown indicates impaired pro-inflammatory polarization. As NF-κB is a key driver of both M1 polarization and osteoclast differentiation (via RANKL-NF-κB-NFATc1 axis), these findings suggest that XT-II may be important for an effective osteoclastogenesis. The impaired differentiation and functionality of osteoclasts observed under XYLT2-deficient conditions may reflect a disturbed ECM-mediated regulation of intracellular signalling. These results provide first indication for the role of XT-II in monocyte-MΦ -driven osteoclastogenesis and bone remodeling.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-07

Exploring the Persistence of Respiratory Virus-Specific Cellular Responses Across the 2024 Summer Season in Germany: A Prospective Study in Healthcare Workers

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Introduction:

T cell-mediated immunity plays a critical role in the control and resolution of acute respiratory infections (ARI). Understanding the stability and seasonal waning of virus-specific cellular responses over time is essential for evaluating long-term immune protection, especially in populations with repeated exposure. This study aimed to assess the persistence of IFN-γ-secreting cellular responses in healthcare workers (HCWs) between the end of the 2023/24 ARI season and the start of the 2024/25 season, focusing on RSV, Influenza A/B, and SARS-CoV-2.

Methods:

As part of the prospective, longitudinal ARIPro cohort study investigating the incidence, risk factors, and preventability of ARI in HCWs, Cell Preparation Tubes (CPT) were randomly collected from participants within the cohort at two time points: After the 2023/24 ARI season (post-season: April 1–30, 2024, n = 45) and before the 2024/25 season (pre-season: October 1–31, 2024, n = 36). Peripheral blood mononuclear cells (PBMCs) were isolated from CPT samples and cellular reactivity was determined using a non-commercial IFN-γ ELISpot assay following stimulation with RSV Antigen (Virion\Serion), a seasonal Influenza vaccine (Influvac Tetra 2024/25, Viatris) targeting Influenza A (H1N1) and B, and the SARS-CoV-2 Spike Ectodomain (S1-S2) Antigen (Virion\Serion). Spot-forming units (SFU) per 10^6 PBMCs were quantified after background subtraction (medium control). Non-parametric analyses were conducted using Mann-Whitney-U test. P-values were corrected according to Krüger and Yekutieli (α=0.05).

Results:

Due to varying cell availability, the number of valid measurements differed between antigens. Cellular reactivity against RSV significantly declined from the post-season to the pre-season 2024 period ($p = 0.001$). A similar not significant trend ($p = 0.24$) was observed in response to Influenza Antigen. In contrast, cellular reactivity targeting the SARS-CoV-2 Spike Antigen remained stable across the same period ($p = 0.98$).

Conclusion:

These findings indicate a marked seasonal decline in virus-specific cellular reactivity to RSV Antigen and to a lesser extent, Influenza A/B Antigens—from spring to autumn, whereas the spike-specific cellular reactivity was maintained. This may in part explain the seasonal risk of acquiring reinfection with RSV or Influenza, particularly as successful immune evasion mechanisms have been described for RSV in literature being successful to underwent cytotoxic T cell-mediated clearance.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-08

Low abundance, high impact: Quantitative Mass Spectrometry for Coagulation Factors

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The treatment and prevention of cardiovascular diseases (CVD) is essential for the well-being of a modern society as these diseases represent the leading cause of mortality worldwide. CVD involve acute thromboembolic events such as strokes or heart attacks – events that can be fatal but are often preventable if the high risk of them is recognized at an early stage. Currently established clinical methods often use indirect approaches or focus mainly on a limited number of factors. Therefore, they often reach limitations regarding specificity, sensitivity as well as the inability to fully elucidate entire mechanisms making them insufficient for a detailed risk assessment.

As part of the blood clotting process (hemostasis) the coagulation cascade is a key player for thrombus formation. In this study we demonstrate the feasibility of a 30-minute Multiple-Reaction-Monitoring LC-MS/MS-Assay consisting of 27 target proteins, compromising the coagulation cascade and other regulatory pathways. Here we optimized the bottom-up proteomics sample preparation and therefore compared three frequently applied protocols (Suspension-Trapping (S-Trap), Filter-Aided-Sample-Preparation (FASP), and In-Solution-Digestion). In addition, two different durations for the tryptic digestion (2 h and 16 h) followed by a micro-flow HPLC coupled to a Triple-Quadrupole Mass Spectrometer were investigated.

We measured a sample cohort consisting of 56 patients with chronic kidney disease at multiple time points over a period of up to 40 weeks resulting in over 850 samples. Serial dilution curves for each peptide for the determination of the figures of merit limit of detection and lower limit of quantitation and equidistant calibration curves for the final quantitation were measured. Dysregulations could be identified by comparing the patient samples to certified plasma controls.

Given the challenge to detect some low abundant proteins, we furthermore tested several different fractionation, enrichment, and depletion methods utilizing both classical acid-based as well as newer nanoparticle-based methods.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-09

Indirekte Referenzintervalle des Parathormons - ein Blick über die Alters- und Geschlechtsabhängigkeit hinaus

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Das Parathormon (PTH) spielt eine entscheidende Rolle für die Kalziumhomöostase des menschlichen Körpers. Trotz der bekannten Abhängigkeit des Knochenstoffwechsels und der Kalziumhomöostase von Alter und Geschlecht, geben viele Testhersteller in ihren Testkit-Unterlagen lediglich ein einheitliches Referenzintervall für alle Patientinnen und Patienten an. Darüber hinaus gibt es bei Methodenvergleichen sowie Ringversuchen erhebliche Messunterschiede zwischen den Geräteplattformen.

Wir verwenden zwei indirekte Methoden zur Überprüfung von Referenzintervallen (reflimR und refineR), um alters- und geschlechtsabhängige Referenzintervalle zu berechnen. Darüber hinaus untersuchen wir die Abhängigkeit der Referenzintervalle von der Nierenfunktion, dem Vitamin-D-Stoffwechsel und der jährlichen Saisonalität.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-10

Seroincidence of RSV, Influenza A/B, and SARS-CoV-2 among Healthcare Workers in the 2024/2025 Winter Season: Insights from a Longitudinal Seroepidemiological Cohort Study

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Introduction:

The incidence of acute respiratory infections (ARIs) requires particular attention in healthcare settings where vulnerable patient groups and highly exposed employees come into contact. The contribution to nosocomial transmission chains and workforce absenteeism poses a significant threat. Therefore, investigating the seroincidence of ARIs is fundamental to better understanding their spread and impact.

Methods:

The prospective ARIPro cohort study assessed the incidence of RSV, Influenza A/B, and SARS-CoV-2 infections among healthcare workers before (October 2024) and after (April 2025) the winter season 2024/25. Per participation per timepoint a serum blood sample combinded with a study questionnaire on ARI infections and vaccinations was collected. SERION ELISA classic Respiratory Syncytial Virus IgG was used to quantify the antibody levels against RSV, using whole virus lysate as target. Individuals vaccinated against RSV were excluded from the analysis due to interference of vaccine-induced antibodies with the immunoassay. The Influenza antibody levels were quantified using SERION ELISA classic Influenza A and B IgG, targeting the nucleo/matrix proteins of Influenza A and B. Roche Elecsys Anti-SARS-CoV-2 antibody test, targeting the

nucleocapsid was used to quantify antibody levels against SARS-CoV-2. Seroconversion was defined as a 2-fold increase of the IgG levels.

Results:

The study cohort consisted of 426 healthcare workers (HCWs) who participated in the ARIPro study in both October 2024 and April 2025. After the 2024/2025 winter season, 7.8% (33/425, 95% CI: 5.6%–¬¬¬¬10.7%) of the study population showed seroconversion to RSV. 7.8% (33/426, 95% CI: 5.5%–10.7%) of HCWs seroconverted to Influenza A and 12.7% (54/426, 95% CI: 9.8%–16.2%) to Influenza B. Data on SARS-CoV-2 incidence will be presented at the conference.

Conclusion:

The assessment of seroincidence is a suitable method to uncover the epidemiology of acute respiratory viruses, regardless of symptomatic manifestation and screening deficiencies. In the present study, Influenza B appeared to be the predominant pathogen during the 2024/2025 winter season, surpassing Influenza A and RSV. This preeminence contrasts with national surveillance data from the Robert Koch Institute (RKI), which reported higher incidences for Influenza A than for Influenza B and RSV. One possible explanation is that Influenza B and RSV are commonly associated with milder symptoms in adults, which may lead to a higher rate of unrecognized infections and, consequently, underreporting in routine surveillance. These findings underline the importance of using seroepidemiology in understanding the dynamics of ARI occurrence.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-11

Evaluation of the Detuning Ratio as a Tool to Detect Potential Interference in LC-MS/MS Analysis

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INTRODUCTION:

While MS/MS is in general a highly specific technique, the presence of unexpected substances in samples can result in interference due to shared mass transitions with target analytes (isomeric/isobaric interferences). Clinical laboratories typically address this issue by monitoring ion ratios (IR) to recognize potential interferences. To supplement this approach, differential tuning effects can be assessed. We aimed to evaluate a complementary method - the detuning ratio (DR) - for its ability to detect isomeric or isobaric interferences.

METHODS:

A DR was based on differential influences of instrument settings of MS systems on the ion yield of a respective target analyte; isomeric or isobaric interferences can lead to a shift of the DR in an affected sample. By quantifying the concentration and determining the IR and DR in samples in which known isomeric interference substances have been spiked to the target analyte, the applicability of DR detection was quantitatively investigated and compared with concentration as well as IR. This experiment was performed for two compound pairs (Cortisone/Prednisolone and O-Desmethylvenlafaxine/cis-Tramadol HCl). In both pairs matching mass transitions can be observed in a collision induced dissociation scan.

RESULTS:

The DR method correctly indicated the presence of isomeric interferences in two independent test systems: Cortisone/Prednisolone and O-Desmethylvenlafaxine/cis-Tramadol HCl. In these two spiking experiments, we observed that the DR and

the IR were both suited to indicate interference in a quantitative model system of interference by known compounds sharing mass transitions with the respective target analyte. DR was found highly reproducible in analytical runs (with a CV ≤5.3%, n=20).

CONCLUSION:

The DR approach provides a valuable supplementary tool for detecting isomeric or isobaric interferences in individual samples analyzed by LC-MS/MS. When used alongside conventional IR monitoring, it can improve the analytical reliability of clinical MS-based assays. A major advantage of DR over IR is its applicability to target analytes with only a single sufficiently abundant mass transition. This is particularly important for immunosuppressants such as tacrolimus and cyclosporine.

The DR principle has a broad potential and can be used in all areas of LC-MS/MS application, even beyond biomedical analyses.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-12

Polyphosphate as a novel biomarker in thrombosis

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Introduction: Polyphosphate (polyP) is a linear polymer composed of three to several thousand phosphate (Pi) residues linked by high-energy phosphoanhydride bonds, and is ubiquitous across biological systems. Upon activation, human platelets release polyP, which remains associated with the plasma membrane as Ca^{2+}-rich nanoparticles. While animal models have demonstrated a critical role for platelet-derived polyP in thrombosis, its function in patients and potential utility as a biomarker remain poorly understood.

Methods: This study aimed to establish a diagnostic assay for detecting polyP in human whole blood. Given the repetitive and non-immunogenic nature of polyP, no antibodies are available for its detection. We therefore generated a recombinant polyP-specific probe (PPBD), derived from the substrate-binding domain of E. coli exopolyphosphatase PPX1, and developed a flow cytometry-based assay using fluorescently labeled PPBD.

Results: In healthy donors, platelet stimulation with collagen-related peptide (CRP-A), adenosine diphosphate (ADP), and TRAP-6 induced dose-dependent increases in polyP surface exposure. The magnitude of polyP signal correlated with platelet activation status, as indicated by P-selectin positivity. In patients receiving the P2Y12 inhibitor clopidogrel, ADP-induced polyP release was significantly reduced, supporting the specificity of our PPBD-Alexa 647 flow cytometry assay. In contrast, TRAP-6-induced polyP exposure remained unaffected by P2Y12 blockade. PolyP signals correlated with standard platelet function assays, including light transmission aggregometry, real-time thrombin generation, and the platelet function analyzer (PFA-100).

Conclusion: We present the first diagnostic assay for the detection of platelet-derived polyP in human blood and demonstrate that polyP is a sensitive biomarker of platelet activation. This assay provides a platform for future translational studies evaluating the role of polyP in thromboinflammatory disorders

DVTA: 03. Aus Wissenschaft und Forschung

P-05-14

The 24/7 implementation of NGAL for early detection of AKIN

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Introduction:

Acute kidney injury (AKI) is defined by the KDIGO criteria as an increase in serum creatinine by ≥26.5µmol/L within 2 days, an increase by ≥1.5-fold within 7 days, or a urine volume of ≤0.5ml/kg/h for 6 hours. AKI is associated with increased morbidity and mortality. It leads to chronic kidney disease in up to 50% of cases. However, urine output is not reduced at the onset of AKI, and creatinine levels do not increase initially, which hampers early diagnosis and treatment. Neutrophil gelatinase-associated lipocalin (NGAL) is a biomarker of proximal tubule damage that rises after just a few hours. NGAL was discovered in research more than 20 years ago, but it has never become a 24/7 available biomarker to protect kidney function. Recently, an NGAL assay became available for clinical chemistry analyzers. We aim to validate the assay with regard to the IVDR.

Method:

We used the NGAL calibrator, controls, and reagents from BioPorto Diagnostics GmbH (Hellerup, Denmark). All experiments were measured on the Atellica (Siemens) analyzer. A total of ten experiments with 1,204 individual measurements were conducted for validation. The testing included precision and accuracy, analytical sensitivity, linearity, freeze-and-thaw cycle, carryover, stability, analytical specificity, recovery rate, high-dose hook effect, and method comparison with cobas (Roche).

Results:

LoB (10.8 and 8.3 ng/mL) and LoD (24.3 and 23.3 ng/mL) is tested for two LOTs. LoQ is at 116.6 ng/mL. The test is linear in the range from 71.8 ng/mL to 3,114 ng/mL with coefficient of variation (CV) below 8%. The sample concentrations are stable for at least 3 days at 20°C , 7 days at 5-8°C, 30 days at -20°C and 60 days at -80°C. NGAL is affected by increased microalbumin (512 mg/L) and hemoglobin (131 – 249 mg/dL) concentrations. Glucosuria have no effects on the NGAL measurements. No high -dose-hook-effect was detectable up to 6,000 ng/mL and with auto-dilution up to 25,000 ng/mL. The sample concentration remains stable over three freeze-thaw-cycles. The average recovery rate is 106%. Method comparison shows a mean coefficient of variation (CV) of 3%. Nevertheless, 23 samples exceed the 10% limit. Comparability is shown between 150 and 400 within the scope of medical decision-making.

Conclusion:

AKI is common. The Institute of Clinical Chemistry and Laboratory Medicine at the UKE provides the first 24/7 NGAL measurements in Germany and the first NGAL measurements on the Atellica in Europe. The NGAL test© provides a rare opportunity to facilitate a promising research biomarker for patient diagnostics. The assay provides technically accurate measurements. We are in contact with the company to improve the precision in the lower measurement range on the Atellica. In collaboration with clinicians, we will verify the clinical benefits on in- and outpatient cohorts.

DVTA: 03. Aus Wissenschaft und Forschung

P-05-13

Vascular Aging and Diabetes Mellitus: Identification and Characterization of High-Risk Phenotypes in a Population-based Cohort

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Introduction:

Lifelong exposure to various known and unknown risk factors gradually alters the structural and functional characteristics of arteries , contributing to the development of vascular aging (VA). Chronological age alone fails to capture the heterogeneity of VA. The concept of Early Vascular Aging (EVA) and Supernormal Vascular Aging (SUPERNOVA) reflects individual differences in arterial aging, with EVA indicating premature vascular decline and SUPERNOVA representing exceptional vascular resilience. The Vascular Aging Index (VAI), as described by Wadström et al. 2019 (1), facilitates a phenotypic classification of vascular aging. It combines carotid-femoral pulse wave velocity (PWV) and carotid intima-media thickness (cIMT). Diabetes mellitus (DM), a key driver of macrovascular damage, often leads to accelerated vascular aging, yet variability exists: some individuals with DM remain cardiovascularly healthy despite long disease duration. Understanding the differing biomarker profiles of individuals on both sides of the vascular aging spectrum may enable early risk prediction and personalized prevention.

Methods:

We analyzed data from the LIFE-Adult study, a population-based cohort in Leipzig, Germany, comprising over 10,000 individuals. EVA and SUPERNOVA phenotypes were defined as individuals in the ≥90th and ≤10th percentile of the VAI distribution, respectively. Mortality was assessed via registry data over a mean follow-up of 145 months. EVA and SUPERNOVA participants with and without DM were selected for in-depth biomarker profiling using the Proximity Extension Assay OLINK CVD III panel. A total of 217 individuals in 4 groups (EVA with DM (n=55); EVA without DM (n=45); SUPERNOVA with DM (n=54); SUPERNOVA without DM (n=63)) were analysed.

Results:

Preliminary survival analyses indicate significant differences in mortality between EVA and SUPERNOVA groups. EVA individuals with DM had the highest all-cause mortality, confirming the compounding risk of metabolic disease and vascular aging. Individuals classified as SUPERNOVA (independent of DM status) and EVA without DM demonstrated superior survival. Preliminary data indicate group differences for e.g. GDF-15 (Growth/differentiation factor-15), especially for EVAs with DM.

Discussion:

Our findings support the clinical relevance of the EVA/SUPERNOVA model for vascular aging. The EVA definition by VAI score identifies high-risk individuals, particularly in the presence of DM, where vascular damage appears to be accelerated. SUPERNOVA individuals, despite risk exposure, exhibit remarkable vascular resilience. Ongoing proteomic analyses using OLINK will explore biochemical signatures of EVA and SUPERNOVA phenotypes, with the goal of developing a laboratory-based scoring system for early risk prediction. This may serve as a basis for individualized prevention approaches, especially in diabetic patients who are often inadequately characterized by standard glycemic markers like HbA1c.

DVTA: 04. Entwicklungsprojekte aus der Laborpraxis

P-05-15

Methodenvalidierung: Cystin in Granulozyten

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Einleitung – Cystinose ist eine seltene angeborene schwere Stoffwechselstörung mit etwa 130 bekannten Fällen in Deutschland. Infolge eines homozygoten Transporterdefekts kristallisiert Cystin in vielen Zelltypen und es kommt zu mannigfaltigen Gewebe- und Organschäden. Unbehandelt verläuft eine Cystinose immer tödlich.

Als Erbkrankheit ist die Cystinose nicht heilbar, mit Cysteamin steht aber ein cystinsenkendes Therapeutikum zur Verfügung. Für die Therapieüberwachung wird routinemäßig die Cystinkonzentration in Leukozyten gemessen. Weil aber Leukozyten heterogen sind, sie unterschiedlich viel Cystin akkumulieren und sich ihre Zusammensetzung ändern kann, ist die Cystinmessung in einem engeren Zellpool eindeutig zu bevorzugen. In dieser Arbeit wird gemäß der S3-Leitlinie Cystinose (2025-05) die Messung in Granulozyten validiert, da diese Cystin am stärksten anreichern.

Methoden – Es werden zwei Isolationsmethoden verglichen, die Dichtegradientzentrifugation und die Negativselektion im Magnetfeld. Nachdem Ausbeute und Reinheit der Isolate bestimmt wurden, werden die Cystinkonzentrationen der Zellen mittels HPLC gemessen und mit den jeweiligen Proteinkonzentrationen normalisiert.

Resultate – Mittels Dichtegradient wurden meist deutlich unter 15% der in der Vollblutprobe enthaltenen Granulozyten gewonnen. Mittels Negativselektion im Magnetfeld konnten hingegen 70-80% angereichert werden, gleichzeitig waren die Zellen sehr rein (99% Granulozyten). Dadurch lässt sich das eingesetzte Probenvolumen von aktuell 7 mL auf 2-3 mL Blut senken, was insbesondere in der Pädiatrie zu begrüßen ist (Patient-Blood-Management). Ein weiterer Nachteil des Dichtegradienten ist das Probenzeitfenster: Bereits nach einem Tag wurden die Granulozyten massiv von Erythrozyten überlagert. Dagegen gelang die Isolation mittels Negativselektion noch mit vier Tage alten Proben verlustfrei, was von großem Vorteil bei externen Einsendungen ist.

Erwartungsgemäß stellen sich die Cystinkonzentrationen in Granulozyten höher dar, als in heterogenen Leukozyten (Faktor 5,5). Für die Umstellung der Cystindiagnostik auf Granulozyten bedarf es eines (laborinternen) Referenzintervalls. Hierfür werden heterozygote Merkmalsträger untersucht, z.B. die Eltern erkrankter Kinder, in deren Zellen Cystin in subklinischen Mengen akkumuliert. Wegen der niedrigen Fallzahlen ist dies jedoch sehr langwierig. In der Normalbevölkerung ist intrazelluläres Cystin nämlich praktisch nicht nachweisbar.

Schlussfolgerung – Ziel der cystinsenkenden Therapie ist es, den Cystinspiegel der Patienten auf das subklinische Niveau von heterozygoten Merkmalsträgern zu verringern. Mit dem Wechsel der Zellaufreinigung hin zur Negativselektion verlängern wir das Probenzeitfenster und reduzieren zugleich das benötigte Blutvolumen deutlich. Darüber hinaus erhöht die Cystinmessung in Granulozyten die Ergebnisqualität und verbessert somit die Überwachung der cystinsenkenden Therapie bei Patienten mit Cystinose.