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DGKL: 01. Autoimmune and Rheumatic Diseases, Allergy, Immunodeficiency

TNF and IFN-γ synergistically induce expression of genes associated with the defense against intracellular bacteria in primary human monocytes

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Introduction

The inflammatory response is predominantly triggered by immune recognition of microbial products or tissue damage and propagated by inflammatory cytokines inducing effector functions such as activation and recruitment of leukocytes. During inflammation, a plethora of secreted cytokines interact in multiple fashions to induce and control an appropriate response against the encountered threat (both the type and strength of the response). Therefore, this study was designed to assess the IFN-γ-mediated modulation of TNF-induced gene expression and the synergistic actions of these two cytokines in co-stimulation in the monocytic cell type.

Methods

Primary human monocytes were isolated from heparinized blood samples obtained from three healthy donors using a negative selection protocol. Monocytes were either long-term pre-incubated ± IFN-γ (10 ng/ml; 48 h) and then short-term stimulated ± TNF (80 ng/ml; 2 h) or co-stimulated with the two cytokines in short- and long-term (2 h and 50 h, respectively). Total RNA was isolated, depleted from rRNA, and sequenced after cDNA library generation on a NextSeq 550 sequencer (Illumina). For normalization and differential expression analysis of the obtained data set, DESeq2 was used.

As a comparison, TNF-induced gene expression in the monocytic cell line THP-1 (pre-stimulated with or without IFN-γ) was analyzed by RT-PCR.

Results

In primary human monocytes, IFN-γ long-term incubation suppressed TNF-induced expression of genes involved in neutrophil recruitment (e.g., CXCL1, CXCL2, CXCL8/IL-8), while strongly inducing genes encoding chemokines mainly recruiting T and NK cells (e.g., CXCL9, CXCL10, CXCL11). Moreover, IFN-γ synergized with TNF to induce genes associated with the defense against intracellular bacteria. A specific subset of these genes was expressed remarkably synergistically, suggesting that their expression in response to IFN-γ alone may remain at an insufficient level, while in combination with TNF, physiologically relevant levels may be obtained. These include the four genes ACOD1, INHBA, UBD, and CLEC6A.

Intriguingly, using a monocytic cell line THP-1, we found that in these cells co-stimulation with IFN-γ and TNF leads to cell death and increased production of the neutrophil-recruiting chemokine IL-8.

Conclusion

Our data suggest that different defense mechanisms against a potential intracellular bacterial threat may be executed after IFN-γ and TNF stimulation. While primary monocytes seem to differentiate into an activated phenotype mobilizing the immune reaction against intracellular bacteria, other cells may just die to leave the threat to recruited neutrophils.

DGKL: 01. Autoimmune and Rheumatic Diseases, Allergy, Immunodeficiency

Differential effects of MARCKS on immunologic functions in the monocytic cell type

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Introduction

The ubiquitously expressed protein MARCKS (myristolated alanine-rich C-kinase substrate) was initially identified as a regulator of actin filament dynamics. Since then, MARCKS was shown to be involved in a variety of cellular processes, including cytokine expression/secretion and intracellular signaling. However, the involvement of MARCKS in immune cell functions is scarcely characterized. Therefore, we aimed at elucidating the molecular mechanisms by which MARCKS regulates immunologic processes in monocytes.

Methods

Monocytic THP-1- and myeloid PLB-985-derived MARCKS WT and knock out (KO) cells were generated using the CRISPR/Cas9 technique. Both cell types were differentiated towards monocytes using calcitriol (100 nM, 4 d). Differentiation was confirmed by detecting surface marker expression (CD14 and CD11b; flow cytometry). Primary human monocytes were isolated from heparinized blood samples by negative selection. Reactive oxygen species (ROS) were detected in luminometer-based time course experiments and phagocytosis was measured based on the uptake of fluorescent-labelled E. coli and zymosan particles ± serum opsonisation.

Results

MARCKS KO in THP-1-derived monocytes strongly reduced ROS production in response to different stimuli (PMA, serum-opsonized (ops.) zymosan, ops. E. coli and S. aureus). Surprisingly, MARCKS deficiency did not affect ROS production in PLB-985-derived monocytes. However, treatment with the MARCKS peptide inhibitor MANS reduced both PMA- and ops. zymosan-induced ROS production in primary human monocytes. Moreover, THP-1-derived MARCKS KO monocytes showed reduced levels of zymosan phagocytosis, whereas phagocytosis of ops. zymosan and E. coli particles was unaltered. Again, no effect on phagocytosis was found in PLB-985-derived MARCKS KO monocytes. Interestingly, MARCKS protein and mRNA levels were significantly higher in THP-1 than PLB-985 cells (despite an increase of MARCKS in both cell types in response to calcitriol), suggesting a MARCKS dose-dependent effect on certain cell functions.

Conclusion and Outlook

Our experiments indicate a differential contribution of MARCKS to ROS production and phagocytosis in the monocytic cell type. Currently, we are analyzing effects of MARCKS on other cellular processes such as migration and cytokine expression/secretion in our established MARCKS WT and KO cell lines to further assess the MARCKS-involving mechanisms. Since MARCKS clusters phospholipids at the plasma membrane thereby regulating intracellular signaling pathways (though it is controversial which pathways are modulated), we will also focus on signaling. Preliminary experiments indicate that MARCKS affects phosphorylation of the major kinase Akt. To identify other MARCKS-regulated signaling kinases and gain global insight in MARCKS' role within the regulation of monocytic immune functions, we will conduct (phospho-) proteome analyses in MARCKS WT and KO cells.

DGKL: 02. Biobanks, International Harmonization

Population- and Disease-orientated Biobanking - Conceptualization at the Heart and Diabetes Centre NRW

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Collection of human biospecimens linked to relevant personal- and health information has become indispensable for developing innovations in biomedical research, making a great impact on disease screening and diagnostics. We present the concept of biobanking at the Heart and Diabetes Centre NRW which was developed across the different facilities of our university hospital. A working group including one staff member of each facility was funded in 2012 which developed standardized processes and documents considering the ethical- and legal requirements for biobanking. A patient consent form according to a template provided by the "Arbeitskreis Medizinischer Ethikkommissionen" e.V. (AKEK) and a handling instruction were prepared according to the needs of each facility willing to establish a biobank. This process was proved through the responsible local ethical committee. Several disease-oriented biobanks and a population-based biobank taking samples from healthy blood donors were built. These biobank samples are used to screen biomarkers for disease susceptibility and are linked to health data such as clinical, lifestyle and environmental data. Acquisition, handling and storage of the acquired samples is conducted by the clinical routine diagnostic laboratory. Future projects deal with improving the logistics and IT-infrastructures of sample documentation by introducing CENTRAXX as sample- and data management system.

DGKL: 02. Biobanks, International Harmonization

Sustainability and biobanking: scrapping of biorepositories or continuing use

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Introduction: Fully automated biorepositories capable of storing cryotubes at -80°C became commercially available approximately 15 years ago. Over this period, biobanking units have evolved in response to changing medical needs. Consequently, some of the initially constructed biorepositories no longer align with the current concept of a biobank unit and are deemed unnecessary. This raises the question: should they be scrapped or continue to be utilized?

Methods: In 2016, the Integrated Research Biobank (IRB) in Greifswald acquired a second Kiwi store from LiCONiC. Although the purchase included all trolleys, it came with a limited number of cassettes to cut costs at the time. Subsequently, the Biobank Graz – a partner of BBMRI.at – decided to dismantle their LiCONiC store, which was originally established in 2012. Through communication between the parties, a decision was made to repurpose the Biobank Graz cassettes in Greifswald.

Results: LiCONiC actively participated in the communication process to assess the compatibility of the cassettes. Following a positive initial check based on engineering drawings, two cassettes were sent to the LiCONiC headquarters, where a hands-on examination also yielded positive results. For safety reasons, all cassettes will undergo a thorough check by LiCONiC before being sent to Greifswald. In addition to technical considerations, financial aspects were negotiated between Biobank Graz and Greifswald, resulting in a mutually beneficial win-win situation.

Conclusion: Scientific infrastructures, such as biobanking units, should enhance their sustainability by promoting the continuous use of components, such as stainless steel cassettes. This can be achieved locally or through collaborations with other biobanking units.

DGKL: 02. Biobanks, International Harmonization

Liquid biobanking: increase of efficiency and performance accompanied by reduced costs and environmental burden

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Introduction: Since 1997 about two million aliquots were collected and stored in the Integrated Research Biobank (IRB), organized by the Institute of Clinical Chemistry and Laboratory Medicine (IKCL) in Greifswald. As the number of stored aliquots is increasing over the time, the storage capacity has to be optimized to save running costs, to avoid additional investments in storage capacity and to improve the picking rate. Against this background, a brainstorm meeting was initiated by the IRB to look for optimizing steps to improve the efficiency and sustainability of the IRB in Greifswald and beyond.

Methods: The scientific exchange focussed on the following topics: application of high density (HD) racks, material specific sorting of cryotubes and application of an automated workbench for these picking procedures at -80°C or below. All these steps need to be steered by our LIMS (CentraXX, Kairos).

Results: First tests with the automated workbench showed, that a reliable picking of small cryotubes needs precise HD racks, which clearly fit with the specific cryotubes. Therefore, a specific HD rack for 250µl jackets cryotubes were designed by LVL. Successful tests were performed by the IKCL, Askion and LiCONiC using their fully automated storage systems and the automated workbench. The implementation of these HD racks increased the storage capacity by more than 40%, leading to significantly reduced running costs and investment per sample. The investments in an automated workbench is covered by saving other costs and will lead to a significant increase in efficiency and a reduced environmental burden in Greifswald and beyond.

DGKL: 02. Biobanks, International Harmonization

Laser-based state of the art cryotube labelling

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Introduction: Cryotubes featuring 2D codes at the vessel bottom prove advantageous for handling within fully automated biorepositories. However, they present a challenge for most laboratory analyzers and technicians receive no human-readable information, complicating manual processing and increasing the likelihood of mix-ups.

Methods: To address these issues, we adopt a dual-labeling approach for our 2D coded cryotubes. In addition to the bottom 2D code, we include a paper-based side code containing a 1D barcode as well as human-readable information such as study name and/or sample material (e.g., EDTA plasma). Due to the increased outer diameter, paper-based labeling can pose challenges during frost or when used with high density racks.

Results: A Tube Laser Marker (TLM) from LVL – a user-friendly tool with customizable programming – allows customers to define the content of the side code without paper. The TLM has proven reliable, exhibiting no failures in its inaugural year and successfully labeling around 10,000 cryotubes. Picking rates remain unaffected and the laser labels demonstrate resilience against mechanical or chemical agitation.

Conclusion: Additional side coding for cryotubes is a well-established practice. The limitations associated with paper-based side barcodes can be entirely overcome by employing laser-based labelling, which boasts impeccable labeling performance, reading characteristics and does not have any adverse impact on picking rates.

DGKL: 02. Biobanks, International Harmonization

Biobank Structure Oldenburg – Building Bridges

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The University Medicine Oldenburg includes four hospitals under different ownership, including one municipal, one private, and two distinct church-affiliated hospitals, each with its own ecclesiastical law, together with the Medical Faculty Oldenburg. This legally and organizationally complex structure complicates the establishment of crucial research infrastructures including a participative biobank structure.

The Biobank Structure Oldenburg (BSO) as a Core Facility is designed to be accessible to all participating hospitals, the faculty and cooperating institutions. The BSO employs digitalized workflows to facilitate standardized and quality-assured bio-sample collection, processing, storage, and distribution for research purposes.

The BSO provides various utilization opportunities, including consultation on sample management, support for collection and processing, and the legal transfer of well-characterized biosamples to the faculty after project completion. It allows targeted sample collection under a Broad Informed Consent. The reuse of biosamples from completed projects, adherence to research data management, and compatibility with national and international research infrastructures underscore its comprehensive approach.

The infrastructure of the biobank relies on a central IT system (CentraXX, Karios) for sample management and workflow provision, with decentralized web-based access. Centralized and decentralized storage capacities, process-oriented sample management, quality assurance, and documentation of clinical data further enhance the facility's efficiency.

The governance structure, embedded in the faculty's concept for central research infrastructures, comprises a user council, a Core Facility leadership, and staff. The user council, inclusive of representatives from all four participating hospitals, research data management, and the Coordination Center for Clinical Studies, plays a vital role in ensuring effective service provision.

DGKL: 02. Biobanks, International Harmonization

iBioTUM - Zentrale Interdisziplinäre Liquid-Biobank des Klinikums rechts der Isar und der Technischen Universität München

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Die Liquid-Biobank der zentralen interdisziplinären Biobank iBioTUM am Universitätsklinikum Klinikum rechts der Isar der Technischen Universität München existiert seit 2020. Die Biobank lagert aktuell rund 80.000 Probenaliquots von rund 5.000 Spendern, wobei 70% der Proben unter einem broad consent gewonnen wurden. Probenmaterialien umfassen Serum, Plasma, periphere mononukleäre Zellen (PBMCs), buffy coat, Urin, Speichel und DNA. Zu den in der Biobank etablierten Methoden gehören die Isolation von PBMCs aus Vollblut sowie die DNA-Extraktion aus buffy coat. Gemeinsam mit der Gewebe-Biobank ist die Liquid-Biobank als iBioTUM Partner in der German Biobank Alliance (GBA).

DGKL: 02. Biobanks, International Harmonization

From population-based towards healthcare integrated biobanking - the journey of the Leipzig Medical Biobank

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Introduction

The Leipzig Medical Biobank (LMB) at the University Leipzig is a state-of-the-art biobank for quality-assured collection, processing, storage and provision of biospecimens. Founded in 2010, mainly liquid samples such as urine, serum, plasma and saliva from epidemiological studies of the Leipzig Research Centre for Civilisation Diseases (LIFE) were processed. In 2017, healthcare-integrated biobanking of oncology patients with solid and haematological malignancies was launched with clinical partners and the Institutes of Pathology and Laboratory Medicine. In 2021, the collection of liquid, tissue and plaque samples from patients with cardiovascular diseases started.

Methods

Samples are processed in a highly standardised and traceable manner and stored at temperatures of -80°C or < -150°C. Storage is performed in strict compliance with an uninterrupted cold chain and constant monitoring. All samples are barcoded and can be linked to quality data and donor clinical data.

Results

More than 1.4 million samples and data sets are available from around 60.000 visits (40.000 donors) for researchers. The biobank has been able to support researchers with around 160.000 samples in > 100 projects. In addition to research on stress, allergy, heart disease and dementia, improvements have also been made in diagnostics. Reference ranges of certain biomarkers covering the whole lifespan have been investigated and are now used at the University Hospital Leipzig.

Conclusion

The LMB is the central biobanking facility of the Medical Faculty of the University Leipzig. Samples are processed according to SOPs using state-of-the-art techniques and equipment. The LMB is open to scientific collaborations with internal and external partners and promotes high-level research.

DGKL: 02. Biobanks, International Harmonization

ibdw – A Centralized, Hospital Integrated Biobank

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Introduction

The ibdw was founded in 2011 as one of five centralized national biobanks in Germany within the framework of the governmental funding program “Nationale Biobank Initiative”. The ibdw is a joint core facility of the University Hospital and the Julius-Maximilians-University of Würzburg and acts as a faculty-wide service provider of human biological material for medical research. From the very beginning, the ibdw has focused on comprehensive automation and seamless integration of sample collection into clinical routine workflows to ensure the highest quality standards.

The ibdw takes a central role in providing services to clinical and biomedical research supporting individual research projects as well as research centers, networks, and infrastructures, such as the “Comprehensive Cancer Center Mainfranken” (CCCM), the “Comprehensive Heart Failure Center” (DZHI), the “Center for Rare Diseases” (ZESE), the “Bavarian center for cancer research” (BZKF), the “National Center for Tumor Diseases” (NCT-WERA), and the “National pandemic cohort network” (NAPKON).

Methods

The ibdw is built on four pillars: The laboratories and sample repositories for body fluids and tissue samples, the biobank information system and sample logistics maintained and operated by the IT department, and the ISO 9001:2015 certified quality management system maintained and developed by the quality managers.

The unique concept of the ibdw is based on three aspects:

- Parallel collection of body fluid and tissue samples from patients

- Collection based on a broad informed consent

- Providing means and tools for seamless integration of sample collection into clinical workflows.

Body fluids are processed semi-automated with a robotic system and stored in two automated storage systems at -80°C. Tissue samples and fluid samples requiring specific handling conditions are processed manually and stored in ULT-freezers or in liquid nitrogen.

Conclusion

By consolidating decentralized structures into a centralized biobank, the ibdw enables comprehensive quality control and standardized procedures, timely adaptation to user needs, and continuous improvement according to state-of-the-art technologies. The flexibility and close interaction of the ibdw with clinical processes allows for short response times and immediate implementation of new requests, such as during the COVID pandemic, where the ibdw started collecting patient samples almost immediately with the onset of the first wave in 2020.

The ibdw considers the provision of high-quality samples, together with comprehensive documentation sufficient to assess the suitability of the samples for use, to be its most important task. To this end, the ibdw participates in national and international proficiency testing schemes, collaborates in national and international biobank networks such as TMF, GBA, ESBB and ISBER, and contributes to developing and establishing national and international standards in DIN, CEN and ISO.

DGKL: 02. Biobanks, International Harmonization

Validation of Models Identifying Advanced Chronic Kidney Injury for Healthcare Integrated Biobanking

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Introduction: Healthcare integrated biobanking (HIB) refers to the collection of surplus clinical specimen for research. The combination of HIB with automated algorithms for clinical phenotype identification facilitates and optimizes sample acquisition within the clinical workflow. In a recent publication, we developed algorithms for identification of advanced chronic kidney disease (CKD) in hospitalized patients. In the present study, these algorithms are validated and adapted for HIB requirements using real-world clinical data. The clinical data rely on research infrastructures of the Medical Informatics Initiative and the University Medicine Network.

Methods: During a pilot phase from September 2020 to December 2021, 635 patients consented to “Broad Consent” at the University Hospital of Jena. Of these, 210 patients (33%) met criteria set between January 2018 and March 2020, including consent for sample preservation or a minimum 3-day hospital stay for accurate sampling. We analyzed electronic health records (EHR) data accessed through the Data Integration Center, following the methodology of the original development study. Due to resource constraints, physician review of records, considered the gold standard, was possible for only 162 patients. To adapt the models for HIB scenarios, we recalculated them with restricted predictor availability in the development study data and validated them in the validation cohort.

Results: The cohort of this validation study was younger with fewer prevalence of CKD and other co-morbidities compared to the development study cohort. Simple rule-based algorithms had lower F1 scores (0.86) and lower positive predictive values (PPV = 0.83) in comparison with the development study, while machine learning-based algorithms performed similarly (F1 score = 0.93; PPV = 1.00). However, these complex ML-algorithms were not suitable for prospective use in HIB due to their reliance on data accessible only after the patient’s discharge from hospital whereas HIB-algorithms necessitate application during patient’s hospital stay. After modifying the algorithms for a HIB scenario F1-score and PPV were 0.86 and 0.81 in the development cohort and 0.79 and 0.74 in the validation cohort.

Conclusion: The study emphasizes the importance of validation studies for algorithm implementation. It also highlights the necessity of considering real-world requirements for HIB before implementation. Furthermore, the study suggests continuous monitoring post-implementation to adapt to overall changes in patient characteristics.

DGKL: 02. Biobanks, International Harmonization

Implementierung eines Biobanking-Konzepts im Rahmen der COPLANT-Studie

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Einleitung/Zielsetzung:

Die COhort on PLANT-based diets (COPLANT)-Studie ist eine multizentrische Kohortenstudie, die pflanzenbasierte Ernährungsformen hinsichtlich des Nährstoffstatus und in Bezug auf ernährungsassoziierte Erkrankungen untersucht. Im Rahmen dieser Studie wird eine zentrale Humanprobensammlung von ca. 6000 Studienteilnehmenden aus acht Studienzentren durch die Integrierte Biobank Jena (IBBJ) mit begleitender Biomarkeranalyse qualitätsgesichert aufgebaut.

Methoden:

Zur Gewährleistung einer standardisierten Präanalytik und hohen Probenqualität wurden definierte Workflows zur Probenentnahme, Präanalytik, zum Probentransport auf Trockeneis und zur begleitenden Biomarkeranalytik in Zusammenarbeit mit der der Studienleitung, dem Datenmanagement und Qualtitätsmanagement am BfR und den lokalen Studienzentren etabliert. Auf der Grundlage von entsprechenden Standard-Arbeitsanweisungen (Standard Operating Procedures, SOPs) wurden Studienzentren z. B. über den Einsatz von Videoanleitungen geschult. Zur Biomarkeranalytik werden die entnommenen Blut- und Urinproben in verschiedene Analysesets unterteilt. Während ein Teil der Bioproben (Set 1) studienzentrumsnah in lokalen Laboratorien direkt analysiert wird, erfolgt in einem weiteren Probenset (Set 2A) eine zentralisierte Probenanalytik kurzfristig nach Probeneingang in die IBBJ. Ein weiteres Probenset (Set 2B) wird zur späteren Analytik längerfristig in die IBBJ eingelagert. Zur Qualitätssicherung werden präanalytische Arbeitsschritte durch Barcodescans und Zeitstempelerfassung dokumentiert. Der Transport der Proben wird durch einen Versanddienstleister sichergestellt und zusätzlich mithilfe eines Temperaturloggers überwacht.

Ergebnisse:

Die Vereinheitlichung präanalytischer Prozessschritte stellt insbesondere in multizentrischen Studien eine große Herausforderung dar. Eine wesentliche Herausforderung stellt hierbei auch die Wahl der Abnahmegefäße dar, die bei verteilter lokaler Laboranalytik auf die individuellen Anforderungen der lokalen Laboratorien abgestimmt werden muss. Zudem müssen auch die unterschiedlichen technischen, organisatorischen und personellen Rahmenbedingungen jedes einzelnen Studienzentrums berücksichtigt werden, die eine Vereinheitlichung der Abläufe erschweren.

Zusammenfassung:

Das Biobanking im Zusammenhang mit multizentrischen Studien hat eine Vielzahl von Herausforderungen zu berücksichtigen. Unter Berücksichtigung lokaler Gegebenheiten an den einzelnen Standorten, stellen der Probentransport und die Gewährleistung einer gewissenhaften standardisierten Präanalytik den Hauptfokus von Qualitätssicherungsmaßnahmen dar.

DGKL: 02. Biobanks, International Harmonization

ZBR – Zentrale Biobank Regensburg

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Seit 2019 ist die Zentrale Biobank Regensburg (ZBR) Partner im Verbund German Biobank Alliance (GBA) und sichert am Standort Regensburg die Sammlung und Lagerung von qualitativ hochwertigen Biomaterialien und den zugehörigen Daten. Sie steht unter der Trägerschaft der Universität Regensburg, der Fakultät für Medizin der Universität Regensburg und des Universitätsklinikums Regensburg.

Die ZBR gliedert sich in zwei Säulen: die Flüssigproben- und Zellbank am Institut für Klinische Chemie und Laboratoriumsmedizin (KCH) des Universitätsklinikums Regensburg (UKR) und die Gewebebank am Institut für Pathologie der Universität Regensburg (UR).

Zu den Aufgaben der ZBR zählen die ausführliche Beratung zu Biobanking, Studien, Probensammelstrategie und Probenlogistik und die fachkundige Probenbearbeitung. Dabei ist eine Ein- und Auslagerung sowie qualitativ hochwertige und sichere Lagerung der Biomaterialien ebenso gewährleistet, wie die fachkundige Dokumentation, Archivierung, Zusammenstellung und Ausgabe von Biomaterialdaten und Biomaterial-zugehörigen Daten.

Das laborinterne Datenmanagementsystem ermöglicht eine elektronische Laboranforderung und erleichtert den Prozess der Probenverwaltung grundlegend.

Primär werden im Rahmen von klinischen Studien Biomaterialien wie Serum, Plasma, Urin und PBMC von Patienten mit kardiovaskulären und onkologischen Erkrankungen gesammelt. Im Falle von Patienten mit onkologischen Erkrankungen verfügt das ZBR zusätzliche über eine Gewebeprobensammlung.

Durch die qualitätsgesicherte Probensammlung, Lagerung und Bereitstellung von humanen Biomaterialien unterstützt die Zentrale Biobank Regensburg die medizinische Forschung und Versorgung. Sie trägt so entscheidend zur Forschung, Entwicklung und Verbesserung von Diagnose- und Therapiemethoden bei.

DGKL: 02. Biobanks, International Harmonization

Establishing a Biobanking Workflow for the IMPULS study cohort

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Introduction: Traditional definitions of life stages are based on chronological age. However, aging as a heterogeneous process demonstrates significant variability among individuals of similar chronological age, influenced by various intrinsic and extrinsic factors including nutrition, lifestyle, and mental health. The IMPULS (“Identifizierung und Manipulation der physiologischen und psychologischen Uhren der Lebensspanne”) research consortium aims to expand upon the already established epigenetic and brain organic clocks by incorporating additional age indicators and elucidating their mutual interactions. The IMPULS study collects psychological and health questionnaires and samples from volunteers aged 40-90 years at baseline with a future follow-up assessment planned.

Methods/Results: For the IMPULS study a biobanking concept was successfully established that allows standardized collection and automated sample processing and storage of serum, plasma, whole blood, urine and saliva samples under highest quality standards at the Institute of Clinical Chemistry and Laboratory Diagnostics (IKCL) and the Integrated Biobank Jena (IBBJ). Moreover, standardized procedures for isolation of peripheral mononuclear blood monocytes (PBMC) and extracting erythrocyte lysates from EDTA-blood and Li-heparin-blood samples were implemented. To explore potential correlations between aging biomarkers and health status of the participants samples from each subject were analyzed for a wide range of clinical laboratory parameters (n=81), including blood lipids, cardiac, kidney, thyroid and liver biomarkers, glucose and iron parameters, minerals, vitamins, hormones, biomarkers of bone metabolism and markers indicating inflammation or allergy. Additionally, SARS-CoV2 antibody titers indicating infection and vaccination were also measured. In total, 7.883 samples from 261 study participants were successfully collected at baseline and more than 21.000 laboratory parameters have already been measured.

Conclusion: The IMPULS study has established a well characterized cohort, providing the basis for further investigations of various aspects of aging.

DGKL: 02. Biobanks, International Harmonization

The Integrated Biobank Jena (IBBJ) – 20 years of high qualitative biobanking to support translational biomedical research

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In 2002, the integrated Biobank Jena (IBBJ) was founded as part of the sepsis research clusters. Since then, the sepsis-focus of the IBBJ has gradually broadened and today the IBBJ serves as hospital-integrated biobank of the Medical Faculty of the Jena University Hospital (JUH). Acting translational, the IBBJ supports biobanking in various research networks, such as Competence Network Sepsis, Centre for Innovation Competence (ZIK) Septomics, Center for Sepsis Control and Care, Research Campus InfectoGnostics, Leibniz-Centre for Photonic in Infection Research, Cancer centre Central Germany and COPLANT. Furthermore, the IBBJ is founding member of the German Biobank Network (GBA/GBN) and engaged member ever since: (a) currently represented in the executive board and (b) by organizing German-wide Round-robin tests since 2019 and even for European Biobanks since 2023 and hence (c) establishing quality management concepts for GBA/GBN biobanks.

The IBBJ is equipped with a fully automated sample store, that allows storage of more than 1.5 million samples at –80°C. In addition, in 2022 two automated cryostores (Askion system) were implemented for long term storage of biosamples in the vapor phase of liquid nitrogen at -150°C. In total, the IBBJ has a capacity of more than 4 million samples (500µL equivalents). Workflows are established ensuring automated liquid handling. The pipetting robots as well as the stores are adjusted to 2D-coded sample tubes and barcoded racks for tracking, storage and retrieval. Corresponding data are integrated in the biomedical research portal CentraXX. IBBJ is constantly expanding the number of it´s collection searchable on the BBMRI-ERIC Sample Locator at https://samplelocator.bbmri.de/. The IBBJ is integrated into the Institute of Clinical Chemistry and Laboratory Diagnostics, which enables, upon request, immediate, standardized pre- and post-biobanking sample preparation (e.g. preparation of PBMCs) as well as analysis (e.g. mass spectrometry).

Currently, the IBBJ hosts approximately 100 sample collections (for project descriptions visit https://www.uniklinikum-jena.de/ikcl/ibbj.html) and comprises more than 905.000 samples from more than 37.000 individuals.

The IBBJ has a comprehensive quality management system and participated regularly at the GBA/GBN Round-robin tests to assure that biological samples are of consistent quality and the right for the intended analyses and study goals are controlled properly. Hence, a major objective is to obtain accreditation according to ISO 20387:2018 in 2024.

Taken together, the IBBJ ensures long-term maintenance and sustainability as well as processing and storage of biomaterials under highest quality aspects.

DGKL: 02. Biobanks, International Harmonization

Refining Biobank Excellence: A Proficiency Test Concept for Elevated Process and Sample Quality

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Introduction: Ensuring reliable access to human biosamples alongside their associated phenotype data is essential for promoting reproducible and high-quality biomedical research, particularly in the realm of omics technologies. Consequently, implementing robust quality control (QC) and quality assurance (QA) measures, as well as standardizing biosample handling procedures, are crucial components within the fields of biobanking and translational research. Quality

management (QM) principles are instrumental in overseeing both sample and process integrity. Proficiency tests (PTs), commonly utilized in laboratory diagnostics within the healthcare sector, serve as well-established tools for assessing analytical proficiency and refining critical procedural steps. Unfortunately, proficiency-testing programs tailored to biobanks and their core processes are only partially developed, primarily concentrating on nucleic acid and peripheral blood mononuclear cells (PBMCs). Fundamental biobanking procedures, such as entry control, aliquoting of bodily fluids, and sample shipment, often escape scrutiny.

Methods: Our proficiency-testing concept, devised within the German Biobank Alliance, attempts to close the gap between existing PTs and the need to regulate central biobanking processes. Thus, we've designed PTs specifically for body fluids to check sample entry control, processing times, homogeneity and volume precision of sample aliquots. Furthermore, we've assessed compliance with international shipping standards regarding proper dry ice temperatures during transit and the secure packaging of potentially hazardous biospecimens. The purpose of the proficiency test concept is to examine fundamental biobank processes and to identify potential process variations at the participating sites. Where such variations exist, the objective is to improve standardization of the workflows investigated through process harmonization.

Results: In this report, we present the results of two PTs conducted across 21 national and 18 international biobanks. We show the challenges in the design and implementation of this PT concept, as well as biobank processes that can lead to major variations and thus need to be standardized.

Conclusion: Our proficiency-testing concept not only facilitates the evaluation of biobank processes but might also contribute to reduce inter-biobank process variability and thereby to improve robustness of observations in multicenter studies.

DGKL: 02. Biobanks, International Harmonization

Biobanking an der Universitätsmedizin Greifswald

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Die Integrated Research Biobank (IRB) ist die Fakultätsbiobank der Universitätsmedizin Greifswald (UMG), die am Institut für Klinische Chemie und Laboratoriumsmedizin (IKCL) angesiedelt ist. Ausgangspunkt für die Sammlung von Bioproben war die 1997 gestartete populationsbasierte SHIP-Studie, die mittlerweile 7 Untersuchungswellen umfasst. Zusätzlich werden 7 Kohorten des GANI-MED-Projekts, 2 Untersuchungswellen der lokalen Probensammlung der Nationalen Gesundheitsstudie (NAKO), Proben der Nationalen Pandemiekohorte (NAPKON, Netzwerk Universitätsmedizin (NUM)) und Bioproben anderer nationaler und internationaler Studien, die für die Analytik bearbeitet werden, gelagert. In erster Linie werden epidemiologische und endokrinologische Fragestellungen sowie Herz-Kreislauf-Themen bearbeitet.

Die Probenlagerung erfolgt in zwei LiCONiC Biobanken mit einer Gesamtkapazität von 3 Mio. Aliquoten bei Verwendung von HD-Racks, die voll automatisiert bei -80°C bearbeitet werden können. Weiterhin wird ein Stickstofflager von ASKION mit 60.000 Stellplätzen betrieben. Eine automatische Workbench ermöglicht das Picken von Aliquoten auf ca. 30 unterschiedliche Racks bei -100°C, wodurch ungewünschte Tau-Frier-Zyklen vermieden werden. Die Cryogefäße werden mit einem Tube Laser Labler spezifisch codiert. Unterstützt werden alle Prozesse durch das LIMS CentraXX (Kairos). Die Probenbearbeitungsprozesse sind in die Routinetätigkeiten des IKCL-Kernlabors durch die Nutzung der Laborstraße (inkl. Zentrifugation), von Pipettierrobotern und Decappern größtenteils integriert. Neben einem umfassenden Panel an Laboranalysen können PBMC, DNA und RNA gewonnen werden.

Aktuell werden ca. 1,8 Mio. Aliquote gelagert, zukünftig ebenso die Proben der DZHK Heart Bank. Jährlich werden im Schnitt 100.000 Proben ein- und 70.000 ausgelagert. Die Expertise wird bei großen nationalen Projekten eingebracht, z.B. im DZHK als Sprecher der wissenschaftlichen Infrastruktur und als Betreiber des LIMS oder bei der NAKO als Sprecher der Expertengruppe

Biomaterialien und Laboranalysen, als Mitglied des Advisory Boards des Biorepositories (Helmholtz Munich) und durch eine enge Beratung bei der (Neu-)Strukturierung des dortigen Biobankings. Darüber hinaus betreibt die IRB das LIMS des NUM.

Die IRB als Teil des Instituts für Klinische Chemie und Laboratoriumsmedizin und zentrale Struktur der UMG betreibt seit fast 30 Jahren ein hochqualitatives und automatisiertes Biobanking, das auch über den Standort Greifswald hinaus Beachtung findet. Für das Biobanking werden stets gesonderte Biomaterialien gewonnen und keine Restmaterialien eingelagert, so dass zu allen Biomaterialen stets hoch standardisiert erfasste klinische Daten vorliegen – eine entscheidende Voraussetzung, um qualitativ hochwertige Biomarkerforschung zu betreiben. Die Anbindung an ein klinisches Zentrallabor erlaubt eine zügige und qualitätsgesicherte Analytik mit integraler Berücksichtigung der Kompetenzen zur Prä- und Postanalytik.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Intensive lifestyle intervention on thrombin generation in individuals at high risk for type 2 diabetes

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Introduction: Prediabetes increases the risk of type 2 diabetes, cardiovascular disease, and mortality and is associated with a prothrombotic state. Therefore we investigated the effect of an intensive lifestyle intervention on thrombin generation and elucidated underlying associations with metabolic variables in individuals at high risk for type 2 diabetes.

Methods: The current analysis included 593 high-risk participants from the multicenter Prediabetes Lifestyle Intervention Study (PLIS). High-risk participants were randomized to a conventional or intensive lifestyle intervention group and were characterized by reduced insulin secretion and/or insulin resistance and elevated liver fat content. Precise metabolic phenotyping was performed before and after the 1-year lifestyle intervention, including oral glucose tolerance testing, MRI-based determination of liver fat content and thrombin generation measurements. Associations and changes in thrombin generation with metabolic variables were evaluated.

Results: At baseline, higher levels of endogenous thrombin potential were significantly associated with younger age and increased body weight and liver fat content. Of 593 high-risk participants, 280 were assigned to the conventional and 313 to the intensive lifestyle intervention group. During the intensive lifestyle intervention, thrombin generation parameters decreased significantly in the high-risk participants. Changes in thrombin generation markers were associated with changes in body weight and liver fat. In the conventional lifestyle intervention group, no significant changes in thrombin generation parameters were observed after the lifestyle intervention.

Conclusions: Thrombin generation markers are strongly associated with body weight and liver fat content in individuals at high risk for diabetes. Lifestyle intervention, especially intensive lifestyle intervention, is able to reduce levels of thrombin generation markers, mediated by reductions in body weight and liver fat content. In addition to the known effects on diabetes risk, these findings highlight the beneficial effects of intensive lifestyle intervention on the hypercoagulable state in high-risk individuals.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Lipoprotein(a) comparison of quantitative determination methods

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Introduction:

Lipoprotein(a) became besides LDL cholesterol one of the most attractive targets for intervention in cardiovascular disease. Since specific Lp(a)-lowering therapies are under clinical investigation, the interest in measuring Lp(a) has markedly increased. The special structure of Lp(a) creates difficulties for an accurate measurement of Lp(a) and has triggered several efforts for a standardization of Lp(a) measurement.

Methods:

We compared the Lp(a) Assay from Beckman Coulter(nephelometry) and Abbott Alinity (immunturbidimetry). We measured over 3000 serum samples.

Results:

The method comparison shows that the Lp(a) Assay from Beckman Coulter has significantly higher values than the assay of Abbott Alinity. The evaluation of the Passing Bablok regression and the Bland-Altmann plot show an average of approximately 60% lower values for the Abbott Lp(a) Assay. The deviation extends over the entire measuring range.

Conclusion:

It is not only important to measure Lp(a) for a better risk stratification but also to identify those patients who might be in most need for a future Lp(a)-lowering therapy like lipidapheresis. There is still room for improvement of the Lp(a) assays in terms of standardization.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Dysregulation of circadian rhythm genes in association with the metabolic memory

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Introduction: Diabetic kidney disease (DKD) is now the most common cause of end-stage chronic kidney disease, affecting 40% of all diabetic patients. The persistence of diabetes-related complications despite improved glycemic control is known as metabolic memory, which has been linked to epigenetic changes, but lacks specific therapeuties. Sodium/glucose cotransporter-2 inhibitors (SGLT2i) represent a promising therapeutic approach for the treatment of DKD. While SGLT2i have shown nephroprotective effects, their ability to reverse metabolic memory remains unknown. The cytoprotective and signaling competent coagulation protease activated protein C (aPC) has been shown to reverse epigenetic changes associated with metabolic memory. The aim of this study was to analyze the effect of SGLT2i and aPC on renal gene expression in association with the metabolic memory in DKD.

Methods: In two independent mouse models of type 1 and type 2 diabetes (streptozotocin (STZ) and genetic db/db (C57BL/KSJ-db), respectively), persistent hyperglycemia was normalized with the SGLT2i dapagliflozin. A subset of mice

was treated with aPC in addition to SGLT2i. After 22 weeks, kidney tissue samples were collected for gene expression analysis.

Results: RNAseq analysis of kidney samples from non-diabetic and diabetic mice with and without SGLT2i treatment was used to identify genes associated with metabolic memory. Nearly half of the induced and repressed genes in DKD remained altered even after normalization of blood glucose levels, reflecting the metabolic memory in SGLT2i treated mice. Pathway analysis of the persistently upregulated memory genes identified circadian rhythm as the most dysregulated pathway. The persistent dysregulation of circadian rhythm genes was confirmed by qRT-PCR analysis of the core clock genes Clock, Bmal1, Per1 and Cry1 in kidney samples from untreated diabetic STZ and SGLT2i treated diabetic mice. The increased expression of Clock and Bmal1 persisted after SGLT2i treatment. snRNAseq analyses using samples from non-diabetic controls (db/m) and diabetic mice (db/db) without or with interventions (SGLT2i) showed a dysregulation of clock genes in podocytes: Clock, Bmal1, Per2 and Cry1 were induced, whereas Per1 was suppressed. Interestingly, additional treatment with aPC normalized the expression of core clock genes.

Taken together, these data suggest that DKD is associated with an altered circadian rhythm in the kidney. While circadian rhythm related genes remained altered after treatment with SGLT2i, additional treatment with the cytoprotective protease aPC was effective in normalizing the expression of these genes in DKD. These studies suggest that dysregulation of circadian rhythm genes is associated with the metabolic memory in DKD. Intervention with aPC, but not with SGLT2i normalizes the expression circadian rhythm genes.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Elevated low-density lipoprotein cholesterol levels in the general patient care – a candidate for the clinical decision support system

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Introduction: Elevated low-density lipoprotein cholesterol (LDL-C) blood level is an established causal risk factor for development of atherosclerosis and cardiovascular disorders, which can lead to myocardial infarction and cause mortality. LDL-C is hence a main therapeutic target in individuals with elevated risk of cardiovascular diseases. However, many patients with very high LDL-C (≥190 mg/dL) can remain undetected and untreated in different clinical settings. The aim of this study was to define proportion of patients with LDL-C serum levels ≥190 mg/dL in general patient care and to develop clinical decision support system which will help clinicians to identify patients at high risk, suitable for medical intervention.

Methods: All patients admitted for LDL-C laboratory examination in 2022 at the Campus Klinikum Lippe, Medical School and University Medical Center OWL, Bielefeld University, were extracted to determine patients with serum LDL-C levels ≥190 mg/dL. Information such as age, sex, cholesterol (CH), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) serum levels, as well as statin therapy and diagnosis were collected.

Results: A total of 337 patients during a period of one year were detected with high LDL-C levels. Median age was 64 years and 33% of patients were male. Supporting serum lipid parameters had a following median and interquartile range: CH 302 mg/dL (284-322 mg/dL), HDL-CH 54 mg/dL (44-69 mg/dL) mg/dL and triglycerides 150 mg/dL (115-209 mg/dL). Patients were from different clinical departments and many were not under statin therapy.

Conclusion: Detecting patients at increased risk of cardiovascular events remains still an unmet clinical need. Clinical decision support system for elevated LDL-C including presentation of specific guideline-guided therapy options is a promising clinical tool which could improve the clinical outcome.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Prospective Associations of Circulating Adiponectin and Chemerin Concentrations with Incident Metabolic Diseases in a Population-Based Study

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Introduction: Various cross-sectional studies have observed associations of the adipokines adiponectin and chemerin with obesity and associated metabolic diseases. However, the actual prognostic value of these adipokines for the development of metabolic diseases is still under debate, as longitudinal data is often missing. Therefore, this study aimed to analyse the prospective associations of adiponectin and chemerin with incident metabolic diseases using data from a population-based study.

Methods: In the Study of Health in Pomerania (SHIP), chemerin was measured in two independent cohorts (SHIP-START-1, SHIP-TREND-0), while data for adiponectin measurements were only available in one of these cohorts (SHIP-TREND-0). SHIP-START-1 and SHIP-TREND-0 participants were followed-up at 5-year intervals for a total of 15 and 5 years, respectively. After exclusion of missing data, 1922 SHIP-START-1 and 2359 SHIP-TREND-0 participants were available for the prospective analyses. Further exclusion criteria were applied separately for each event (missing data, having an event at SHIP-START-1 or SHIP-TREND-0). The associations between adiponectin/chemerin and incident metabolic syndrome, its components, diabetes mellitus, and cardiovascular events (cardiac infarction, heart surgery, stroke, pacemaker, cardiovascular mortality) were analysed using separate multivariable Cox proportional hazard regression models.

Results: Each increase of adiponectin per one standard deviation (SD) was associated with a lower risk of developing metabolic syndrome (hazard ratio (HR) = 0.56; 95%-confidence interval (CI) = 0.44-0.71), while the opposite effect was observed for the increase of chemerin per one SD (HR = 1.19; 95%-CI = 1.07-1.32). A closer look at the results for the different components of the metabolic syndrome showed that both adipokines were able to predict abdominal obesity, reduced HDL-cholesterol, and elevated glucose, but not elevated blood pressure. Furthermore, adiponectin and chemerin concentrations were associated with incident diabetes mellitus, but again in different directions (adiponectin: inverse, chemerin: positive). A significant association with the development of cardiovascular events was only found for adiponectin (HR = 0.63; 95%-CI = 0.48-0.83).

Conclusion: The analyses revealed that both adipokines have a prognostic value for the development of metabolic diseases, but their effects run in different directions. A high adiponectin concentration was associated with a lower risk of metabolic syndrome, diabetes mellitus, and cardiovascular events, while a high chemerin concentration was associated with a higher risk of metabolic syndrome and diabetes, but not cardiovascular events. The analyses underline the potential role of these biomarkers for the prediction of metabolic syndrome and its associated diseases.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Leptin, but not ghrelin, is associated with food addiction scores in a population-based subject sample

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Background

Ghrelin and leptin are both peptide hormones and act as opposing players in the regulation of hunger, satiety and energy expenditure. Leptin reduces appetite and feelings of hunger and is secreted mainly by adipocytes, while ghrelin increases appetite and food intake and reduces metabolic rate. Both hormones have been implicated in addictive disorders. Ghrelin was shown to have pro-addictive effects while leptin's role in addiction yields more conflicting results. Their involvement in the regulation of both food intake and addictive behaviors make them interesting candidates when investigating the regulation of food addiction. However, only few human studies have been performed and large-scale studies are lacking to date. We aimed to investigate the association between total ghrelin and leptin serum levels with scores in the Yale Food Addiction Scale (YFAS).

Methods

Subjects were recruited in the LIFE Adult cohort. 909 subjects were included in the analysis and we performed univariate multiple linear regression models, adjusted for age, sex (in total group analyses only), alcohol consumption, smoking status, BMI scores, cortisol concentrations, Center for Epidemiological Studies Depression Scale (CES-D) and the 7-item Generalized Anxiety Disorder Scale (GAD-7) sum scores. The dependent variable was the YFAS score.

Results

In men, leptin serum levels showed a significant positive association (standardized $\beta = 0.146$; $p = 0.012$) with the YFAS score. This finding was confirmed in an extreme-group comparison: men in the highest quartile of leptin levels had significantly higher YFAS sum scores than men in the lowest quartile (1.55 vs. 1.18; $p = 0.00014$). There was no association with YFAS sum score in the total group (standardized $\beta = -0.002$; $p = 0.974$) or in women (standardized $\beta = -0.034$; $p = 0.674$). Total serum ghrelin showed no association with YFAS sum score neither in the total group (standardized $\beta = -0.043$; $p = 0.196$) nor in men ($n = 530$; standardized $\beta = -0.063$; $p = 0.135$) or women ($n = 379$; standardized $\beta = -0.035$; $p = 0.494$).

Conclusion

Our findings are in line with previous literature and suggest that total ghrelin serum levels are not associated with food addiction scores. Leptin had been previously shown to be associated with food addiction and we confirmed this finding for men in a large, population-based approach.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

PTP1B and TCPTP as Potential Targets in Inflammatory Insulin Resistance and Intensive Care Unit-acquired Weakness (ICUAW)

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Introduction

Intensive care unit-acquired weakness (ICUAW) is caused by critical illness and affects up to 90% of patients with severe sepsis. The main hallmark of ICUAW is muscle atrophy, reduced muscle strength and weakness, contributing to longer hospitalization, higher morbidity and mortality. Treatment options are limited. Due to many risk factors (e.g., hyperglycemia), deciphering the pathological mechanism is difficult and individual for each patient. Especially pro-inflammatory cytokines, such as interleukin 6 (IL-6), that are elevated during sepsis affect muscle biology on multiple levels, and might interfere with insulin signaling as well as protein homeostasis. Protein tyrosine phosphatases (PTPs) that regulate insulin signaling and inflammatory responses display a potential link between risk factors and disease outcome.

Methods

To investigate the function of PTPs during inflammation, biopsies of M. vastus lateralis of ICUAW patients or patients who underwent elective orthopedic surgery (controls) were used to quantitate the gene expression of PTPs and insulin signaling components. In addition, C57Bl/6J mice were exposed to polymicrobial sepsis induced by cecal ligation and puncture (CLP) surgery. Sham-operated mice served as controls and skeletal muscles were dissected for further analyses. In vitro, differentiated C2C12 murine muscle cells were treated with IL-6, IL-6/IL-6R or vehicle for mechanistical analyses. Pharmacological inhibition and knockdown of Ptpn1 or 2 was conducted to investigate their impact on insulin signaling and glucose uptake in vitro.

Results

PTP1B/Ptpn1 and TCPTP/Ptpn2 were significantly upregulated in M. vastus lateralis of ICUAW patients and skeletal muscle of CLP animals when compared to control patients or sham animals, respectively. The gene expression of Insulin Receptor/Insr was enhanced and a downregulation of Insulin Receptor Substrate-1/Irs1 was observed in the same comparisons indicating inflammatory insulin resistance. In vitro, IL-6 increased protein amounts of PTP1B and TCPTP time-dependently, while tyrosine-phosphorylation of the insulin receptor was reduced by 15% after insulin treatment (10 nM, 15 min). Pharmacological inhibition of PTPs, using broad-spectrum individual PTP-antagonists, enhanced insulin signaling in C2C12 cells, quantified by increased phosphorylation of the Insulin Receptor and its downstream effector AKT. In addition, knockdown of Ptpn1 or Ptpn2 in vitro increased glucose uptake in C2C12 cells, indicative for improved insulin sensitivity.

Conclusion

A dysregulation of the insulin-signaling pathway mediated by inflammatory processes might contribute to ICUAW. Our data suggest that PTP1B and TCPTP are hitherto unrecognized mediators that regulate peripheral insulin resistance in sepsis-induced inflammatory muscle atrophy. These results provide new insights into targeting PTPs during systemic inflammation to reduce complications caused by an imbalance in insulin signaling.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

An optimised methylglyoxal scavenging peptide lowers free- and tissue bound advanced glycation end-products in mice with diet induced obesity and improves insulin sensitivity in mice

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Introduction:

Methylglyoxal (MG) is a dicarbonyl compound that reacts with basic amino acid side chains of arginine and lysine to form advanced glycation end products (AGEs), including MG-hydroimidazolone 1 (MG-H1) and Carboxyethyllyine (CEL). AGEs have been associated with diabetes and implicated as causative factors in diabetic complications. Here, we report the development of a pharmacokinetically optimised peptide-based MG scavenger (Dap3) as a novel pharmacological intervention. We aimed to investigate the effect on insulin- and glucose tolerance as well as MG scavenging activity in mice.

Methods:

Dap3 was created by solid-phase peptide synthesis. MG scavenging in vitro was quantified by HPLC. Biodistribution of 68-Ga labelled peptides was analysed using PET. Efficacy of Dap3 was investigated in mice with diet-induced obesity (DIO). Mice were kept on a 60 % high fat diet or formulated control food for 25 weeks before starting treatment with Dap3 (3 µmol/kg, ip) for 4 weeks. Glucose (ip GTT) and insulin tolerance test (ip ITT) were conducted, and fasting insulin levels were quantified by ELISA at the end of the treatment period. Peptide plasma levels as well as plasma and tissue levels of MG and AGEs were determined by LC-MS/MS.

Results:

The cyclic peptide Dap3 contains diaminopropionic acid as active site and hexadecanedioic acid to prolong its plasma half-life. Half-life of Dap3 in lean mice was 7.2 h by LC-MS/MS and little to no kidney uptake was seen by PET imaging. MG scavenging kinetics of Dap3 (MG $t_{1/2}$=0.18 h) in vitro were comparable to aminoguanidine (MG $t_{1/2}$=0.21 h). Application of Dap3 to DIO mice resulted in improved ITT (AUC 224 vs 173; $p < 0.0001$) and GTT (AUC 480 vs 389; $p < 0.05$). Fasting insulin levels were elevated in placebo-treated DIO mice compared to lean mice (7.7 vs 0.7 ng/ml; $p < 0.0001$). Dap3 partially normalised fasting insulin levels (4.1 vs 7.7 ng/ml; $p < 0.0001$) compared to untreated DIO mice. Plasma-MG levels were elevated in placebo- (133 vs 103 nM; $p < 0.01$) but not in Dap3-treated DIO mice (123 vs 103 nM) compared to lean mice. Plasma free MG-H1 (178 vs 99 nM; $p < 0.0001$) and free CEL levels (85 vs 73 nM $p < 0.05$), and protein-bound MG-H1 (15.4 pmol/mg vs 10.8 pmol/mg; $p < 0.05$) and CEL levels in muscle (5.3 vs 1.5 pmol/mg; $p < 0.0001$) were lowered by treatment with Dap3. MG-modified Dap3 reached concentrations of 2 µM 3 h after administration to DIO mice confirming scavenging activity in vivo.

Conclusion:

Here we describe the development of a MG scavenging peptide. Unlike previous strategies to target MG, the molecule combines a long plasma half-life with excellent MG scavenging properties. Treatment of mice resulted in improvements in glucose tolerance, insulin tolerance, and fasting insulin levels, alongside reductions in plasma and tissue-bound AGE levels. We believe this approach presents a promising strategy to address a pathomechanism not yet targeted for insulin resistance and diabetes.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Antikörpermessungen im Rahmen der Diabetesabklärung – Qualitätseinbußen bei der Umstellung von ELISA auf CLIA?

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Einleitung: Autoantikörper gegen Strukturelemente des Pankreas sind ein wesentlicher Bestandteil bei der labordiagnostischen Abklärung des Diabetes mellitus. Die Methodenheterogenität und mangelnde Standardisierung schränken die Inter-Assay Vergleichbarkeit von Messergebnissen ein und erschweren somit einen Methodenwechsel im Labor. Wir haben die Vergleichbarkeit von Autoantikörpern gegen Glutamat-Decarboxylase 65 (GAD65), Tyrosinphosphatase (IA-2) und Zinktransporter 8 (ZnT8) an drei kommerziell verfügbaren Geräteplattformen evaluiert.

Im Rahmen der zunehmenden Automatisierung und des beständigen Zeit- und Kostendrucks stellen viele Laboratorien die Quantifizierung der Autoantikörperdiagnostik gegen Glutamat-Decarboxylase 65 (GAD65), Tyrosinphosphatase (IA-2), sowie Zinktransporter 8 (ZnT8) von immunologischen Methoden wie „Enzyme (Linked) Immuno(sorbent) Assays“ (ELISA/EIA) auf „Chemi Lumineszenz Immuno Assays“ (CLIA) um. Diese Arbeit soll die Vergleichbarkeit einiger derzeit auf dem Markt befindlicher Testsysteme evaluieren.

Material und Methoden: Die Antikörper (AK) gegen GAD65 (n=70), IA-2 (n=54) und ZnT8 (n=32) wurden mittels ELISA auf dem Euroimmun Analyzer 1 (EUROIMMUN) sowie mittels CLIA auf Maglumi 800 (SNIBE) und iFlash 1800 (YHLO) gemessen. Für alle Parameter und jedes Gerät wurde die Intra- und Inter-Assay Präzision bestimmt. Weiterhin wurden die Linearitäten mittels Verdünnungsreihen überprüft.

Ergebnisse: Für iFlash sowie Maglumi zeigte sich bei der GAD65-AK-Messung eine gute Inter- und Intra-Assay-Präzision und Richtigkeit. Die Präzision des iFlash war hierbei deutlich schlechter (Faktor 1,5-3) und die des Maglumi deutlich besser (Faktor 0,3), als vom Hersteller angegeben.

Die Methodenübereinstimmung des EIA für GAD65-AK lag bei 51,5% (iFlash, n=68), bzw. 75,4% (Maglumi, n=69). Nur 38% (iFlash, n=50) bzw. 71% (Maglumi, n=51) der im EIA positiv gemessenen Proben zeigten sich auch im CLIA positiv.

Die Methodenübereinstimmung des EIA für IA2A-AK lag bei 68% (iFlash, n=50), bzw. 81,1% (Maglumi, n=50). Nur 68% (iFlash, n=37), bzw. 70% (Maglumi, n=40) der im EIA positiv gemessenen Proben zeigten sich auch im CLIA positiv.

Die Methodenübereinstimmung des EIA für ZnT8-AK lag bei 58,1% (Maglumi, n=31). Nur 54% (Maglumi, n=31) der im EIA positiv gemessenen Proben zeigten sich auch im CLIA positiv.

Die Methodenübereinstimmungen der CLIA-Methoden lag bei 67,2% (GAD65, n=67) beziehungsweise 70% (IA2A, n=50).

Diskussion: Im Methodenvergleich zwischen EIA und CLIA lässt sich ein deutlicher, inkonsistenter Sensitivitätsverlust von bis zu 62% darstellen. Im Rahmen steigender Spezifität der molekularen Targets der labormedizinischen Diagnostik sollte eine kritische Evaluation der Referenzgruppe und ihrer ethnogenetischen Varianzen erfolgen, die als Grundlage der Testvalidation durch den Hersteller dienen. Unsere Ergebnisse decken sich mit vorbeschriebenen, substanziellen Unterschieden der Referenzwerte für unterschiedliche ethnische Gruppen.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Die Assoziation zwischen der intradermalen Ablagerung von Advanced Glycation Endproducts und der strukturellen Integrität des Nervus ischiadicus bei Personen mit Typ-2-Diabetes

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Fragestellung: Die Haut-Autofluoreszenz (sAF) als Marker für die intradermale Ablagerung von Advanced Glycation Endproducts (AGEs) prognostiziert mikroangiopathische Komplikationen bei Typ-2-Diabetes (T2D), wie die distale sensomotorische Polyneuropathie (DSPN). Diese Studie zielte darauf ab, zu untersuchen, ob Veränderungen der strukturellen Integrität des Nervus ischiadicus mit intradermaler Ablagerung von AGEs zusammenhängen.

Materialien und Methoden: 62 Personen mit T2D (20 Frauen, 42 Männer), 29 mit DSPN (7 Frauen, 22 Männer), 33 ohne DSPN (nDSPN) und 10 gesunde (HC) Teilnehmer unterzogen sich klinischen, neurologischen und serologischen Untersuchungen sowie der Diffusionstensor-Bildgebung des rechten Nervus ischiadicus, um die Nervenintegrität durch fraktionale Anisotropie (FA) des Nervus ischiadicus zu quantifizieren. Die arterielle Steifigkeit wurde mittels Pulswellengeschwindigkeit (PWV) gemessen.

Ergebnisse: sAF (HC 2,1±0,25 [arbitrary unit], nDSPN 2,3±0,47, DSPN 2,6±0,43; p=0,005) war bei Individuen mit DSPN höher im Vergleich zu HC (p=0,010) und nDSPN (p=0,035). Die partielle Korrelationsanalyse der FA des Nervus ischiadicus kontrolliert für die eGFR und Alter ergab eine negative Korrelation mit sAF bei allen Individuen mit T2D (r=-0,30, p=0,039). Bei DSPN korrelierte sAF positiv mit dem hochsensitiven TNT (r=0,58, p=0,005) und mit der PWV (r=0,52, p=0,007). Bei DSPN korrelierte die FA des Nervus ischiadicus negativ mit PWV (r=-0,47, p=0,010).

Schlussfolgerung: Diese Studie ist die erste, die zeigt, dass die intradermale Ablagerung von AGEs und arterielle Steifigkeit mit der strukturellen Integrität proximaler peripherer Nerven bei Personen mit T2D zusammenhängt.

DGKL: 03. Diabetes, Metabolic Diseases, Endocrinology, Lipid Metabolism Disorders

Eine Beeinträchtigung der Nierenfunktion korreliert mit einer reduzierten strukturellen Integrität des Nervus ischiadicus bei Personen mit Typ-1- und Typ-2-Diabetes

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Fragestellung: Die distale symmetrische Polyneuropathie (DSPN) und diabetische Nephropathie wurden bisher als mikroangiopathische Komplikationen klassifiziert. Heute wird jedoch das Konzept einer gemeinsamen Pathogenese mit zentraler Rolle der vaskulären Funktion hinterfragt. Unser Ziel war es, die Beziehung zwischen der strukturellen Integrität des Nervus ischiadicus und der Nierenfunktion bei Individuen mit Typ-1-Diabetes (T1D) und Typ-2-Diabetes (T2D) zu untersuchen.

Materialien und Methoden: Insgesamt 56 Personen mit T2D (18 Frauen, 38 Männer) und 14 Personen mit T1D (8 Frauen, 6 Männer) wurden hinsichtlich klinischer Parameter für die Glukosekontrolle, Nierenfunktion und Neuropathie untersucht, sowie mit MR-Neurographie (MRN) des Nervus ischiadicus. Die MRN erfolgte mittels Diffusionstensor-Bildgebung, und die fraktionale Anisotropie (FA) wurde als Marker für die strukturelle Integrität gemessen.

Ergebnisse: Bei Personen mit T2D und DSPN waren sowohl die glomeruläre Filtrationsrate (GFR) (DSPN: 82,2 ± 14,9 ml/min/1,73 m^2; ohne DSPN: 93,1 ± 12,2; p=0,011) als auch die FA des Nervus ischiadicus (DSPN: 0,40 ± 0,05; ohne DSPN: 0,45 ± 0,05; p=0,001) niedriger im Vergleich zu denen ohne DSPN. Jedoch wurde kein Unterschied im Albumin-Kreatinin-Ratio zwischen Personen mit und ohne DSPN in der T2D-Gruppe beobachtet. Unter Personen mit T1D wurde eine positive Korrelation zwischen der FA des Nervus ischiadicus und der GFR gefunden (r=0,55, p=0,042). Darüber hinaus zeigte eine partielle Korrelationsanalyse kontrolliert für Alter eine positive Korrelation zwischen der FA des Nervus ischiadicus und der GFR bei Personen mit T2D (r=0,29, p=0,034) sowie gemeinsam in Individuen mit Typ-1- und Typ-2-Diabetes (r=0,33, p=0,005).

Schlussfolgerung: Sowohl in Individuen mit Typ-1-Diabetes als auch mit Typ-2-Diabetes lässt sich ein Zusammenhang zwischen der Nierenfunktion und dem Grad der peripheren Nervenschädigung nachweisen. Da Personen mit schwerer Niereninsuffizienz von der Studie ausgeschlossen wurden, implizieren die Ergebnisse, dass gemeinsame pathogenetische Mechanismen, die periphere Nerven und Glomeruli betreffen, bei Personen mit Diabetes bereits vor der Entwicklung einer diabetischen Nephropathie identifiziert werden können.

DGKL: 04. Genetic Diagnostics, Molecular Diagnostics, Immunogenetics, Pharmacogenetics

Hemoglobin C: A distinction of most frequent genotypes considering clinical and laboratory findings

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Introduction:

Hemoglobin C (Hb C) is a structural variant of the main hemoglobin fraction A (Hb A) and is characterized by an amino acid substitution at position six of the β globin chain (β6Glu>Lys). Humans who carry two gene copies of Hemoglobin C (Hb CC) are affected by Hb C disease clinically presenting splenomegaly, jaundice, and mild chronic hemolysis, while individuals with a single Hb C gene variant (Hb AC) typically do not exhibit any symptoms. However, in patients suffering from compound heterozygous hemoglobin genotypes such as Hemoglobin S (Hb S)/Hb C (Hb SC) as well as Hb C/beta thalassemia, a single Hb C variant has significant impact on clinical courses. Moreover, the level of Hb C is impacted by the co-existence of alpha thalassemia. Thus, differentiation of Hb C genotypes and combinations thereof is important for patient management and prediction of clinical outcomes.

Aims and Methods:

The primary objective of the study is to compare the genotypes for Hb C disease, considering the co-inheritance with alpha thalassemia, Hb SC disease, and compound heterozygosity with beta thalassemia. A retrospective data analysis from 36 patients with Hb C variants will be carried out. Correlation studies for genetic, routine laboratory and clinical data will be performed. Blood count analyses will be normalized by z-transformation, as their reference intervals are highly age-dependent.

Results:

Three groups of patients (17 female, 19 male), age ranging from 1 month to 61 years are studied: Hb C trait (Hb AC; n=10), Hb C trait with coexisting alpha thalassemia (n=7), and compound heterozygosity for Hb C/Hb S and Hb C/beta thalassemia (n=19), respectively. Laboratory findings of hematological, as well as hemolytic biomarkers in all three groups are used for statistical analysis. Finally, clinical phenotypes are analyzed regarding their respective genotypes.

Conclusion: Presentation of genetic Hb C combinations results in heterogeneous laboratory and clinical findings, and that should be taken into account for patient management. Thus, our data support the need for differentiating between Hb C trait, Hb C trait with co-existing alpha thalassemia, and compound heterozygous Hb C diseases (Hb SC and Hb C/beta thalassemia).

DGKL: 04. Genetic Diagnostics, Molecular Diagnostics, Immunogenetics, Pharmacogenetics

Borrelien und FSME-Virus in der Zecke: Effiziente, automatisierte RealTime-PCR

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Zecken sind in ganz Deutschland und Europa weit verbreitet und können während des Blutsaugens Bakterien und Viren übertragen. Der Mensch wird am häufigsten durch den Gemeinen Hausbock (Ixodes ricinus) gestochen. Folgeinfektionen

durch Borrelia burgdorferi spielen die größte Rolle, bedeutsam sind aber auch die vor allem in Süddeutschland verbreiteten Viren der Frühsommer-Meningoenzephalitis (FSME). Etwa 20 % aller Zecken tragen Borrelien in sich, mit einer Keimdichte von zumeist zwischen 1.000 bis 100.000 Borrelien pro Zecke, ausnahmsweise sind bis zu 10.0000.000 möglich. Das Übertragungsrisiko steigt mit dem Keimbefall und der Kontaktzeit bis zur Entfernung der Zecke. Doppelinfektionen mit dem FSME-Virus sind möglich. Zur Vermeidung einer Infektion sollte die Zecke so schnell wie möglich komplett und ohne Quetschen von der Haut entfernt werden (am besten mit einer Zeckenpinzette). Viele Menschen möchten wissen, ob von „ihrer" Zecke ein Infektionsrisiko ausgeht.

Seit über 10 Jahren bietet das Medizinische Labor Bremen dafür den Nachweis von Borrelien und FSME-Virus in Zecken mit der RealTime-PCR an, dem besten verfügbaren Verfahren. Der Test ist bei frischen und alten, eingetrockneten Zecken gleichermaßen anwendbar. Für große Durchsatzzahlen und eine schnelle Ergebniserstellung im Labor sollten die für die Analyse erforderliche Homogenisierung der Zecke, Extraktion der Nukleinsäuren und die eigentliche PCR möglichst automatisiert ablaufen. Bei unserem Verfahren werden die Zecken in PBS-Puffer in einer stark agitierten Kugelmühle (MagNA Lyser, Roche) zerkleinert. Dies gewährleistet eine vollständige, hoch effiziente und immer gleichbleibende Zerkleinerung der Zecken, die z.B. einer manuellen Aufarbeitung mit dem Mikropistill weit überlegen ist. Das Zeckenlysat wird anschließend im cobas® 6800 System (Roche) vollautomatisch extrahiert und mit einer RealTime-PCR analysiert (Nachweisgrenze: ca. 10 Borrelien/Zecke). Die Ergebnisse werden online ins Laborinformationssystem übertragen. Durch diesen automatisierten Workflow sind wir in der Lage, auch in Spitzenzeiten bei hohen Anforderungszahlen sehr schnell ein sensitives, sicheres und reproduzierbares Ergebnis zu liefern. Am häufigsten erhalten betroffene Menschen nach Zeckenstich Erleichterung durch ein negatives Ergebnis. Im Fall eines Keimnachweises kann schnell und auf rationaler Grundlage ärztlicher Rat zum weiteren Vorgehen eingeholt und ggf. unverzüglich gehandelt werden.

DGKL: 05. Gender Medicine, Pediatric Laboratory Medicine, Decision Limits, Reference Intervals

Exploring different sources of statistical data on age- and sex-based variation in natural killer cell recirculation

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Introduction

Natural killer (NK) cells are continuously generated in the human bone marrow in healthy patients. Upon entry into the blood stream, NK cells can recirculate in the intravascular space and through lymphoid organs or home into inflamed tissues (Crinier et al., 2018). While human blood NK cells are routinely measured by flow cytometry as part of the immune status (consensus phenotype typically CD3- CD56+CD16+/-), data on reference intervals (RI), mean or median absolute NK cell counts per µl are heterogeneous or lacking. We are testing different indirect methods to characterize NK cell counts in healthy adults using different sources of secondary data and different statistical protocols.

Methods

We collected published research data that contained NK cell measurements using flow cytometry of clinically healthy adults and contacted the authors to ask for the primary data. Additionally, to compare the results of this literature review with our measurements, we screened the NK cell concentration data from the routine clinical laboratory for healthy individuals with normal CBC and serum CRP levels. Both the extracted published data and the measured data were stratified by age and sex. Individuals younger than 20 and older than 80 years were excluded due to small sample sizes in both datasets.

Results

We found the published data to be very heterogeneous. We contacted 16 authors and received four datasets that met the inclusion criteria, creating a joint dataset of 514 individuals in the age range of 20 to 79 years. Searching our clinical laboratory database from June 2022 to April 2024, 442 cases met the inclusion criteria. Overall, the median NK cell concentration in healthy subjects was stable over all analyzed age groups, in accordance with a steady state of NK cell egress from the bone marrow in the absence of severe infectious or hematological disease. Further data analysis and extraction of clinically usable RI are ongoing.

Conclusion

While our current sample size does not suffice to determine an RI using classical protocols (Jones et al., 2019; Meyer), we were able to find similar central tendencies and preliminary age trends between two groups of secondary data, suggesting further potential of the method. We will continue to collect both patient and published data to increase the sample size and to compare statistical protocols for secondary RI determination. By exploring these diverse approaches, we hope to clarify the underlying features of NK cell counts in healthy adults. In addition, we contribute to the field of RI determination by establishing workflows for immune cell marker analysis from pooled data from statistical and wet-lab data sources. However, additional data for very young and very old age groups and for numerous special populations of interest are needed to better understand how a normal NK cell compartment can be reliably quantified.

DGKL: 05. Gender Medicine, Pediatric Laboratory Medicine, Decision Limits, Reference Intervals

Assessing the Validity of Revised Therapeutic Reference Ranges for Psychotropic Drugs: A Comparative Analysis of Updated Ranges and Real-world Distributions in a Medical Laboratory Setting

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Introduction: The value and utility of blood level monitoring, referred to as Therapeutic Drug Monitoring (TDM), remain subjects of ongoing debate within the medical community. Regrettably, its importance is frequently undervalued by clinicians. One of the challenges faced by TDM is the absence of clear reporting of drug-specific therapeutic reference ranges in the literature. To address these challenges, the existing AGNP TDM guidelines are undergoing revisions to improve the methods used for establishing therapeutic reference ranges.

Method:

First, we developed a systematic methodology on how to find therapeutic reference ranges for psychotropic drugs [1].

Second, serum level data was collected anonymized from a routine drug monitoring service for aripiprazole (N=3169), olanzapine (N=5657), and venlafaxine (N=6332) irrespective of clinical settings such as dosing or diagnosis. We calculated data distributions and derived preliminary reference ranges using the 25th to 75th interquartile range (IQR). The focus of this work is to compare the ranges defined by systematic protocol with real-world data collected in a TDM routine laboratory.

Results:

Recently, we have published several studies introducing updated therapeutic reference ranges in adults for a series of psychotropic drugs [2-5]. For the antipsychotic drugs olanzapine and aripiprazole, and for the antidepressant drug venlafaxine, we could define following valid therapeutic reference ranges: i) 120-270 ng/mL for aripiprazole, ii) 20-40 ng/mL for

olanzapine, and iii) 140-600 ng/ml for the active moiety of venlafaxine. Findings from dopamine receptor occupancy studies support these novel ranges.

In the real-world data sets, none of the analyzed drug data followed a gaussian distribution. Our suggested preliminary reference ranges (IQR) are as follows: 99-273 ng/mL for aripiprazole, 20-52 ng/mL for olanzapine, 115-274 ng/mL for o-desmethylvenlafaxine, and 176-411 ng/mL for the sum of o-desmethylvenlafaxine plus venlafaxine. The revised reference ranges align well with real-world data, as we have reported.

Conclusion: The relationship between the concentration of many psychotropic drugs and their therapeutic effects is not always well-defined. In such cases, preliminary reference ranges can be established using real-world data from routine laboratories. The updated reference ranges will be a valuable tool for clinicians, supporting the integration of TDM in patient care.

DGKL: 05. Gender Medicine, Pediatric Laboratory Medicine, Decision Limits, Reference Intervals

Plasma reference values for C19 oxy-steroids, 11-keto testosterone and 11-keto androstenedione in a paediatric cohort

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Introduction

Rege et al (2018) showed that 11-keto-testosterone (11KT) is the dominant androgen in girls during adrenarche. Claahsen-van der Grinten et al (2022) mention C19 oxysteroids as a possible parameter for therapy control in congenital adrenal hyperplasia (CAH). Turcu et al. (2016) measured significantly increased C19 oxysteroids in patients with 21-hydroxylase deficiency (21OHD). To date, C-19 oxysteroids are not routinely measured and there are no paediatric reference values.

Method

An LC-MS/MS method was developed to determine 11KT and 11-keto-androstenedione (11KA4). Serum and plasma samples (0.1 mL) were extracted using solid phase extraction (SPE). The study included 414 healthy children (140 males, 274 females, aged 0-18 years) using residual material from blood checks. A complete steroid hormone profile was available for all children, with BMI known for 412 and Tanner stage B for 251 girls and Tanner stage G for 107 boys.

Results

The method was linear from 0,5 nmol/L up to 50 nmol/L for both hormones. The lowest limit of quantification was 36 pmol/L. The coefficient of variation was highest for 11KT at 9% and for 11KA at 11%.

Prepubertal (< 8 years) girls (Mean 0.49 nmol/L) have significantly higher 11KT values than boys (Mean 0.19 nmol/L) ($p < 0.01$). With the onset of thelarche, 11KT values in girls increase significantly from Mean 0.52 nmol/L (Tanner B1) to Mean 0.93 nmol/L (Tanner B2-5) ($p < 0.0001$). In boys, 11KT increases significantly with the onset of gonadarche from MW 0.53 nmol/L (Tanner G1) to MW 0.89 nmol/L (Tanner G2-5) ($p < 0.001$). Obese girls (pBMI>97th) have significantly higher 11 KT levels than normal weight girls (pBMI < 90th) (Mean 0.95 nmol/L vs. 0.62 nmol/L; $p < 0.01$).

Conclusion

Age- and gender-specific reference values for 11KT and 11KA4 have been established. These values can serve as a foundation for future assessments of C19 oxy steroids. The data confirm the significance of C19 oxy steroids during physiological development. It has been demonstrated that the concentration of 11KT increases in both boys and girls with the onset of puberty. The data from the CAH cohort confirm that 11KT levels can be elevated in 21OHD. Further studies are required to determine the extent to which this is dependent on the therapy setting.

DGKL: 06. POCT, Global Health, Mobile Laboratories

Utilization of laboratory point-of-care testing in the outpatient sector: An analysis of reimbursement data from Thuringia, Germany

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Introduction: Point-of-care tests (POCTs) are laboratory procedures that can be performed within practices and typically yield results in less than 15 minutes. Although a wide range of different POCTs are available, surprisingly little is known about their utilization in German outpatient care. This study aims to investigate the current use and trends of POCT use by outpatient physicians in Germany.

Methods: We conducted a retrospective consecutive, cross-sectional study by analysing outpatient healthcare claims data (2017 – 2022) provided by the Thuringian Association of Statutory Health Insurance Physicians. These data include all POCT uses by outpatient physicians offering care to statutory health insurance patients in Thuringia (91% of the total Thuringian patient population).

Results: We will present results on the utilization of POCTs outpatient physicians in Thuringia. This will include results on frequently used POCTs and whether there have been any changes in POCT utilisation over time, including the effects of the COVID-19 pandemic. Moreover, we will discuss the role of POCT diagnostics within outpatient practices in relation to initiated external laboratory service. Our analyses will also reveal POCT utilisation in different outpatient physician groups, such as general practitioners, paediatricians and gynaecologists. Additionally, we will report which characteristics of physicians (i.e. age, sex, practice type, community type of practice location) and patients (age, sex) are associated with POCT utilization.

Conclusion: Our study represents the first systematic investigation of POCT utilization by outpatient physicians in Germany. Our findings will contribute to an understanding of recent trends in POCT use in German outpatient care. The results will provide empirical evidence for physicians, researchers and stakeholder of the German healthcare system.

DGKL: 06. POCT, Global Health, Mobile Laboratories

Kennzeichnung pathologischer BGA-Werte für mehr Patientensicherheit / Welche Möglichkeiten bietet POCT?

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Zielsetzung: Die Kennzeichnung pathologischer POCT-BGA-Werte unterstützt den Kliniker in der Beurteilung der Messergebnisse und erhöht die Patientensicherheit. Hierzu bedarf es der Berücksichtigung altersabhängiger Referenzbereiche je Parameter. Falls die POCT-BGA-Geräte nicht über das Laborinformationssystem (LIS) angebunden sind, bestehen aufgrund

Gerätesoftware und/oder Middleware oft erhebliche Limitationen und es können oftmals Referenzbereiche erst ab dem 18. Lebensjahr angegeben werden. Um pathologische BGA-Messergebnisse unterhalb des 18. Lebensjahres ohne LIS-Anbindung zu kennzeichnen, bedurfte es eines neuen Ansatzes.

Methoden: Die Limitation der BGA-Systeme bzgl. der Referenzbereiche in den von uns betreuten Kliniken liegt in der Gerätesoftware. Hier besteht nur die Möglichkeit der Eingabe von altersabhängigen Referenzbereichen für 10 Altersstufen, wobei erschwerend zu berücksichtigen ist, dass für alle BGA-Parameter die gleichen Altersstufen anzuwenden sind.

Ergebnis und Schlussfolgerung: Auf Basis einer Literaturrecherche für BGA-Referenzbereiche wurde eine Tabelle entwickelt und in der BGA-Gerätesoftware eingepflegt, welche diese Vorgaben berücksichtigt. Dabei war allerdings zu berücksichtigen, dass z.B. der Parameter Hämoglobin mehr als 10 Altersstufen für die altersabhängigen Referenzbereiche umfasst. Es konnte erreicht werden, dass an den POCT-BGA-Systemen bereits ab dem sechsten Lebensmonat altersabhängige Referenzbereiche angezeigt und damit auch pathologische Werte gekennzeichnet werden. Dies unterstütz die Kliniker in der Beurteilung der Messergebnisse und verbessert die Patientensicherheit.

Auszug der Liste altersabhängiger BGA-Referenzbereiche

Parameter	Einheit	arteriell, kapillär				
Alter	*bis 1 Jahr	bis 2 Jahre	bis 6 Jahre	bis 12 Jahre	bis 18 Jahre	
pH	mmHg	7,38-7,45				
Alter	*bis 1 Jahr	bis 2 Jahre	bis 6 Jahre	bis 12 Jahre	bis 18 Jahre	
PCO2 m	mmHg	27,0-39,8				
PCO2 w	mmHg	27,0-39,8				
Alter	*bis 1 Jahre	bis 2 Jahre	bis 6 Jahre	bis 12 Jahre	bis 18 Jahre	
tHB m	g/dl	10,2-13,4	10,2-13,4	10,7-13,9	11,2-14,6	12,5-16,6
tHB w	g/dl	10,2-13,4	10,2-13,4	10,7-13,9	11,2-14,6	12,0-15,4

*diese Werte gelten ab 6 Monate

Auszug der Liste altersabhängiger BGA-Referenzbereiche

Parameter	Einheit	arteriell, kapillär				
Alter	bis 20 Jahre	bis 60 Jahre	bis 65 Jahre	bis 90Jahre	bis 120 Jahre	
pH	mmHg	7,37-7,45	7,37-7,45	7,37-7,45	7,37-7,45	7,37-7,45
Alter	bis 20 Jahre	bis 60 Jahre	bis 65 Jahre	bis 90Jahre	bis 120 Jahre	
PCO2 m	mmHg	35-46	35-46	35-46	35-46	35-46
PCO2 w	mmHg	32-43	32-43	32-43	32-43	32-43
Alter	bis 20 Jahre	bis 60 Jahre	bis 65 Jahre	bis 90Jahre	bis 120 Jahre	
tHB m	g/dl	13,5-17,0	13,5-17,0	13,5-17,0	12,5-17,2	12,5-17,2
tHB w	g/dl	12,0-15,6	12,0-15,6	12,0-15,6	11,8-15,8	11,8-15,8

DGKL: 06. POCT, Global Health, Mobile Laboratories

Etablierung eines neuen POCT BGA-Ausfallkonzepts / Besser aktiv als passiv

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Hintergrund: Angesichts der hohen klinischen Relevanz und Dringlichkeit der patientennahen BGA-Messung bedarf es eines Ausfallkonzepts für den Fall von Gerätestörungen. Vielfach sieht das Ausfallkonzept die Nutzung von BGA-Geräten auf Nachbarstationen, die BGA-Messung im Labor oder die Bereitstellung von POCT-BGA-Geräten aus dem Labor vor (passives Konzept). Dies führt jedoch immer wieder zu Problemen bei der Nutzung der BGA-Geräte oder zu erheblichen Zeitverzögerungen aufgrund langer Laufwege oder Vorbereitungszeiten bis die POCT-BGA-Geräte aus dem Labor

messbereit sind. Hieraus ergab sich die Notwendigkeit der Etablierung eines an die Strukturen und Erfordernisse der Kliniken angepassten BGA-Ausfallkonzepts.

Methoden: Entsprechend der strukturellen Aufteilung der von uns betreuten Kliniken in drei Campi wurde pro Campus ein BGA-Ausfallgerät in zentralen Bereichen aufgestellt. Diese Bereiche sind jeweils bereits mit einem BGA-Gerät ausgestattet und nutzen darüber hinaus die BGA-Ausfallgeräte für Patientenmessungen (aktives Konzept). Um bei einer Gerätestörung maximale Flexibilität zu bieten, sind die BGA-Ausfallgeräte auf einem stabilen Rollwagen montiert und verfügen über eine zusätzliche Stromversorgung via USV (Unterbrechungsfreie Stromversorgung). Zusätzlich sind die Austauschgeräte über eine WLAN-Bridge ins Netzwerk eingebunden, um bei Nutzung in anderen Bereichen eine schnelle Übertragung der Patientenmessergebnisse ins Krankenhausinformationssystem zu gewährleisten.

Ergebnisse und Schlussfolgerung: Mit Hilfe der IT-Abteilung und der Herstellerfirma der BGA-Geräte wurde dieses Ausfallkonzept entwickelt, getestet und implementiert. Durch die routinemäßige Nutzung der Austauschgeräte im patientennahen Bereich, wurde ein kosteneffektives, sicheres und zeitsparendes Austauschkonzept geschaffen, welches bei den Mitarbeitern der Kliniken zu einer hohen Akzeptanz geführt hat.

DGKL: 06. POCT, Global Health, Mobile Laboratories

SARS-CoV-2 Antigen Rapid Detection Tests: test performance during the COVID-19 pandemic and the impact of COVID-19 vaccination

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Introduction

SARS-CoV-2 antigen rapid detection tests (RDTs) emerged as point-of-care diagnostics alongside reverse transcription polymerase chain reaction (RT-qPCR) as reference. This study investigates RDT long-term performance in large-scale, clinical screening use during the COVID-19 pandemic up to endemic transition, amidst virus variants of concern and increasing vaccination rates.

Methods

In a prospective performance assessment from 12 November 2020 to 30 June 2023 at a single centre tertiary care hospital, RDTs from three manufacturers (NADAL®, Panbio™, MEDsan®) were compared to RT-qPCR with standardised viral load among patients, accompanying persons and staff aged ≥ six month. Regression models were used to assess influencing factors on RDT performance.

Results

Among 78,798 RDT/RT-qPCR tandems analysed, 2,016 (2.6%) tested SARS-CoV-2 positive, with an overall sensitivity of 34.5% (95% CI 32.4-36.6%). A logistic regression revealed a significant decline of typical COVID-19 symptoms over the pandemic course and lower rate of individuals with a typically symptomatic infection among vaccinated. The lasso regression model indicated only higher viral load and typical COVID-19 symptoms as significantly increasing the likelihood of a positive RDT result in the case of a SARS-CoV-2 infection directly.

Conclusions

Viral load and COVID-19 symptoms directly influence RDT performance, while the effects of vaccination and Omicron VOC on RDT performance are mediated by these factors. RDTs remain valuable for detecting SARS-CoV-2 in symptomatic individuals and offer potential for detecting other respiratory pathogens in the post-pandemic era, underscoring their importance in infection control efforts.

DGKL: 06. POCT, Global Health, Mobile Laboratories

Fäkales Calprotectin gemessen mit einem App-basierten Heimtest im Vergleich zu einer Labor-Standardmethode

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Zielsetzung

Fäkales Calprotectin (FC) dient als nicht-invasiver Marker für die Beurteilung der Krankheitsaktivität bei Patienten mit chronisch-entzündlichen Darmerkrankungen (CED). Gemessen wird in der Regel mit immunologischen Methoden wie dem Enzyme-linked Immunosorbent Assay. Es sind allerdings auch quantitative Heimtests auf Basis der Lateral-Flow-Technologie mit Smartphones als Auslesegeräte kommerziell erhältlich. Das Ziel der Studie war der Vergleich der quantitativen und qualitativen Leistung zwischen dem FC-Heimtest Preventis SmarTest® Calprotectin Home und dem in unserem Labor verwendeten immunologischen Tests (Eurospital Calprest® Turbo).

Methoden:

45 Routineproben wurden parallel mit beiden Tests gemäß den Anweisungen der Hersteller analysiert. Die Auslesung des Heimtests wurde mit zwei Smartphones (Apple iPhone 14 Pro und Samsung Galaxy XCover 5) durchgeführt. Die qualitative Interpretation (positiv, negativ, Graubereich) wurde unter Verwendung der vom Hersteller bereitgestellten Cut-offs durchgeführt.

Ergebnisse:

Statistisch signifikante Korrelationen zwischen Heimtest und Laborstandardmethode wurden für beide Smartphones ermittelt (Spearman’s rho 0.703 und 0.715, alle $p < 0.005$). Der Heimtest zeigte systematisch höhere Konzentrationen im Vergleich zum Routinetest. Es zeigte sich eine minimale qualitative Übereinstimmung zwischen den beiden Tests (Cohen’s Kappas (κ) = 0.323 und 0.300; p = 0.003 und 0.005), insbesondere fand sich mit dem Heimtest eine niedrigere Rate an Positiven. Beide verwendeten Smartphones zeigten eine gute quantitative und qualitative Übereinstimmung.

Diskussion und Schlussfolgerung:

Die Tests sind quantitativ nicht austauschbar. Aufgrund der guten Korrelation ist der Heimtest jedoch prinzipiell gut für das Follow-up-Management von Patienten mit bekannter CED anwendbar. Die höhere Rate an Proben, die mit dem Heimtest als negativ eingestuft wurden, könnte zu einer Unterschätzung der tatsächlich betroffenen Patienten bei erstmaliger Durchführung des Tests führen.

DGKL: 06. POCT, Global Health, Mobile Laboratories

Revolutionizing Urine Analysis: AI-Powered Digital Holographic Microscopy in a Handheld Device

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Introduction

The ECLM European Urinanalysis Guideline has recently undergone comprehensive revision, highlighting, in addition to the increasing prevalence of renal diseases, the significance of effective and early diagnosis of urinary system impairments. Mostly the analysis is based on the urine stick, but 10-30 % of the results are abnormal and require further investigation. Digital holographic microscopy (DHM), which does not require centrifugation, offers a precise alternative to sediment analysis. The device presented herein is unique in its kind, employing a combination of DHM and an AI-based algorithm in a handheld format, thereby bringing advanced urine analysis directly to the patient.

Method

Digital holographic microscopy (DHM) is an innovative combination of holography and microscopy. By illuminating the uncentrifuged and homogenized urine sample with coherent light, microscopic objects present in the urine will diffract the light. Together with the unaltered reference light, a complex interference pattern is generated, defined as a hologram. The dataset of this pattern contains information about both, the intensity and the phase of the light waves. Advanced image analysis software based on the principles of diffraction and interference is used to reconstruct a 3D image. Subsequently, a trained machine-learning algorithm detects and categorizes the objects.

Results

Automatic image analysis based on DHM-generated images in a handheld format is unique to date. It is remarkable that no urine labeling or centrifugation is required for particle analysis. Compared to other, more complex devices, these differences will not lead to any significant loss of performance. Existing data demonstrate precision for pathological samples of 4.3 % for erythrocytes and 6.2 % for leukocytes. Comparative measurements with already established devices on the market (UriSed mini and Sysmex i500) are currently ongoing but will be finalized for poster presentation.

Conclusion

Based on current results, the performance of the device can be expected to be comparable with much more complex systems available on the market. A precise comparison will reveal slightly larger ranges of variation. However, these must be considered in the context of the significant advantages of the device, which are mainly due to the use of DHM in combination with an AI-based algorithm. Together, they provide new possibilities in the field of effective urine analysis. Immediate availability reduces the hurdles and shortens the time between sampling and analysis, which can have a significant impact on the analysis of urine samples. Thus, the device can make a crucial contribution to timely diagnosis of urinary system disorders.

DGKL: 06. POCT, Global Health, Mobile Laboratories

Reliability of co-oximetry versus SLS hemoglobin determination in elderly patients at University Hospital Bonn

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Introduction

In elderly patients, diagnosis of anemia often occurs (WHO anemia definition: hemoglobin (Hb) male < 13 g/dl, Hb female < 12 g/dl). Furthermore, the frequency of transfusion increases with age. During a patient's first medical examination at the emergency department of the supra maximal care University Hospital Bonn, hemoglobin determination is performed with SLS as well as co-oximetry determination. Given the importance of determining anemia correctly, the following questions arise: Do the two methods yield comparable results in elderly (65-84 years) and suprageriatric (> 85 years) patients? Are both methods equally appropriate for diagnosing anemia / critical anemia in these critical patient collectives?

Methods

This single-center retrospective observational study was performed at the central laboratory and the interdisciplinary emergency center of the University Hospital Bonn, Germany (UKB).

Age 65-85 years, n = 6500 patients (male 3677, female 2823, mean age 74.8 years) and for age > 85 years, n = 1481 (male 627, female 854, mean age 88.9 years) patients were included. For each patient, point of care co-oximetry Hb concentration was determined with RapidLab 1265 (Siemens Healthineers), and central laboratory SLS Hb concentration was determined with XN1000 (Sysmex). Results were compared and statistical analyses were performed in "R" regarding diagnosis of anemia (WHO definition)/ critical anemia (Hb < 8 g/dl).

Results

Classification of WHO anemia differed for both age groups as revealed by McNemar tests (65-85 years: all $p < 0.001$, and age > 85 years: all $p < 0.001$). Regarding classification of critical anemia Hb < 8 g/dl, the two methods differed depending on age group. For age group 65-85 years, point of care co-oximetry and central laboratory SLS Hb concentrations differed significantly in their classification of critical anemia ($p = 0.025$), whereas for age > 85 years, there was no significant difference between the two methods ($p = 0.054$).

Conclusion

Overall, in elderly patients point of care co-oximetry and central laboratory SLS Hb concentrations yielded relevant differences regarding diagnosis of anemia. Hb concentrations determined with SLS are more likely to yield a diagnosis of WHO anemia compared to Hb determined with point of care co-oximetry at University Hospital Bonn. In contrast to age group 65-85 years, suprageriatric patients showed no differences between the two methods regarding critical anemia.

DGKL: 07. Hematology, Hemostasis

Evaluation of the Sysmex CN-6000 coagulation analyzer for routine and specialized coagulation testing in a central laboratory

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Introduction: The Sysmex CN-6000 is a fully automated high-throughput coagulation analyzer. The objective of this study was to evaluate the analytical performance of the analyzer for routine and special coagulation testing in a high-throughput central laboratory of a university hospital.

Methods: The intra- and between-day precision and accuracy of 29 coagulation parameters were evaluated on the Sysmex CN-6000 using commercially available quality control materials. Patient plasma samples were used to compare results of coagulation measurements between the Sysmex CN-6000 and the Atellica COAG 360 including plasma samples with visual interference. The sample throughput of both analyzers was compared using plasma samples from healthy volunteers.

Results: Intra- and between-day coefficients of variation were acceptable for all assays tested on the Sysmex CN-6000. High correlation and good agreement were observed when comparing coagulation results from the Sysmex CN-6000 and the Atellica COAG 360. Samples with visual interference showed comparable coagulation results between the two analyzers with slightly better detection by the Sysmex CN-6000. The sample throughput per hour for analysis of a panel of five coagulation parameters was higher with the Sysmex CN-6000 compared to the Atellica COAG 360 (247 vs. 193 tests).

Conclusions: The Sysmex CN-6000 demonstrated excellent analytical performance for a large number of coagulation parameters and has a high throughput capacity, ideal for the needs of a central laboratory with a high volume of routine and specialized coagulation testing.

DGKL: 07. Hematology, Hemostasis

Testing factor VIII activity in patients with haemophilia A: a comparison of the results of a one-stage clotting and a chromogenic substrate assay.

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Introduction:

Haemophilia A is a congenital bleeding disorder caused by deficiency or dysfunctionality of clotting factor VIII (FVIII), leading to spontaneous or prolonged bleeding and in particular cases to severe haemorrhage. Accurate measurement of FVIII activity is crucial for diagnosis, classification, treatment monitoring, and therapy decision-making in these patients. Several laboratory test assays are available for FVIII activity measurement, of which the most commonly used are the one-stage clotting assays (OSA) and the chromogenic substrate assays (CSA). However, result deviations obtained from these assays are frequent and may lead to over- or underestimation of haemophilia severity, especially in patients classified with mild or moderate haemophilia. The analytical difference between these assays can be attributed to genetic FVIII variations or specific replacement therapies with plasma-derived or recombinant FVIII concentrates. Despite these challenges, a comprehensive understanding of specific factors contributing to inter-assay discrepancies is still lacking. Additionally, limited laboratory access to various FVIII activity assays further complicates an optimized assessment of FVIII activity in clinical settings. A standardized laboratory approach including dual FVIII activity testing might help to improve the diagnosis and therefore treatment of patients suffering from haemophilia A.

Aims and Methods:

This study aims to investigate analytical differences of FVIII activity testing comparing laboratory findings obtained from OSA and CSA. We conduct a retrospective analysis for patients suspected of or diagnosed with haemophilia A, who were presented to a coagulation outpatient department or for hospital treatment. Patient samples for FVIII activity measurement were collected between 2022 and the present, being simultaneously tested by OSA and CSA. Patients treated with Emicizumab are excluded from the study.

Results:

Our analysis will systematically correlate the results of FVIII activity testing received from OSA and CSA. We will identify the frequency and magnitude of assay discrepancies between the two laboratory tests and evaluate their implications for the classification of haemophilia A severity.

Conclusion:

A standardized dual laboratory approach for FVIII activity testing with OSA and CSA could enhance the accuracy of diagnosis and treatment decision-making in patients with haemophilia A. Such an approach may lead to reduced treatment costs and improved quality of life for affected individuals.

DGKL: 07. Hematology, Hemostasis

Raman-spektroskopische Charakteristika von Zellen im Blutausstrich

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Der Raman-Effekt basiert auf der inelastischen Streuung von Licht an Materie. Durch applizieren von Strahlungsenergie (monochromatischem Licht) kann es zu Energieübertragung zwischen Photon und Molekül kommen und das gestreute Licht somit eine höhere oder niedrigere Frequenz als der einfallende Lichtstrahl besitzen. Diese Energie bzw. Frequenz-Verschiebung kann als Raman-Spektrum dargestellt werden und ist spezifisch für das mit dem Laser wechselwirkende Molekül.

Für die Untersuchung zirkulierender Blutzellen und deren Differenzierung („großes Blutbild“) spielt die mikroskopische Betrachtung gefärbter Blutausstriche in der Routine-Labordiagnostik eine bedeutende Rolle. Physiologische sowie pathologische Veränderungen der verschiedenen Zellreihen können bewertet werden. Die Analytik setzt insbesondere bei bestimmten Pathologien eine große Erfahrung der untersuchenden Person voraus und untersucherabhängige Abweichungen in der Bewertung sind nicht auszuschließen.

Wir untersuchen mittels Raman Spektroskopie charakteristische „Fingerabdrücke“ der verschiedenen Zelltypen, welche deren molekulare Komposition insgesamt abbilden. Hierfür werden von Blutausstrichen Raman-Spektren entlang der Oberfläche aufgenommen und mittels Raman-Bildgebung als zweidimensionales Bild dargestellt, wobei jedes Pixel die Information des korrespondierenden Einzelspektrums enthält.

Ziel ist in einem ersten Schritt die verschiedenen Zelltypen anhand ihrer Spektren (mithilfe statistischer Verfahren, “KI”) zu charakterisieren und sauber voneinander unterschieden zu können. In einem nächsten Schritt sollen inflammatorisch veränderte Leukozyten charakterisiert werden. Hierfür werden sowohl Raman-Spektren von Mitogen-aktivierten Lymphozyten als auch von inflammatorischen Patientenproben untersucht. Perspektivisch ist ein Ziel, die inflammatorische Genese (z.B. infektiologisch vs. autoimmun) mittels spezifischer Unterschiede in den Raman-Spektren identifizieren zu können.

DGKL: 07. Hematology, Hemostasis

Influence of coagulation proteases on cognitive function

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Background

A role of coagulation proteases, their receptors (including protease activated receptors, PARs) and fibrinolysis in the central nervous system (CNS) in health and disease is established. One important regulator of coagulation protease activity and signaling is thrombomodulin (TM), a mostly endothelial expressed protein, which mediates activation of the anticoagulant and cytoprotective protease activated protein C (aPC). TM expression and function is impaired in vascular disease and vascular aging, the latter often being accompanied by cognitive impairment.

Aims

We aim to answer the question whether TMPro/Pro mice (a Glu404Pro point mutation reduces TM-thrombin-dependent aPC generation by >90%) show phenotypical alterations in the brain and if yes, whether this is related to hypercoagulability or loss of aPC generation.

Methods

We will use mouse models with different genotypes and interventions to investigate coagulation-dependent impairment of cognition using tests for locomotor activity, fear-related exploratory behavior and learning patterns.

Results

We show that mice with a partial loss of TM-function (TMPro/Pro mice) have impaired cognition and altered gene-expression in various cell-types in the CNS (snRNAseq results). Surprisingly, we could show that the cognitive impairment in TMPro/Pro mice was restored when crossing the TMPro/Pro mouse with a mouse carrying a hyperactivatable protein C transgene (aPChigh)

Conclusion

We suggest that loss of TM-function, as observed in vascular disease, may impair CNS function. These results extend previous work by the Isermann group showing an impaired myelination and increased ROS generation in the CNS in TMPro/Pro mice. We further aim to answer whether interventions restoring specific TM-functions may rescue the phenotype and whether an acquired loss of TM-function causes structural CNS-defects and impairs cognition. Answering these questions will provide important insights into the TM-function for CNS-related disease processes and provide important mechanistic hints.

DGKL: 07. Hematology, Hemostasis

Implementation of a Semi-Automated Multimer Analysis of the von Willebrand Factor

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Von Willebrand syndrome (vWS) is a common bleeding disorder, and its diagnosis requires specialised methods. Multimer analysis detects qualitative and structural changes in vWF and thus allows the classification of vWS as part of a staged diagnosis. This complex analysis is often performed manually using agarose gel electrophoresis. A commercial assay kit suitable for in-vitro diagnostics, Hydragel vWF multimers from Sebia, has been available for semi-automated analysis for a few years. To date, there are no reference ranges for this technique in Germany.

In addition to samples from healthy volunteers, we acquired data from vWS patients with different subtypes. We demonstrate our improved gel image analysis and the validation of this assay system according to CLSI guidelines. Our data will contribute to improving the diagnosis of vWS patients through standardised tests and faster diagnosis.

DGKL: 07. Hematology, Hemostasis

Mentzer- and Huber-Herklotz-index are not sensitive enough to reliably predict alpha-thalassemia in a large real-world cohort

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Introduction

Alpha-thalassemia is usually not detected by hemoglobin separation techniques like HPLC or electrophoresis, and a definite diagnosis can only be made by molecular genetics methods. Different erythrocyte index formulas have been described to predict (alpha-) thalassemia. Two frequently used formulas, especially in Germany, are the Mentzer- and Huber-Herklotz-index (HH).

In the present study, we sought to evaluate these prediction methods in a routine clinical laboratory setting using real- world data.

Methods

In the period 2014-2024, consecutive samples from patients sent for hemoglobinopathy work-up at two large European laboratories (Fürst Medical Laboratory, Oslo, Norway, and MLL Munich Leukemia Laboratory, Munich, Germany) were included. Alpha-thalassemias were detected by multiplex gap-PCR or MLPA. Hemoglobin electrophoresis was performed on SEBIA Capillarys. Erythrocyte parameters and ferritin were measured on Sysmex XN-1000 and SIEMENS Advia or Atellica instruments, respectively.

The Mentzer-index was calculated by Mentzer = MCV (fL) / RBC (T/L), the Huber-Herklotz-index by HH = (MCH (pg) x RDW (fL)) / (10 x RBC (T/L)) + RDW (fL).

Results

In samples from 18,928 individuals, alpha- and beta-thalassemia were detected in 4,216 and 3,435 individuals, respectively.

Applying the Mentzer-index to patients with microcytosis, only 1,691 samples from patients with alpha-thalassemia showed a value below 13 indicating thalassemia, whereas 2,519 patients with alpha-thalassemia had a Mentzer-index above 13 or no microcytosis. Thus, the sensitivity of the Mentzer-index to detect alpha-thalassemia was 40.1%. The overall specificity of the Mentzer index was 94.5%.

HH was applicable to 1017 alpha-thalassemia cases and showed a value below 23 (indicating alpha-thalassemia) in 703 cases (69.1%). By definition, the HH cannot be applied when MCH is above 27 pg, which was the case in 99 samples with alpha-thalassemia. Thus, HH with a cut-off value of 23 showed an overall sensitivity of 63.0% and a specificity of 55.1%. With a cut-off value of 20, HH had a sensitivity of 15.9% and a specificity of 89.6%.

Conclusions

Applying the Mentzer- and the Huber-Herklotz-index to real world data showed that these formulas exhibit rather low sensitivities to predict alpha-thalassemia missing about half of all patients. Thus, molecular genetic testing should be performed in combination with hemoglobin separation techniques when samples are investigated for thalassemia in a routine clinical laboratory setting.

DGKL: 07. Hematology, Hemostasis

FVIII und Emicizumab: Möglichkeiten der differenzierten Messung

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Einleitung: Emicizumab bindet als bispezifischer humanisierter rekombinanter Antikörper FIXa und FX und ahmt dadurch die Gerinnungsaktivität des FVIIIa nach. Klinische Studiendaten haben die Sicherheit und Effektivität von Emicizumab zur

Behandlung von Patienten mit neutralisierenden anti-FVIII Antikörper (Inhibitoren) bei erworbener Hämophilie A gezeigt1). Die Emicizumabtherapie stellt die labormedizinische Hämostaseologie vor Herausforderungen, da die korrekte Bestimmung von Emicizumab in der Gegenwart von FVIII, zum Beispiel bei therapeutischer Gabe von porcinem FVIII (susoctocog alfa, rpFVIII) oder ansteigenden FVIII Spiegeln in der Remission, in den bisherigen Labortesten erschwert ist. In gleicher Weise kann Emicizumab in der Bestimmung der FVIII Restaktivität interferieren und in den Standardtesten zu falsch erhöhten Werten führen.

Methoden: Labormedizinische Analysen zur Messung der Emicizumabkonzentration im Plasma wurden etabliert (chromogene FVIII Aktivitätsbestimmung mit humanen Gerinnungsfaktoren; Emi-CSA, modifizierter Clot-Assay; EMI-CBA). Die residuale FVIII Aktivität wurde mithilfe von spezifischen anti-FVIII Antikörpern eliminiert. Mithilfe von anti-Emicizumab Fab Fragmenten konnte die Interferenz von Emicizumab im Test der aktivierten partiellen Thromboplastinzeit (APTT) und Testen, die auf der APTT basieren, eliminiert werden.

Ergebnisse: Sowohl der EMI-CSA als auch der EMI-CBA sind geeignet Emicizumabkonzentrationen in Plasmaproben von Patienten und in Proben, die mit Emicizumab versetzt wurden, zu detektieren. Die untere Nachweis- und Bestimmungsgrenze war im EMI-CSA etwas niedriger im Vergleich zum Emi-CBA. Beide Teste zeigten eine akzeptable intra- und inter-Assay Varianz von < 15%. Sowohl rekombinanter humaner als auch porciner FVIII interferiert mit der Emiczumab Aktivität im EMI-CSA und EMI-CBA. Dieser Störfaktor des residualen FVIII konnte durch die Präinkubation mit anti-FVIII Antikörpern beseitigt werden. Demgegenüber war die Neutralisierung von Emicizumab durch die Verwendung von zwei spezifischen Fab Fragmenten gegen Emicizumab effektiv und eröffnet damit die Möglichkeit der Verwendung von APTT-basierten Testen (FVIII Aktivität, Bethesda Assay zur Bestimmung von Inhibitoren).

Fazit: Die modifizierten hämostaseologischen Teste ermöglichen die differenzierte Messung von Emicizumab, FVIII Aktivität und Inhibitorkonzentration in Patienten mit erworbener Hämophilie A und damit sowohl ein Monitoring der Emicizumabtherapie, der rpFVIII Substitution als auch des Verlaufs der Erkrankung (FVIII, Inhibitor).

DGKL: 07. Hematology, Hemostasis

Characterization of platelets in critically ill patients with COVID-19 viremia

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Introduction

Platelets are small, non-nucleated cells that play a central role in haemostasis. They are also important in supporting the immune system during viral infections. Overactivation of platelets has been reported in severe coronavirus disease 2019 (COVID-19), resulting in the release of granules, exacerbating inflammation and contributing to a cytokine storm. The aim of this study was to comprehensively investigate the role of platelets in COVID-19 progression and to identify predictive biomarkers of disease outcome (Wolny et al., 2023). Furthermore a higher amount of immature platelet fraction (IPF) was observed in patients with severe COVID-19 disease. These occur when platelets are consumed, and are a measure of increased thrombopoiesis . These IPF cells are characterized by their comparatively larger size, high level of alpha and dense granules, RNA content. They are hyperreactive and possess prothrombotic activity (Wolny, et al. 2023).

Methods

Mass spectrometry-based proteomic profiling of highly purified platelets from critically diseased COVID-19 patients with different outcomes (survivors and non-survivors) and age- and gender-matched controls was performed. Potential

biomarker candidates were further validated in both platelets and plasma using two independent methods including targeted mass spectrometry based on parallel reaction monitoring (PRM) and ELISA. For the IPF investigation the XN-1000 (Sysmex) analysis was used in patient cohort. Platelet functional test was done with light transmission aggregometry and flow cytometry was used for platelet activation marker analysis.

Results

Platelets from severe COVID-19 patients showed significant differences in the abundance of proteins associated with protein folding, degranulation, cytokines and cell signaling (e.g. NFκB pathway). In addition, carbonic anhydrase 1 (CA-1) was identified as a potential marker protein in platelets and showed a significant increase in COVID-19 patients. In a second study, we determined the IPF of 77 hospitalized patients. Platelet count is significantly lower in the severe group than in the mild and moderate group and IPF is increased in severely ill patients.

Conclusion

In conclusion, we have shown that platelets from patients with severe COVID-19 diseasehave a specific proteomic profile compared to healthy controls and surviving patients. Among the identified differential proteins, CA-1 emerged as the most compelling biomarker candidate. Another study showed that platelet count is lower and IPF is higher in severely ill patients than in less severely ill patients. In addition, platelet function is also impaired in these patients.

DGKL: 08. Infectiology, Infection Serology, Microbiology

C-terminal alpha-1-antitrypsin peptides (CAAPs) represent substrates of the serine protease TEMPRSS2 and reduce cytopathic effect of SARS-CoV-2 delta infection

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Introduction

Infection with respiratory viruses, such as influenza A or SARS-CoV-2, depends on the proteolytic pre-processing of viral surface proteins to enable target cell entry. For SARS-CoV-2, proteolytic activation of the viral spike protein, specifically the cleavage of the S1-S2 boundary, by cathepsin L or the transmembrane serine protease 2 (TMPRSS2) facilitates ACE2 receptor binding and cellular entry through endosomal or plasma membrane fusion mechanisms. Thus, regulating protease activity potentially attenuates SARS-CoV-2 infection, particularly when targeted in the respiratory system.[1] In the lung, proteases regulate several processes such as pathogen defense and regeneration, and several antiproteases controll pulmonary protease activity. The SERPIN alpha-1-antitrypsin (AAT) is considered a crucial inhibitor for maintaining pulmonary health, and AAT deficiency due to hereditary mutations in the SEPINA1 gene lead to early onset of COPD with emphysema. Furthermore, AAT inhibits TMPRSS2 activity, thus reducing SARS-CoV-2 infection in vitro.[2] So a geographical overlap between SARS-CoV-2 infection and AAT deficiency has been discussed and studies indicate a higher risk of severe COVID-19 outcome if patients with a SERPINA1 variant and low AAT plasma level.[3,4] Recently, we discovered elevated concentrations of AAT proteolytic cleavage products, known as C-terminal AAT peptides (CAAPs), in the plasma of patients with severe SARS-CoV-2 infection, suggesting their potzential suitable as a predictive biomarkers.[5] Here we observed the proteolytic degradation of AAT and corresponding CAAPs by TMPRSS2 and found an improved efficiency of CAAPs to reduce SARS-CoV-2 infection.

Methods

Incubations of purified TMPRSS2 and human AAT were analysed with MALDI-TOF-MS and LC-MS/MS to investigate AAT degradation and CAAP production. Furthermore, incubations of single CAAPs, C36 and C42, and TMPRSS2 were examined by LC-MS/MS to reveal potential peptide-protease interactions. Afterwards, the inhibition of cytopathic effect by

SASRS-CoV-2 delta variant infection was investigated with WST-1 assay in Vero-E6 cells treated with AAT, CAAPs or control peptides (10-100 µM each).

Results

The MALDI-TOF-MS analysis of AAT/TMPRSS2 incubations indicates a time and concentration dependent degradation of the antiprotease. In addition, LC-MS/MS analysis revealed changes in CAAP concentrations, with specific production of peptide C22 and an approx. 50% decrease of C36. By LC-MS/MS, a degradation of peptides C36 and C42 (10 µM each) by TMPRSS2 (2 µM) could be observed, that associates with generation of C22. Treatment of Vero-E6 cells with C36 or C42 (1µM) one hour before infection with SARS-CoV-2 delta variant improved the cell viability significantly ($p=0.002$ and $p=0.023$) and cytopathic effects could reverse completely using higher peptide concentrations (100 µM). In contrast, treatment with AAT, C22 or control peptides were not effective at the used concentrations.

Conclusions

Here we indicate the degradation of AAT by TMPRSS2, which result in CAAP C22 generation and protease inhibition. Furthermore, the COVID-19 associated CAAPs C36 and C42 retain the interaction with TMPRSS2, generating C22. Furthermore, both peptides possess improved efficiency to inhibit cytopathic effects of SARS-CoV-2 delta variant infection, thus potentially demonstrating their importance in COVID-19 disease progression.

DGKL: 08. Infectiology, Infection Serology, Microbiology

Malaria diagnostics in the Berlin metropolitan area: a comparative analysis of conventional microscopy versus fluorescence flow cytometry.

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Introduction:

The World Health Organization proclaimed approximately 249 million malaria cases and 608,000 deaths in 2022. Thus, malaria remains a significant global health burden. Globalization and migration trends further corroborate the medical impact of malaria also in non-endemic regions including Europe. Moreover, climate change might pose an additional future risk factor for spreading of mosquito-borne diseases. Hence, accurate and fast laboratory malaria diagnostics is decisive for effective patient treatment and clinical management.

Conventional manual microscopy of stained blood smears still represents the diagnostic gold standard. It is a widely accessible and cost-efficient diagnostic method to detect and quantify Plasmodium ssp. infections. However, the limit of detection, i.e. the sensitivity of laboratory methods, is impacted by the microscopist's experience, morphological variations, and low parasitemia levels. Additionally, the preparation of blood smears and the microscopy process itself are time consuming. Fully-automated hematology analytics might represent a feasible approach to circumvent these obstacles.

Aims and Methods:

Here we analyze the performance of the automated fluorescence flow cytometry-based approach applying the Sysmex XN-31 analyzer in comparison to the results of conventional manual microscopy. Determination criteria focus on sensitivity, accurate species identification, calculated parasitemia levels, and on time efficacy.

Results:

Patient samples are subjected to comparative measurements using both laboratory methods (manual vs. automated). Fluorescence flow cytometry results are visualized in two-dimensional scattergrams providing information about distinct

morphological characteristics of plasmodium species and developmental stages. The level of parasitemia is quantified for infections with Plasmodium falciparum. Further, time efficacy for diagnosis is evaluated by comparisons of turn-around-times.

Conclusion:

Malaria diagnostics performed by automated fluorescence flow cytometry may offer a precise, faster, and less laborious diagnostic approach compared to conventional manual microscopy. The automated method might serve as a supportive tool in the context of shortage of trained personnel and increasing needs for standardized laboratory processes.

DGKL: 08. Infectiology, Infection Serology, Microbiology

Bioanalytik für den Gesundheitsschutz – Untersuchungen von pathogenen Bakterien in Lebensmitteln

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Bioanalytik für den Gesundheitsschutz – Untersuchungen von pathogenen Bakterien in Lebensmitteln

Hintergrund: Salmonella, Listeria monocytogenes und EHEC/STEC sind die drei bedeutsamsten Zoonoseerreger in Lebensmitteln. Grundsätzlich sind Zoonoseerreger in Lebensmitteln unerwünscht, denn sie können eine Infektionsquelle für lebensmittelbedingte Erkrankungen sein. Ziel der Untersuchungen ist es die Positivraten der genannten Bakterien in Fleisch, Milch und entsprechenden Erzeugnissen zu ermitteln. Insgesamt wurden (von 2013-2023) 282.435 Lebensmittelproben untersucht und Ergebnisse von 50.954 Hygieneproben ausgewertet.

Methoden: Verfahren zum Nachweis von Salmonellen mittels real-time-PCR1; Horizontales Verfahren zum Nachweis, zur Zählung und zur Serotypisierung von Salmonellen2; Verfahren zum Nachweis von Listeria monocytogenes mittels real-time-PCR3; Horizontales Verfahren für den Nachweis von L. monocytogenes4; Horizontales Verfahren für den Nachweis von Shiga-Toxin bildenden Escherichia coli (STEC)5; Qualitativer Nachweis von Shiga-Toxin bildenden Escherichia coli (STEC) in frischen pflanzlichen Lebensmitteln - Multiplex real-time PCR-Verfahren6.

Ergebnisse: Bei der Anzahl der Untersuchungen (n) wurden die Positivraten (%) ermittelt (keine Veränderung über den Untersuchungszeitraum). Lebensmittel, allgemein: Salmonella PCR1, n=199.233, (0,7%); Salmonella kulturell2, n=34.535, (0,8%); L. monocytogenes PCR3 n=20.178, (3,1%); L. monocytogenes kulturell4, n=15.088, (1,9%); STEC5, STEC6, n=13.401, (7,5%). Fleisch und Fleischerzeugnisse: Salmonella PCR1, n=78.498, (1,1%); Salmonella kulturell2, n=12.490, (1,2%); L. monocytogenes PCR3, n=3.340, (6,5%); L. monocytogenes kulturell4, n=1.426, (5,7%); STEC5n=2.898, (10,9%). Milch, Rohmilch, Erzeugnisse daraus: L. monocytogenes PCR3 n=11.089, (1,8%); L. monocytogenes kulturell4, n=10.601, (1,1%); STEC5, n=1.977, (13,2%). Lebensmittel, frisch, pflanzlich: STEC5, n=2.979, (13,1%). Hygiene: Salmonella PCR1, n=30.435, (0,04%); L. monocytogenes PCR3 n= 20.519, (2,7%).

Diskussion und Schlussfolgerung: Die Ergebnisse unterstreichen die Notwendigkeit der Untersuchungen von Lebensmitteln auf pathogene Bakterien zum Schutz der Verbraucher. Insbesondere rohe Lebensmittel wie Fleisch, Rohmilchkäsen und frische pflanzliche Lebensmittel, können mikrobiologische Gesundheitsgefahren für Menschen bergen. Dies wird durch die ermittelten STEC Positivraten von über 10 % bei den untersuchten Lebensmitteln verdeutlicht. Salmonellen sind in den untersuchten Proben verhältnismäßig seltener nachzuweisen als L. monocytogenes. Auch bei den Resultaten der Hygieneproben spiegelt sich dies wider. Salmonellen sind optimal auf warmblütige Wirte angepasst, befinden sich dort im Darm und können z.B. bei der Schlachtung und Zerlegung auf Fleisch gelangen. L. monocytogenes hingegen können als Umweltkeime in Lebensmittelbetrieben in einem breiten Temperaturbereich überleben und auf unterschiedlichste Weise Lebensmittel kontaminieren.

DGKL: 08. Infectiology, Infection Serology, Microbiology

Intensive Care Infection Score (ICIS) – Frühzeitige Unterscheidung zwischen infektiösem und nicht-infektiösem Zustand bei Vorliegen unterschiedlicher Grunderkrankungen

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Zielsetzung

Unterschiedlichste Grunderkrankungen können mit systemischen Entzündungsreaktionen (SIRS) einhergehen. Hierbei ist es insbesondere bei intensivpflichtigen Patienten herausfordernd zwischen einem infektiösen und einem nicht-infektiösen Zustand zu differenzieren. Unter Einbeziehung des ICIS, der über eine gewichtete Punktzahl die Entzündungsreaktion charakterisiert (Score zwischen 0 und 20) ist das Ziel anhand des ICIS-Wertes die Therapie infektiöser Geschehen zu monitoren. In diesem Zusammenhang werden die Patienten basierend auf der vorliegenden Grunderkrankung kohortiert, um auf diese Weise potenzielle Unterschiede im Verlauf der ICIS-Werte identifizieren zu können. Darüber hinaus wird der Verlauf der ICIS-Werte im Verhältnis zu dem Verlauf der gängigen Infektionsparameter (CRP, PCT, Leukozyten) betrachtet, um somit möglicherweise ein frühzeitigeres Eingreifen in Therapiestrategien hinsichtlich erforderlicher medikamentöser Umstellungen zu ermöglichen.

Methoden

Die ICIS Studie wird auf den Intensivstationen des Klinikum Passau durchgeführt. Eingeschlossen werden Patienten und Patientinnen von 18 bis 99 Jahren, bei denen an mindestens 10 aufeinander folgenden Tagen eine Blutabnahme durchgeführt wurde. Der ICIS, der sich aus Parametern des großen Blutbildes und des Retikulozyten-Hämoglobin-Äquivalent zusammensetzt wird in Zusammenschau mit dem klinischen Verlauf und der zu Grunde liegenden Erkrankung beurteilt. Hierbei werden neben den Infektionsparametern auch die medikamentösen Therapien einbezogen.

Ergebnisse

Die ersten Zwischenanalysen zeigen bereits ein früheres Ansprechen des ICIS auf eine bakterielle Infektion, als dies bei den üblichen Infektionsparametern der Fall ist. Die bisherigen Ergebnisse zeigen, dass der Einsatz des ICIS ein wertvoller Parameter ist um unter anderem frühzeitig medikamentöse Therapien zu steuern. Darüber hinaus deuten die Ergebnisse bisher darauf hin, dass unterschiedliche ICIS Verläufe je nach Grunderkrankung vorliegen, so dass möglicherweise eine Anpassung der Grenzwerte in Erwägung gezogen werden kann. Es bleibt abzuwarten, ob sich bei größeren Kohorten die ersten Tendenzen signifikant bestätigen.

DGKL: 08. Infectiology, Infection Serology, Microbiology

Humoral and cellular immune responses in fully vaccinated individuals with or without SARS-CoV-2 breakthrough infection: Results from the CoV-ADAPT cohort

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Despite recent advances in prophylactic vaccination, SARS-CoV-2 infections continue to cause significant morbidity. A better understanding of immune response differences between vaccinated individuals with and without later SARS-CoV-2 breakthrough infection is urgently needed. CoV-ADAPT is a prospective long-term study comparing humoral (anti-spike-RBD-IgG, neutralization capacity, avidity) and cellular (spike-induced T-cell interferon-γ [IFN-γ] release) immune responses in individuals vaccinated against SARS-CoV-2 at four different time points (three before and one after third vaccination). In this cohort study, 62 fully vaccinated individuals presented with SARS-CoV-2 breakthrough infections vs 151 without infection 3-7 months following third vaccination. Breakthrough infections significantly increased anti-spike-RBD-IgG ($p < 0.01$), but not spike-directed T-cell IFN-γ release (TC) or antibody avidity. Despite comparable surrogate neutralization indices, the functional neutralization capacity against SARS-CoV-2-assessed via a tissue culture-based assay-was significantly higher following breakthrough vs no breakthrough infection. Anti-spike-RBD-IgG and antibody avidity decreased with age ($p < 0.01$) and females showed higher anti-spike-RBD-IgG ($p < 0.01$), and a tendency towards higher antibody avidity ($p = 0.051$). The association between humoral and cellular immune responses previously reported at various time points was lost in subjects after breakthrough infections ($p = 0.807$). Finally, a machine-learning approach based on our large immunological dataset (a total of 49 variables) from different time points was unable to predict breakthrough infections (area under the curve: 0.55). In conclusion, distinct differences in humoral vs cellular immune responses in fully vaccinated individuals with or without breakthrough infection could be demonstrated. Breakthrough infections predominantly drive the humoral response without boosting the cellular component. Breakthrough infections could not be predicted based on immunological data, which indicates a superior role of environmental factors (e.g., virus exposure) in individualized risk assessment.

DGKL: 09. IVDR, In-house Validation

Effiziente Umsetzung der IVDR-Anforderungen für Labore und POCT – Wege aus dem Dschungel

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“Zielsetzung”

Auch zwei Jahre nach Einführung der IVDR stehen Labore und POCT-Hersteller weiterhin vor großen Herausforderungen. Seien es die Anforderungen an das QM-System (EN ISO 15189) oder an die Erfüllung der Grundlegenden Sicherheits- und Leistungsanforderungen; hier ist insbesondere das Kapitel Leistungsbewertung hervorzuheben.

Einerseits herrscht bei vielen Laboren und POCT-Herstellern nach wie vor Unklarheit bezüglich der Auslegung der IVDR; andererseits sehen sie sich konfrontiert, die Umsetzung der Anforderungen im Arbeitsalltag mit dem vorhandenen Personal zu stemmen.

Auch wenn durch das Proposal der EU-Kommission, das wahrscheinlich bis zur Europawahl im Juni positiv beschieden wird, weitere Übergangsfristen verlängert werden, sollte dies nicht darüber hinwegtäuschen, dass diese Fristverlängerungen nur für bereits im Markt befindliche Produkte gelten. Damit weiterhin innovative Produkte gemäß der IVDR entwickelt werden, bedarf es einer genauen Analyse und Umsetzung der Anforderungen.

“Methoden/Ergebnisse”

In dem Vortrag soll anhand von Praxisbeispielen erläutert werden, wie die gestiegenen Anforderungen für Labore bzw. POCT-Hersteller effizient umgesetzt werden können. Es werden Stolperfallen und häufig gemachte Fehler beleuchtet und wie sie umgangen werden können. Außerdem werden pragmatische Ansätze zur Ermittlung von Leistungsdaten für Labortests bzw. POCT vorgestellt.

Des Weiteren wird anhand der 2022 überarbeiteten Version der EN ISO 15189 gezeigt, wie Labore die Übergangsfrist bis Dezember 2025 sinnvoll nutzen können. Auch vor dem Hintergrund, dass hier nun auch Anforderungen an die Qualität und Kompetenz für POCT berücksichtigt werden, die bisher in der ISO 22870 festgelegt waren.

DGKL: 09. IVDR, In-house Validation

Validierung zur Bestimmung diagnostisch relevanter Analyte aus Punktatmatrices an Advia Chemistry XPT Systemen.

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Zielsetzung

Zum momentanen Zeitpunkt gibt es keine CE-zertifizierten Reagenzien, die eine Bestimmung von Albumin, Amylase, Cholesterin, Glucose, Totalprotein, Triglyceriden und Laktatdehydrogenase aus Punktatmaterial an Siemens Advia Chemistry XPT Systemen ermöglichen. Vor diesem Hintergrund war das Ziel, die CE-zertifizierten Reagenzien, zugelassen für humanes Serum auf Tauglichkeit zu überprüfen und entsprechend zu validieren.

Methoden

Im Zuge der Methoden Validierung wurden die Leistungsmerkmale Präzision, Richtigkeit, Linearität, analytische Sensitivität und eine mögliche Verschleppung für die Punktatmatrix ermittelt. Die statistische Auswertung erfolgte basierend auf den geltenden Qualitätsvorgaben der RiliBÄK.

Ergebnisse

Für die Parameter Cholesterin, Glucose, Triglyceride und das Totalprotein konnten valide Leistungsmerkmale erzielt werden, so dass eine Umsetzung in der Routine möglich erscheint. Für die weiteren Parameter Albumin, Amylase und die Laktatdehydrogenase sind weitere Messungen ausstehend um eine abschließende Beurteilung treffen zu können.

DGKL: 09. IVDR, In-house Validation

Fehlgeschlagene Etablierung eines IVD- und CE-zertifizierten Tests zum Nachweis von Benzolmetaboliten im Urin mittels LC/MS.

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Einleitung: Mit dem Inkrafttreten der In Vitro Diagnostic Regulation (IVDR, europäischen Verordnung 2017/746) haben sich weitreichende Veränderungen für medizinische Laboratorien ergeben. Während dieser Transition finden entsprechend den im Dezember 2021 verlängerten Übergangsfristen weiterhin noch bis zum Jahr 2028 Tests mit einer IVD-Zertifizierung Anwendung. Ein Vorschlag der Europäischen Kommission zur erneuten Verlängerung liegt seit Januar 2024 vor und soll die Fristen bis 2030 ausweiten. Vor diesem Hintergrund erfolgte der Versuch, einen IVD- und CE-zertifizierten Test auf Benzolmetabolite im Urin zu etablieren, was uns auch nach intensiven Optimierungsversuchen nicht gelungen ist.

Material und Methoden: Es erfolgte eine simultane Bestimmung von S-Phenylmercaptursäure (SPMA) und t,t-Muconsäure (t,t-MA) im Urin mittels LC-MS/MS. Hierzu fand die in-house Validation eines IVD- und CE-zertifizieren Kits (t,t-Muconic and s-Phenylmercapturic Acids in urine by LC/MS, Eureka) statt. Hierbei wurden eigene Daten mit den vom Hersteller angegebenen Werten zu Masseübergängen, Cone-Spannung (V) sowie Kollisionsenergie verglichen. Weiterhin erfolgten Messungen zu Intra-/ und Inter-Methoden-Präzision sowie Unrichtigkeit.

Ergebnisse: Bei dem Analyt t,t-MA zeigten sich multiple, grobe Abweichungen der Herstellerangaben zu unseren eigenen Ergebnissen. Betroffen waren Angaben zu Chromatographie, Masseübergängen und 6-Punkt-Kalibration. Insbesondere eine unzureichende chromatographische Auftrennung bei den vorgegebenen Bedingungen erschwerte eine Quantifizierung mittels Peakfläche erheblich. Bei dem Analyt SPMA zeigten sich ebenfalls Abweichungen der Herstellerangaben zu unseren

eigenen Ergebnissen. Diese betrafen die Parametereinstellungen des Massenspektrometers (Cone-Spannung und Kollisionsenergie). Allerdings war die inter-day Präzision mit > 15% unbefriedigend.

Diskussion und Schlussfolgerung: Da die Etablierung des IVD- und CE-zertifizierten Testkits gemäß den Herstellerangaben aufgrund der unzureichenden Abdeckung des klinisch relevanten Bereiches nicht zielführend war, wurde der Versuch einer Etablierung außerhalb der Zweckbestimmung unternommen. Dieser ist nicht gelungen. Auch eine versuchsweise Anpassung zur Optimierung der Testcharakteristika ist uns nicht gelungen. Eine Anwendung des Kits in der Routinediagnostik war daher nicht möglich. Eine kritische Prüfung auch grundlegender Testcharakteristika ist auch bei IVD- und CE-zertifizierten Tests weiterhin dringend angeraten.

DGKL: 10. Cardiology, Kidneys, Lungs, Biomarkers for Risk Prediction

The real-world rate of false negative troponin tests

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The measurement of cardiac troponin T is a central part in the diagnosis of acute myocardial infarction (AMI). Troponin T is rapidly released in the bloodstream after ischemia.

Due to the extreme sensitivity of the assay, the troponin T test is vulnerable to interfering antibodies. Direct comparison between the last and the newest generations of the cardial tests shows an increase of this effect.

The common observation of an incorrect test result is a deviation of over 30% from the real value. According to literature 10-20% of measurements give inaccurate results. This rate is higher in the most important risk group, patients who already had an AMI. The development of troponin T antibodies is an example of “autoimmunization” due to altered self. However, also antibodies interfering with the test matrix have been found.

We have screened samples with low troponin T values to identify masked test results. First, clinical documentation and other laboratory parameters were evaluated to confirm an AMI, following a PEG precipitation to remove antibodies and retest the sample for troponin T. A differing result indicated test matrix interference. In addition, we spiked the sample with a defined amount of recombinant troponin T and tested for recovery. Here a differing result indicates troponin T (analyt) interference.

Samples showing antibody interference will be tested on peptide microarrays to identify the epitopes detected by the autoantibodies.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Possible applications of clinical decision support systems to compensate for the weaknesses of current laboratory information systems

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Today’s laboratory information systems often lack the necessary capabilities to use the constantly growing knowledge about various clinical pictures in the sense of precision medicine for the care of patients in the outpatient sector.

For example, unavailable mathematical operations hinder the introduction of new calculation parameters or the inability to automatically query a patient’s previous values and include them in the current diagnosis prevents the implementation of

complex decision trees. Modern diagnostic approaches such as "intelligent Liver Function Testing" (iFLT) can therefore only be established on a broad scale at great expense. Clinical Decision Support Systems (CDSS) can also be used to carry out more complex calculations in addition to the classic field of application of implementing and automatically executing diagnostic algorithms. Using the example of the conversion of the eGFR calculation from the CDK-EPI formula (2009) to the EKFC formula, we show how CDSS can help to take over limited functionalities of today's laboratory information systems. In addition, we identify further use cases that have hardly been used in the outpatient sector in Germany to date.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Analysis of Adolescent Calcium Blood Levels over 24 Years

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Laboratory test results play a significant role in clinical decisions for individual patients. Analysing these results over large populations and extended periods could offer a wealth of additional insights. The UKE has collected lab results for 24 years, and hundreds of thousands of patients; however, this fortune of data has yet to be explored.

In this study, we have taken a glimpse at the development of population medians of the calcium blood levels of adolescents in a sliding window over 24 years. Decomposing the factors contributing to the observed variance beyond the specifics of the individual case presents a formidable task. Data science techniques are essential in identifying long-term trends, technical discontinuities, and seasonal and circadian variations, shedding some light on artificial sources of apparent effects.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Comparison of different definitions for electronic acute kidney alerts in hospitalized patients

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Introduction: Electronic alert systems (e-alerts) for in-hospital acute kidney injury (AKI) are used to identify patients at risk of AKI-related adverse outcomes. E-alerts that are mostly relying on the current KDIGO definition for AKI are criticized for delayed diagnosis and underdiagnosis in female patients. Recently, the AACC proposed a novel definition of AKI – the 20/20 AACC AKI criteria. This study compares e-alerts based on KDIGO or AACC criteria, focusing on their association with adverse outcomes.

Methods: A retrospective comparison of e-alert systems based on KDIGO and AACC criteria was conducted over a one-year period in a tertiary university hospital. Adverse outcomes included in-hospital mortality, new dialysis procedures (D/M), and a compound endpoint (CE) combining D/M with a decline in renal function between admission and discharge. This decline was defined by the current KDIGO criteria for acute kidney disease.

Results: Among 14,719 hospitalized patients, KDIGO-based e-alerts triggered in 2,598 patients, with AACC-based alerts firing in 2,554 of these and exclusively in another 1,138. Demographics and comorbidities were similar across both e-alert groups. 745 patients were dialyzed or died during hospital stay, 1304 patients experienced the compound endpoint that includes a

decline of renal function during hospital stay. AACC-based e-alerts showed slightly higher sensitivity for D/M (71.5% vs. 68.3%) and CE (81.4% vs. 74.0%) but lower specificity (77.4% vs. 85.1% for D/M, 80.4% vs. 88.8% for CE). KDIGO criteria but not AACC criteria were significantly more sensitive for adverse outcomes in male patients (71% for D/M, 78% for CE) than in female patients (63% for D/M, 69% for CE). AACC criteria are met earlier in a fifth of patients that also fulfill KDIGO criteria. This subgroup has a higher prevalence of adverse outcomes (22.9% for D/M and 42.7% for CE) than the subgroup with KDIGO and AACC based e-alerts firing at the same time (17.7% for D/M and 35.1% for CE). Patients exclusively flagged by AACC based e-alerts had a lower prevalence of adverse outcomes (3.1% for D/M and 9.6% for CE) compared to patients detected by both e-alerts, but were still more at risk compared to patients without any e-alert (1.8% for D/M and 2.1% for CE).

Conclusion: AACC-criteria based e-alerts fire partly earlier and markedly more often than KDIGO-criteria based e-alerts, without significant bias against female patients. Even patients exclusively flagged by AACC criteria exhibit increased risk, suggesting AACC-based e-alerts as a promising alternative. Further validation in diverse cohorts and long-term outcomes assessment is warranted.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Quantifying Disease Severity in Amanitin-Intoxicated Patients by Fusing Common Laboratory Values

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Introduction

Amanitin intoxication caused by the ingestion of poisonous mushrooms can lead to acute hepatic and renal failure (1,2). Current methods to detect amanitin intoxication and predict the clinical course include measuring amanitin levels in urine and assessing liver enzymes, liver-derived proteins, creatinine, and LDH (3,4). Early detection of amanitin intoxication has been reported to improve clinical outcomes (5). Precise quantification of disease severity in intoxicated patients can lead to a better understanding of disease progression. We here use an adapted version of a statistical tool called Patient Vital Status (PVS) (6) to systematically analyze the dynamics of common laboratory values after ingestion of the toxin. Such systematic statistical analysis might help to optimize early detection of severe cases to optimize patient outcomes.

Methods

The PVS originates from the RELSA score, initially for severity assessment in laboratory animals (7). Given its customizability, this scoring system was here adapted to dynamically analyze laboratory measurements such as prothrombin time, ALT, AST, bilirubin, and LDH instead of vital signs to generate a fusion score, which allows for comparative severity assessment, especially in the case of amanitin poisoning. Severity quantification involves comparison with a reference cohort of mildly intoxicated patients, aiding in severity clustering.

Results

The patient cohort contained amanitin-intoxicated patients and a control group. Clinical outcomes were consistent with PVSmax severity clustering showing that the fused results could reliably show the overall health status of the cases.

Each patient's last PVS was evaluated for outcome severity. In the patient with the most severe outcome, the last PVS was considerably higher than in the less severely affected patients in both groups. Both the PVSmax and the mean PVS were significantly higher in intoxicated patients than in the control.

The only severe PVS peaks occurred within four days after intoxication, while the control group remained non-elevated. There was no significant relationship between the multidimensional PVS and urinary amanitin concentration.

Conclusion

The PVS showed significant performance in quantifying disease severity in amanitin-intoxicated patients compared to a healthy control group. The clustering of severity groups with PVSmax also corresponded to the actual clinical course. The PVS of individual patients adequately reflected the current vital status over time. We found that the disease severity is unrelated to the amanitin level in the urine, as expected and in accordance with the test specifications. Further, the case with the most severe outcome had a significantly higher last PVS than the other patients. Thus, real world data support the usefulness of our statistical score and further studies will evaluate the PVS dynamics in other medical conditions.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Marker Importance Analysis for Machine Learning Based Wrong Blood in Tube Error Detection

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Wrong blood in tube (WBIT) errors refer to the situation in medical laboratories, where the patient-name on the blood sample tube does not match the person that the blood in the tube actually belongs to. WBIT are serious errors that can have heavy consequences such as delays in correct diagnosis and/or start of treatment. Misdiagnosis and mistreatment (wrong treatment, no treatment) can occur possibly resulting in serious harm and even death of the patient. WBIT-errors happen more often than one might think, with an estimated rate between every 1300-3500 samples [1] and a great likeliness of underreporting.

Despite the critical impact of WBIT, existing measures like staff training and validation of the results by medical staff are shown to be insufficient to prevent their occurrence. Electronic solutions that reduce the human intervention in the blood analysis process have been significantly successful but come with high costs.

Clinical decision support systems (CDSS) [2] are tools that assist healthcare professionals in making timely and informed decisions by providing relevant patient-specific information and knowledge. In the context of laboratory medicine, they have been implemented as alarm mechanisms [3], detecting critical conditions or pre-analytical errors using the analytes measured in laboratory tests. Accordingly, automated analysis of blood analytes as in [4], such as complete blood count components and laboratory medical parameters of organ function, can potentially detect WBIT errors early, offering a cost-effective and highly accurate alternative to manual methods.

This study extends existing research by employing state-of-the-art machine learning techniques to implement a real-time WBIT error detection as a component of a CDSS. This involves investigation of highest performing preprocessing techniques and classification models tailored for blood test analyte data. Specifically, the importance of different analytes for the classification of WBIT errors are analyzed, considering the availability of marker combinations in different clinical centers. The study investigates various marker combinations and sorts them by their contribution. Additionally, experimented feature extraction methods such as PCA and NCA have been employed to boost performance. Visual analysis of 2D and 3D marker combinations has provided insights into the data and enhanced the detection accuracy of WBIT errors.

We argue that such a solution as a part of CDSS will serve as an efficient, low-cost alarm mechanism, supporting medical professionals in timely decision-making and prevention of the adverse consequences of WBIT errors. By leveraging the models trained on different marker combinations which are applicable to various clinical settings, this study aims to set a new standard in the detection and management of WBIT errors, potentially transforming practices in laboratory medicine and enhancing patient safety.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Automatisierte Auswertung von Immunfixationen mit tiefen neuronalen Netzwerken

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Zielsetzung: Die zuverlässige Auswertung der Immunfixationselektrophorese ist Teil der Labordiagnostik des Multiplen Myeloms. Bislang erfolgte dies routinemäßig durch die subjektive Beurteilung durch qualifizierte Labormitarbeitende. Die Möglichkeit von subjektiven Fehlern und relativ hohe Kosten bei langer Personalbindung sind die Herausforderungen dieses heute üblichen Ansatzes.

Methoden: Deep Convolutional Neural Networks werden auf die Auswertung von Immunfixationsbildern angewendet. Zusätzlich zu „Standard“ monoklonalen Gammopathien (IgG-Kappa, IgG-Lambda, IgA-Kappa, IgA-Lambda, IgM-Kappa und IgM-Lambda) werden auch bi- oder oligoklonale Gammopathien, Leichtketten-Gammopathien, nicht-pathologische Fälle und Fälle mit unklarem Befund festgestellt. Die Zuordnung zu einer dieser 10 Klassen ist mit einem Konfidenzwert versehen.

Ergebnisse: Bei einem Testdatensatz mit über 4.000 Bildern werden etwa 25% der Fälle als nicht schlüssig oder aufgrund geringer Konfidenz für die anschließende manuelle Auswertung aussortiert. Bei den verbleibenden 75%, etwa 3.000 Fälle, wird nicht ein einziger als falsch positiv und nur einer als falsch negativ eingestuft. Die verbleibenden wenigen Abweichungen der automatischen Bewertung von den manuell von Experten vergebenen Klassifizierung sind Grenzfälle oder können anderweitig erklärt werden. Als eine Software, die auf einem normalen Desktopcomputer läuft, benötigt das Deep Convolutional Neural Network weniger als eine Sekunde für die Beurteilung eines Immunfixationsbildes.

Diskussion und Schlussfolgerung: Die Unterstützung der Laborexperten bei der Beurteilung von Immunfixationsbildern kann eine nützliche Ergänzung zur Labordiagnostik sein. Die Entscheidungskompetenz sollte jedoch immer beim befundenden Arzt verbleiben, der für den Befund verantwortlich ist.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

A Novel Statistical Approach for the Verification of Reference Intervals from Real-World Data

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Introduction

Laboratories are required to verify their reference intervals (RIs) used in routine on a regular basis. However, the established statistical test for verifying RIs as recommended in the Clinical and Laboratory Standards Institute (CLSI) guideline EP28-A3c has several limitations: It cannot detect too wide RIs, it has a high statistical uncertainty and it requires collection of

samples from apparently healthy subjects. So far, no rigorous statistical approach exists that employs real-world data (RWD) from laboratory routine for verification of RIs.

Methods

We have developed a novel statistical approach for verification of RIs, which compares a candidate RI with the RI estimated by an indirect method (refineR algorithm) using RWD. Acceptance criteria of the statistical approach are derived from the statistical uncertainty of RI estimation based on N = 120 samples and from the difference in positivity rates between the two RIs calculated for the lab-specific population. For the novel statistical approach, operating characteristic curves were generated based on simulated test sets of nine biomarkers mimicking routine measurements. These test sets comprise normal and skewed non-pathological distributions with varying sample sizes and added pathological samples. For these pathological distributions, the fraction of samples, location and extent of overlap with the non-pathological distributions were varied.

Results

The operating characteristic curves of the novel statistical approach demonstrate comparable performance for different shapes of the non-pathological distribution (normal and skewed). The statistical power of the approach to detect large discrepancies between RIs is considerably increased when compared to the binomial test based on N = 20 samples as recommended in CLSI EP28-A3c. In contrast to the latter, the novel approach is able to detect too wide RIs.

Conclusion

The developed statistical approach enables verification of RIs based on already available RWD. It shows better statistical power than the test recommended in the CLSI EP28-A3c guideline and does not require collection of samples from apparently healthy subjects. It may serve as a viable alternative to the recommended test for the task of verifying RIs for lab-specific populations.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Raman Spektroskopie als neue Form der labormedizinischen Diagnostik

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Klassisch fordern Behandelnde Laborbefunde gezielt aufgrund einer Verdachtsdiagnose an. Für jeden angeforderten Marker wird ein individueller labormedizinischer Test durchgeführt. Hierbei können eine Reihe von Problemen auftauchen: Proben können verzögert, verwechselt und wichtige diagnostische Tests von Behandelnden nicht angefordert werden, um nur einige zu nennen.

Die Raman Spektroskopie dagegen liefert einen Fingerabdruck, welcher die Proben in ihrer molekularen Komposition insgesamt abbildet. Mithilfe statistischer Verfahren ("KI") können aus den Spektren Informationen über den Gesundheitszustand der Patienten gewonnen werden.

Wir entwickeln mithilfe der Raman-Spektroskopie eine multiparametrische Diagnostik, welche neben individuellen Markern und Krankheitsbildern zum einen kritische Zustände wie den der Sepsis, zum anderen präanalytische Fehler wie Patientenverwechslungen identifiziert.

In einer ersten Studie konnten wir für eine Leberzirrhose-Kohorte eine ganze Reihe von Markern in guter bis hervorragender Qualität aus Raman Spektren bestimmen. In einer zweiten Studie konnten wir aus den Raman Spektren bestimmen, ob Proben liegengeblieben waren - wofür bislang eine Nachweismethode fehlte, obwohl dies zu deutlich veränderten Laborwerten führen kann.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Ki-gestützte Prozesse in Primärsystemen der Gesundheitsversorgung

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Zielsetzung:

Diese Studie untersucht die Implementierung von KI-gestützten Prozessen in Primärsystemen der Gesundheitsversorgung für die Bereiche Auftragssteuerung, medizinische Validation und Abrechnungssysteme. Das Hauptziel besteht darin, die Effizienz, Genauigkeit und Qualität dieser Prozesse zu verbessern und damit die Patientenversorgung zu optimieren.

Methoden:

Die Studie basiert auf einer umfassenden Recherche sowie der Analyse von Best Practices im Bereich der KI-Anwendungen im Gesundheitswesen. Es wurden Daten über die Anforderungen und Herausforderungen der Auftragssteuerung, medizinischen Validation und Abrechnungssysteme in Primärsystemen gesammelt. Anschließend wurde eine detaillierte Bewertung der Funktionalitäten von KI-gestützten Prozessen durchgeführt.

Ergebnisse:

Die Integration von KI-gestützten Prozessen in Primärsystemen der Gesundheitsversorgung ermöglicht eine automatisierte, präzise und kontextorientierte Auftragssteuerung, eine medizinische Validation im Kontext anamnestischer Informationen und eine optimierte Abrechnung. Durch den Einsatz von KI-Technologien werden Fehler reduziert, Bearbeitungszeiten verkürzt und die Gesamtqualität der Prozesse verbessert. Die Studie zeigt, dass KI-gestützte Prozesse einen bedeutenden Mehrwert für die Primärsysteme der Gesundheitsversorgung bieten können und die Patientenversorgung auf ein neues Niveau heben können.

Fazit:

Die Ergebnisse dieser Studie unterstreichen die Bedeutung von KI-gestützten Prozessen in Primärsystemen der Gesundheitsversorgung für die Optimierung der Auftragssteuerung, medizinischen Validation und Abrechnungssysteme. Die Integration von KI-Technologien trägt zur Effizienzsteigerung und Qualitätssicherung bei und stellt einen wichtigen Schritt zur Weiterentwicklung der Primärsysteme im Gesundheitswesen dar. Zukünftige Forschung und Implementierung auf diesem Gebiet sind entscheidend, um die Potenziale der KI in Informationssystemen voll auszuschöpfen und die Patientenversorgung weiter zu verbessern.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Volldigitaliserung im medizinischen Labor von der standardisierten papierlosen Anforderung bis zur ePA.

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Zielsetzung:

Dieser Use-Case zielt darauf ab, die vollständige Digitalisierung des Datenflusses im medizinischen Labor von der standardisierten papierlosen Anforderung bis zur elektronischen Patientenakte (ePA) aufzuzeigen. Besonderes Augenmerk liegt

auf den Funktionen des Laborinformationssystems (LIS) bzw. des Order Entry-Systems, die Patienten, Einsendern und medizinischen Laboren gleichermaßen eine barrierefreie Auftrags- und Ergebniskommunikation ermöglichen.

Die Integration von MIO-Laborbefund und die Verwendung einer KI-gestützten LOINC-Codierung der Leistungskataloge sind zentrale Aspekte dieses Use-Cases.

Methoden:

Dieser Use-Case basiert auf einer umfassenden Recherche sowie der Analyse von Best Practices im Bereich der schon fortschreitenden Digitalisierung im Gesundheitswesen. Es wurden im Vorfeld Daten über die spezifischen Anforderungen und Herausforderungen des Datenflusses im medizinischen Labor gesammelt und anschließend eine detaillierte Bewertung der Funktionalitäten des LIS bzw. des Order Entry-Systems durchgeführt. Die Einbindung von MIO-Laborbefund und die Verwendung einer KI-gestützten LOINC-Codierung wurden dabei besonders berücksichtigt.

Ergebnisse:

Die Implementierung einer standardisierten papierlosen Anforderung und die Integration der ePA in das Laborsystem ermöglichen eine effiziente und nahtlose Kommunikation zwischen Patienten, Einsendern und medizinischen Laboren. Die Einbindung von MIO-Laborbefund und die Verwendung einer KI-gestützten LOINC-Codierung führen zu einer Standardisierung und Automatisierung von Prozessen, wodurch die Genauigkeit und Effizienz des Datenflusses im medizinischen Labor verbessert werden.

Schlussfolgerung:

Die vollständige Digitalisierung des Datenflusses im medizinischen Labor bietet erhebliche Vorteile für die Auftrags- und Ergebniskommunikation sowie für die Gesamtqualität der Patientenversorgung. Die Integration von MIO-Laborbefund, KI-gestützten LOINC-Codierung der Laborleistungskataloge und der ePA in das LIS stellt einen wichtigen Schritt zur Verbesserung der Effizienz und Genauigkeit von Laborergebnissen dar. Zukünftige Forschung und Implementierung auf diesem Gebiet sind entscheidend, um die Potenziale der digitalen Transformation im Gesundheitswesen voll auszuschöpfen.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Utilizing ChatGPT to Program an App for Laboratory Medicine

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Introduction: Digital progress has opened up new perspectives for research and process optimization in laboratory medicine. In this transformation process, programming skills are becoming increasingly important for finding digital solutions, conducting efficient and reproducible research, and developing automation tools. These tools are instrumental in streamlining routine tasks, thereby allocating more resources to complex analytical work. Despite the recognized relevance of programming skills in modern laboratory medicine, its integration into the field's educational and practical frameworks remains limited. This gap highlights the need for innovative tools that support the learning and application of programming skills in laboratory medicine.

Methods: The objective of this research was to create an automation program with a Shiny User Interface in R, using ChatGPT (GPT-4) and basic programming skills. This application was designed to facilitate intra-assay and inter-device precision testing for immunophenotyping at the University of Cologne, incorporating necessary automation and statistical analysis components. The development process involved crafting a procedural program, which was then refined through iterative interaction with ChatGPT, including prompt adjustments and continuous evaluation of the AI-generated output within the R environment.

Results: The collaboration with ChatGPT resulted in the development of a fully functional application within two weeks. ChatGPT provided valuable debugging assistance, clear explanations, and helpful code annotations. However, there were instances where ChatGPT generated faulty code, lacked reproducibility, and offered complex and misleading solutions.

Conclusion: In general, ChatGPT can assist individuals with basic programming skills in the development of software tools for laboratory medicine, while also potentially enhancing their coding proficiency. However, the efficacy of ChatGPT as a standalone tool for novices is compromised by its frequent inaccuracies, lack of reproducibility and its complex problem-solving approaches. Therefore, further studies are needed to determine potential integrations of ChatGPT into digital laboratory medicine as well as its education in the future.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

Tackling the implementation hurdle: A machine-learning decision support tool for the screening of mild bleeding disorders

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Introduction: The medical laboratory is an ideal candidate to pioneer machine-learning algorithms (MLA) in medicine. In contrast to radiology, however, very few MLAs have been implemented yet. We aimed to develop, externally validate, and implement an easy-to-use, machine learning-based decision support tool for screening for mild bleeding disorders (MBD), a challenging clinical situation for which no practical tools exist.

Methods: Detailed clinical and laboratory data were collected in two independent prospective cohort studies including consecutive patients referred for a suspected MBD to specialized outpatient units (n = 555, training cohort; n = 217, external validation cohort). The diagnostic workup was done following current guidelines and an expert panel established the final diagnosis. In the training cohort, the items of the ISTH-BAT were simplified by grouping levels with similar response patterns. Predictors were selected using the “Boruta” algorithm and focus group discussions. Multiple machine-learning algorithms were fitted to the data and five algorithms were further tuned. The best-performing model was externally validated. To assess user-friendliness, we developed a survey platform comprising a demographic questionnaire, four case vignettes, and the system usability scale (SUS), a validated questionnaire for software applications. The survey was sent out to surgeons, anesthesiologists, and hematologists, and various background data were collected.

Results: The following predictors were selected: (a) activated partial thromboplastin time, (b) PFA-200 closure time (epinephrine/collagen cartridge), (c) sex, and (d) a streamlined bleeding history (surgery, tooth extraction, epistaxis, minor wounds, cutaneous bleeding, oral bleeding, postpartum hemorrhage, and menorrhagia). In the validation cohort, 87.5 % of patients with MBD were correctly identified (sensitivity; 95% confidence interval [CI]: 79.9, 93.0), and 54.3 % (specificity; 95% CI: 44.3, 64.0) of patients without MBD would have been correctly excluded from further work-up. The

AUROC was 0.86 (95% CI: 0.81, 0.90), in contrast to existing diagnostic instruments 0.81 (ISTH-BAT, 95% CI: 0.75, 0.87), 0.73 (PFA-200, 95% CI: 0.65, 0.79), and 0.49 (INR, 95% CI: 0.42, 0.55). The decision support tool was implemented on an easy-to-use web application (https://toradi-hit.dbmr.unibe.ch/mbdcheck/). Thirty-three surgeons, 29 anesthesiologists, and 24 hematologists participated in the survey; most physicians had 10-14 years of experience in their respective fields (29.0 %). The median time needed to fill out the tool was 72 seconds (interquartile range [IQR]: 49.0, 79.5). The median SUS score was 82.5 (IQR: 72.5, 90, > 80 = Software with excellent usability). In conclusion, we were able to develop, externally validate, and implement an effective decision-support tool for screening of patients with MBD.

DGKL: 11. Artificial Intelligence/ AI, Medical Informatics, Digitalization, MIO/LOINC

kc.uol.de - Comprehensive Online Tools for the Clinical Laboratory

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Background: Efficient and accurate data management is paramount for clinical laboratories to ensure high quality patient care. However, accessing and utilizing appropriate tools can be challenging, e.g. when extensive technical expertise is necessary or due to economic reasons. To address this issue, we present a collection of free-to-use online tools designed to streamline processes and aid in data management problems common for the clinical laboratory.

Methods: R Software for Statistical Computing in conjunction with the Shiny framework was used to generate the interactive web applications. The applications were tailored to be readily accessible via web browser, eliminating the necessity for cumbersome software installation. The tools were designed with user-friendliness in mind enabling laboratory staff at all skill levels to use them. The tool collection includes multiple functions to address common challenges encountered in clinical laboratory settings.

Results: The currently featured tools include a method comparison app for evaluating assay agreement utilizing Passing and Bablok regression scatterplots and a difference plot, a calculation sheet for quality control assessments, a unit conversion tool, and a utility for estimating reference intervals from real-world data using different approaches (RefineR, TML, ReflimR). All tools aim at providing accurate results efficiently, enhancing the productivity and reliability of laboratory operations.

Conclusion: The online tools presented here offer a simple and easy to use way for clinical laboratories to optimize their workflows and improve data management.

DGKL: 12. Guidelines, Diagnostic Pathways, HTA, Clinical Epidemiology

Why most diagnostic studies overestimate test performance: Evidence from a large-scale meta-analysis

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Background: Misperceptions of diagnostic test performance may lead to incorrect medical decisions, heightened patient anxiety, increased healthcare expenses, and misguided public health decisions. To avoid this, knowledge about bias introduced in studies evaluating diagnostic tests is essential. Utilizing all studies evaluating the diagnostic performance of SARS-CoV-2 immunoassays, we assessed the direction and extent of bias caused by study design and patient characteristics.

Methods: Following a detailed protocol (PROSPERO CRD42023343656), we conducted a large-scale systematic review and meta-analysis (MEDLINE, EMBASE, and iSearch portfolio) including all studies assessing the diagnostic performance of SARS-CoV-2 immunoassays, thus addressing a common diagnostic question and biological target. A broad range of study design characteristics and 2 x 2 tables were retrieved. We performed a three-level meta-analysis to control for multiple tests within one primary study. The relative effect of suboptimal design characteristics on the reported diagnostic odds ratios (DOR) was assessed by a meta-regression controlling for the type of immunoassay, target epitope, target immunoglobulin, and time from start of symptoms.

Results: Overall, 18’092 articles were identified and 1’766 were assessed in full text. In the current analysis, we eventually included 641 primary studies comprising 3’124 study groups and 1’384’556 test results. Suboptimal design characteristics were associated with a two- to five-fold increase in DOR in most domains, e.g. case-control design (55% of studies; relative DOR [RDOR] 4.4; 95% confidence interval [CI] 3.1, 6.2), unclear patient selection (65%; RDOR 3.7, 95% CI 2.5, 5.5), industry funding (4 %; RDOR 4.6; 2.3, 9.2), unclear blinding of index test interpretation (70%; RDOR 2.8; 1.9, 4.1), and different reference standard for controls (73%; RDOR 3.6; 2.7, 4.7). Few characteristics were associated with a decrease in DOR, e.g. inappropriate early timing of index test (RDOR 0.2; 0.1, 0.3), and symptoms as a reference test for COVID-19 positive patients (RDOR 0.1; 0.0, 0.6). Widely used study designs resulted in a substantial overestimation of SARS-CoV-2 immunoassay test performance. Our data suggest that overestimation is a common problem and carefully designed studies are needed to establish unbiased performance measures.

DGKL: 12. Guidelines, Diagnostic Pathways, HTA, Clinical Epidemiology

Publication success and impact of studies evaluating diagnostic tests

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Introduction: Contemporary research is based on the assumption that a project’s quality determines whether resources are allocated, whether it is published successfully, and how far it impacts the entire field. This assumption holds particular significance in the context of studies that assess diagnostic tests, as they are the essential first step in patient management.

To test this hypothesis, we utilized all studies assessing the diagnostic performance of SARS-CoV-2 immunoassays, examining the geographic distribution, predictors of publication success, importance in the scientific network, and policy impact.

Methods: Large-scale systematic review and meta-analysis (PROSPERO CRD42023343656; MEDLINE, EMBASE, and iSearch portfolio) including all studies assessing the diagnostic performance of SARS-CoV-2 immunoassays, thus benefiting from a common diagnostic question and biological target. The methodological quality of the primary studies was assessed using the QUADAS-2 tool, and various study characteristics were retrieved. Additionally, measures of publication success (impact factor [IF], total citations, relative citation ratio), importance in the scientific network (pagerank), and policy impact (PlumXs guideline and policy citations) were retrieved. Box-Cox transformed linear regression, quasi-Poisson regression, or zero-inflated Poisson regression was applied.

Results: Out of 18'092 articles screened and 1'766 assessed in full text, 782 were included for this analysis. Studies from African, South American, and Asian countries were disproportionately underrepresented. Measures of publication success were significantly associated with the H-index of the last author, the year of publication (YOP), and extreme diagnostic accuracy measures. The importance in the scientific network was determined by the IF of the journal, the H-index of the last author, and YOP. IF, YOP, and high applicability were significantly associated with the number of policy citations. Other associations of QUADAS-2 scorings with any of the success measures were not found.

Our data indicate that publication success and impact of studies evaluating diagnostic tests depend more on the prestige of the journal and the senior author than on the methodological quality of the study.

DGKL: 14. Liquid Biopsy, Oncology, Personalized Diagnostics and Therapy

Design and characterization of a new site-specific glycosylation procedure of proteins for the functionalization of nanobodies

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Glioblastoma multiforme (GBM) poses a significant therapeutic challenge due to aggressive nature and limited treatment options. Conventional antibody-drug conjugates (ADCs) showed promise in treating various cancer types but their efficacy against GBM is limited due to their incapability to penetrate the blood-brain barrier. Compared to monoclonal antibodies (~150 kDa), nanobodies are smaller in size (~15 kDa), which offers a potential solution to enhance ADC efficacy in brain tumors. However, nanobodies lack native glycosylation sites that can be used for site-specific modification. The aim of the here presented project focuses on the development of a simple strategy to functionalize nanobodies. Previously, an antibody directed against the calcitonin receptor, which is expressed by ~80% of GBM biopsies, was used [1]. The concept of conjugation via a glycosylation site is now be transferred to nanobodies.

The in vitro glycosylation is realized by a specific amino acid sequence that can be attached N- or C-terminally to the protein and is recognized and glycosylated by the native glycosylation machinery of the yeast Pichia pastoris KM71H. Ten sequences were tested, five tags each for the N- and C-terminus of a model protein. In the tag, the motif NXS/T is flanked by five amino acids on each side to create an environment that is independent of the corresponding protein. The glyco-tags were designed by using the "NetGlycServer" software [2]. For transfection the plasmid pPICZα A is used, allowing modification and amplification in E. coli (DH5α). Tumor necrosis factor-α (TNFα) serves as a first model protein, but studies will be completed by comparing the glycosylation efficacy of the glyco-tags using other proteins. The glycans are enzymatically truncated to N-acetylglucosamine and modified with N-azidoacetylgalactosamine to allow selective modification via click chemistry. The resulting ligand can be clicked to an effector or equipped with dyes for diagnostic features.

Ten different glyco-tags were produced and tested for product identity, yield, and completeness of the reaction to optimize the glycosylation motif and the reaction conditions. Glycosylated TNFα was successfully isolated and verified by Western blot via Myc- and His-tag. A lectin blot and a mobility shift after glycan removal proved the successful glycosylation. Further analyses will include MALDI-MS and chromatography.

We successfully glycosylated a first model protein by attaching a glyco-tag C-terminal to the original protein sequence. Testing all glycotags is still ongoing to evaluate the glyco-tag with the highest glycosylation efficacy. Analyzing other model proteins including nanobodies against GBM will provide insights into the versatility and efficiency of the approach and offer potential diagnostic applications through dye conjugates.

DGKL: 14. Liquid Biopsy, Oncology, Personalized Diagnostics and Therapy

Engineering of glyco-tags for site-specific coupling of ligands to bottle-brush copolymers to create a targeted carrier system

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Gene transfer lies at the heart of many modern therapeutic approaches to different types of diseases. Genetic material introduced into a cell can lead to the repair of defective genes, the production of antigens triggering an immune response, or cell death via suicide genes. The ability to utilize the genetic code for specific cells enables individual therapeutic and diagnostic applications. The carrier delivering this genetic material faces the challenge of protecting the cargo while being able to efficiently release it unharmed inside the cell. Hence, a carrier system based on bottle-brush copolymers is being developed to encapsulate the genetic material with cationic side chains and protect it with uncharged hydrophilic side chains, forming micellar virus-like particles. A universal and site-specific strategy to conjugate this carrier system to receptor-targeting ligands is needed to deliver these particles to specific cells. To achieve this goal, we constructed a set of ten different glyco-tags consisting of defined amino acid sequences that can either be attached N- or C-terminally to the sequence of any protein. These tags can be recognized by the host system that then post-translationally N-glycosylates them by the natural glycosylation machinery. These N-glycans are now accessible for modification by a glycosyltransferase to introduce a strained alkyne for click chemistry. This approach allows for the attachment of any protein (not having N-glycans critical for its biofunctionality) to virus-like particles. To test the general applicability of this modification system, we are analyzing the expression and glycosylation efficiency of epidermal growth factor (EGF) and interleukin 2 (IL-2) – proteins that target receptors, which are overexpressed in certain cancers – in Chinese hamster ovary (CHO) cells. By comparing the amount of glycosylated protein for the same glyco-tags but on different proteins, we can assess the ability of each tag to induce N-glycosylation in the CHO expression system. Transfer of a strained alkyne to the single N-glycosylation site enables us to orthogonally couple a dye to the respective protein and use the fluorescence to analyze its ability to bind to the intended receptor on cell surface via flow cytometry. When we click the alkyne of EGF or IL-2 with an azide on the surface of the virus-like particles, we can guide them to the target cells of interest. This cannot only be used for therapeutic applications. The inside of the virus-like particle can be equipped with any diagnostic probe, as long as it can be complexed with the cationic bottle-brush chains. Targeted systems are increasingly used in the pharmaceutical industry to specifically address cells for diagnosis or treatment. Coupling a targeting moiety to an effector is still challenging due to stoichiometric chemistry resulting in heterogeneity. We present a novel glyco-tag for site-specific coupling and production of homogeneous and functional products.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Klinische Anwendung und diagnostische Fälle der Elementspeziesanalytik

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Zielsetzung

Die Elementanalytik zur Quantifizierung von Metallen bei chronischen Belastungen, Vergiftungen oder zur Diagnostik des Spurenelementestatus wird zunehmend erweitert durch die Elementspeziesanalytik, die die chemische Bindungsform differenziert und die Verteilung in den Organen berücksichtigt. Die klinische Bedeutung in Arbeitsmedizin, Umweltmedizin, Forensik und Toxikologie wird anhand aktueller Anwendungen und klinischer Beispiele dargelegt.

Methoden

Die Massenspektrometrie in Kombination mit chromatographischen Methoden eröffnet herausragende Möglichkeiten zur Elementspeziesanalytik für arbeits- und umweltmedizinische Fragestellungen und bei klinischen Vergiftungsfällen.

Ergebnisse

Über die Bestimmung der Arsenspezies wird eine Unterscheidung von toxikologisch wenig bedeutsamem Arsenobetain aus Fischverzehr und den toxischen und karzinogenen Spezies anorganischen Arsens (III) und (V) und deren Metaboliten Methylarsonat und Dimethylarsinat ermöglicht und an Fallbeispielen demonstriert.

Die Bestimmung von intra- und extrazellulärem Chrom im Blut differenziert das hochtoxische Chrom (VI) vom allergisierend wirksamen Chrom (III). Ein klinischer Fall mit akzidenteller Doppelvergiftung mit Chrom und Arsen wird präsentiert, für den Lebertransplantation und Erythrozytenapherese lebensrettend waren.

Die Bestimmung des Selens am Transportprotein Selenoprotein P gibt für die Versorgung mit diesem essentiellen Spurenelement wesentliche Aussagen, da hierbei die Transport- Speicher- und Verteilungsfunktionen des Selens und damit die Organversorgung direkter beurteilt werden als durch die Gesamt-Selenbestimmung.

Methylquecksilber wird durch (GC-MS) bestimmt und spielt umweltmedizinisch (Minamata-Krankheit durch belasteten Fisch) und forensisch eine Rolle. Durch Differenzierung des intra- und extrazellulären Anteils vom Quecksilber im Blut lassen sich auch mit der ICP-MS Rückschlüsse auf die Bindungsform des Quecksilbers ziehen. Anhand zweier akuter Vergiftungsfälle wird gezeigt, wie sich anorganisches Quecksilber und noch stärker toxisches Methyl-Quecksilber im Blut verteilen.

Tributylzinnoxid (TBTO) ist als Biozid und als PVC-Stabilisator unverzichtbar und wird als endokriner Disruptor reguliert. Zur Arbeitsplatzüberwachung und in der Umweltoxikologie ist spezifisches Biomonitoring unerlässlich, wie an Mitarbeitern eines TBTO-verarbeitenden Betriebes demonstriert wird.

Diskussion und Schlussfolgerungen

Die Bindungsform bestimmt die toxischen Eigenschaften. Die Speziesanalytik erlaubt Rückschlüsse zur Toxikokinetik und bessere pathophysiologische Einordnung, wie für Arsen, Chrom, Selen, Quecksilber und Zinn dargelegt. Weitere aktuelle Anwendungen für Elementspeziesanalytik für die Elemente Thallium, Jod, Nickel, Mangan, Antimon, Gadolinium, Brom und Blei liegen vor und werden weiterentwickelt.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Quantitative LC-MS/MS analysis of an acute apixaban overdose in human plasma samples

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Introduction: Apixaban, a selective, reversible direct factor Xa inhibitor, is used in clinical routine as an anticoagulant for the prophylaxis and treatment of thromboembolism and non-valvular atrial fibrillation. The side effect profile of apixaban includes severe and non-severe bleeding, vestibular symptoms and hypersensitivity rash. Several case reports have described the toxicokinetic of apixaban overdose.

In the case of acute overdose, the central problem of all anticoagulants is systemic bleeding. We present the case of a 59-year old male Caucasian, who was admitted to the intensive care unit of University Hospital Regensburg with an acute apixaban overdose and fulminant bleeding complications. Blood plasma concentrations were subsequently quantified by LC-MS/MS.

Methods: Apixaban was extracted by protein precipitation from plasma samples. Chromatographic separation was achieved on the AcquityTM UPLC (Waters, MA, USA) equipped with a C18 Column employing a gradient elution technique. Mass

analysis was conducted employing the Xevo TQS Triple Quadrupole Tandem Mass Spectrometer (Waters, MA, USA). Quantification of apixaban was performed by isotope dilution using [13C12H8]-apixaban. The analytical method underwent comprehensive validation procedures in accordance with the guidelines outlined by the European Medicines Agency for bioanalytical method validation.

Results: The patient presented in this case survived after several weeks in intensive care with severe bleeding complications. Half-life of apixaban in blood was reported to be about 19.7 h. In this study we analyzed the apixaban plasma levels over a period of 12 days. Interpretation of the results will be completed soon and reported at time.

Conclusion: We hereby report a case of apixaban overdose with significant bleeding complications. Apixaban was quantified by stable-isotope-dilution-assay using LC-MS/MS and [13C12H8]-apixaban as stable isotope-labelled internal standard over a period of 12 days. Due to the precise monitoring of apixaban plasma level by LC-MS/MS analysis, the applied method demonstrates a supplemental analyzing tool to conventional coagulation diagnostics (as anti-factor Xa activity and aPTT time) with regard to treatment response and prognosis of an acute overdose of apixaban.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Rare Cases in a Routine TDM Laboratory: Intoxications by Superwarfarin Rodenticides

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Introduction: LC-MS/MS instrumentation, mostly triple quadrupoles, is nowadays an inevitable tool in every therapeutic drug monitoring (TDM) laboratory. Specialized assays covering both therapeutic drugs and serious toxins of the same mode of action can support clinicians in identifying the cause of a medical issue such as an impaired coagulation of unclear origin.

Our rodenticide or vitamin K antagonist screening in plasma by LC-MS/MS comprises 10 (super)warfarins (warfarin, phenprocoumon, coumatetralyl, coumachlor, acenocoumarol, flocoumafen, difenacoum, bromadiolon, difethiazon, and brodifacoum). Superwarfarin (second generation) rodenticides were developed in the 1980s due to upcoming resistance of rodents to first generation anticoagulants such as warfarin. Brodifacoum is the most widely used rodenticide worldwide. Its biological half-live in humans is several weeks 20 – 62 days [1], while its elimination kinetics is still matter of scientific discussion.

Methods: For routine screening of urine samples, we use the ToxTyperTM (Bruker Daltonics). However, this method does not detect rodenticides. Consequently, upon indication of an impaired coagulation, we perform an additional screening in plasma looking for the above mentioned 10 (super)warfarins. The measurement is performed after protein precipitation with acetonitrile/methanol and reversed phase chromatographic separation in electrospray ionization negative mode on an API4000 triple quadrupole in multiple reaction monitoring (MRM) mode (Agilent HPLC, AB Sciex) within 5 min.

Results: In the last 10 years, we performed 244 rodenticide screenings in samples from humans and animals leading to 112 positive findings (73 phenprocoumon, 27 brodifacoum, 12 other). Brodifacoum positive samples may derive from intentional and unintentional ingestion. Two cases are matter of discussion in this presentation. The first case is from patients presenting in hospital with impaired coagulation. Screening the initial urine samples using the ToxTyperTM yielded identification of patients' known medication, but failed to detect any oral anticoagulants. We identified brodifacoum in plasma samples using the targeted MRM method. Concentrations exceeded 500 µg/L upon admission, and samples from time point t+26 d and t+42 d allowed for an estimation as zero order elimination kinetic. No clinical information was available in the second case. Initial brodifacoum concentrations in plasma were 900 µg/L and elimination kinetic was first order.

Conclusion: Our fast target screening for (super)warfarines allows for an easy integration into our routine workflow. Fourty six percent of rodenticide requests yielded positive results. Out of these, 65 % were phenprocoumon positive samples while we identified brodifacoum in 24 % of the samples.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Measurement of the anticoagulant bivalirudin in human plasma using automated online solid-phase extraction combined with ultra-performance liquid chromatography-tandem mass spectrometry mass spectrometry

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Introduction:

During cardiac surgery, heparin is generally used as an anticoagulant during the extracorporeal circulation period. The need for cardiac surgery in patients with heparin-induced thrombocytopenia (HIT) is therefore problematic. In these cases, a blood thinner such as bivalirudin, which can bind thrombin quickly and reversibly, would be a good alternative. Direct measurement of bivalirudin in the plasma of patients would provide significant support in setting the individual dosage of the drug.

Methods:

A 50 µl volume of blank plasma, calibrator standards, quality control (QC) samples and plasma samples were precipitated with 200 µl methanol containing bivalirudin-13C6,15N as internal standard (IS). After centrifugation the clear supernatant was further treated and thereafter subsequently used for bivalirudin measurements using an automated online solid-phase extraction method coupled with ultra-performance liquid chromatography electrospray ionization-tandem mass spectrometry (online SPE-UPLC-MS/MS). Run time was 5.5 minutes per injection.

Results:

Best results for bivalirudin, as well as its corresponding 13C6,15N isotope were achieved by monitoring the fragmentation of double-charged molecule ions with m/z transfers of m/z 1090.8 > 650.7 and m/z 1094.2 > 657.6, respectively. A second mass transition was used for verification. Bivalirudin-13C6,15N as IS was used to compensate matrix effects. The calibration curve of bivalirudin was linear at least over 3 orders of magnitudes. Limits of detection (LOD) in the plasma matrix was < 0.01 µg/L. The intraassay and coefficient of variation (CV) for bivalirudin were < 9% for clinically relevant concentration range.

Conclusion:

We successfully developed an online SPE-UPLC-MS/MS method for fast, sensitive, and specific measurement of the anticoagulant bivalirudin in human plasma using bivalirudin-13C6,15N as appropriate IS.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Platelet lipidomics indicates enhanced thrombocyte activation in APS in vivo

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Introduction

The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the presence of anti-phospholipid antibodies (aPL) in patients with thromboembolic events and/or pregnancy complications. These aPL comprise anti-

cardiolipin antibodies, anti-β2-glycoprotein I antibodies and the functionally measured lupus anticoagulant. In particular, the aPL-mediated platelet activation appears to play a central role in this pathophysiological process.

Methods

In this study, platelets were isolated from blood samples of triple positive APS patients (n=20), patients affected by thromboembolism without APS (n=20) and healthy volunteers (n=16) by centrifugation/washing. With quantitative mass spectrometry-based lipidomics, we analyzed lipid profiles of platelets before and after thrombin induced activation.

Results

Lipid analysis revealed an increase in lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) species in platelets from APS patients. These lipids are products of phospholipase A2 (PLA2), suggesting increased arachidonic acid (AA) production in APS platelets in vivo. To obtain further evidence for PLA action and also for AA generation, we calculated the ratio of PLA2 and phospholipase A1 (PLA1)-derived lysophospholipid species to AA-containing phosphatidylethanolamine (PE) and phosphatidylcholine (PC) precursors. These ratios indicate higher PLA activity in thrombin-activated APS platelets. In addition, the PLA2 surrogate ratios correlate with serum levels of anti-β2-glycoprotein I antibodies and allow excellent discrimination of APS patients from patients with other causes of thromboembolism and healthy volunteers.

Conclusion

In conclusion, using a platelet lipidomics-based approach, we provide strong evidence for aPL-mediated platelet activation of PLA2 and AA production in vivo.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Making Use of Non-Destructive 1H-NMR Quantification: Re-Measurement of Prepared Plasma Samples applying different interim storage conditions

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Making Use of Non-Destructive 1H-NMR Quantification: Re-Measurement of Prepared Plasma Samples applying different interim storage conditions

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Introduction: Quantitative 1H Nuclear Magnetic Resonance (NMR) spectroscopy holds great promise for metabolomics studies, providing rapid and non-destructive analysis of biological samples in clinical settings. Spectral consistency and accurate quantification are essential for reliable results, but various physico-chemical parameters can affect the protonation state of sample molecules, thereby influencing metabolite signals by inducing chemical shifts in the spectrum. The non-destructive nature of NMR is advantageous, particularly in clinical practice where sample reusability may be vital for subsequent requests by clinicians. This study aimed to assess the impact of post-analysis sample storage on blood plasma 1H-NMR spectra integrity, shedding light on the reliability of re-measurements of prepared samples.

Methods: EDTA plasma samples from 68 unselected subjects (anonymized, leftover material) were analyzed using a 600 MHz NMR instrument (Bruker), to record 1D 1H-NMR spectra. The samples were subsequently stored, either for 24 hours at 4 °C or for 10 weeks at -20 °C, in open capillary NMR samples tubes before remeasurement. The concentrations of 17 metabolites were determined using the manufacturer's quantification method and displayed by box plots for each measurement time point.

Results: Storage of prepared samples after first measurement at 4 °C for 24 hours and at -20 °C for 10 weeks resulted in gradual changes in concentrations of certain analytes over the duration of storage. Overall, larger concentration changes were also associated with increased imprecision. Storage affected the measurement quality of the analysis, although this was not equally pronounced for all metabolites. The size of the observed changes was much more pronounced after 10 weeks than after 24 h. Furthermore, the percentage of samples with missing results, e.g. due to concentrations below the limit of detection, also increased over time for many metabolites compared to the first measurement prior to storage.

Conclusion: Re-measurement of prepared NMR-samples after 24 hours at 4°C yields similar results for most analytes. After 10 weeks at -20°C the number of unavailable results increases together with a decreased recovery of results which indicates that this time span and storage conditions are not suitable for re-measurement of NMR samples.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Novel quantification method improves 1H NMR measurement of plasma creatinine

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Introduction

Plasma creatinine is one of the most frequently requested measurands in laboratory medicine as it is the primary biomarker for the evaluation of kidney function. For creatinine determination in clinical routine, enzymatic or Jaffe methods are typically employed.

1H nuclear magnetic resonance spectroscopy (NMR) is one of the key techniques in metabolomics, but yet remains confined to research context and isn't utilized in clinical settings. NMR offers notable advantages, including non-destructive, robust and reproducible measurement with minimal preparation requirements. Also, a large panel of metabolites may be determined within one measurement. Up to now, automated quantification by Bruker B.I.QUANT PS of creatinine from NMR spectra is based on the NOESY experiment (Nuclear Over Hauser Enhancement Spectroscopy) or a novel quantification method based on the JRES experiment (coupling constant J RESolving Spectroscopy). In our study, we compared both quantification methods to a standard enzymatic method used in clinical routine.

Methods

Plasma creatinine levels of 744 patients with dyslipidaemia were measured on a 600 MHz NMR platform (Bruker Biospin, Ettlingen, Germany) and quantified with the conventional and advanced quantification method. Results were compared to creatinine levels determined with a standard enzymatic method (Dimension Vista, Siemens Healthineers, Eschborn, Germany) as reference by Passing-Bablok analysis and Spearman correlation coefficients. Further, coefficients of variation (CV%) were calculated from daily measured pool samples to evaluate imprecision.

Results

Creatinine levels determined with the advanced JRES-based NMR quantification method showed considerably less bias to the enzymatic method and correlation higher than the conventional NOESY-based quantification (slope = 1.08 and slope = 1.50; r = 0.9 and r = 0.65 for the advanced and conventional method, respectively). Also, imprecision was lower for the advanced

quantification method (CV = 6.8% and CV = 12.1% for the advanced and conventional method, respectively; CV = 4.6% for the enzymatic method).

Conclusion

The advanced NMR quantification method demonstrates considerably improved agreement with the reference method than the conventional NOESY-based quantification method. As a result, we strongly recommend the adoption of the novel JRES-based quantification approach over the previous method for determining plasma creatinine from NMR spectra. Furthermore, our findings may be regarded a step toward integrating NMR methods into clinical practice.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Proteomic profiling of microglial membrane microdomains following plant sterol treatment

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Introduction:

Plant sterols derived exclusively from diet were previously shown to have anti-inflammatory effects. In contrast to cholesterol, plant sterols are able to cross the blood-brain barrier and accumulate in the brain [1], where they may affect signaling of membrane microdomains. These specific subdomains in cellular membranes are enriched in cholesterol and sphingolipids and contains phytosterols (PS) with 1% of the total sterol content. To study whether the changes in cell membrane PS content influence membrane protein distribution, we investigated cell membranes of SIM-A9 mice microglia cells enriched with PS.

Methods:

For that, we established a detergent-free OptiPrepTM-based isolation of lipid rafts from membranes by gradient ultracentrifugation [2]. The membranes were separated into 16 fractions and the first six were confirmed to contain the cell membrane microdomain marker flotillin by western blot. Microglia SIM-A9 cells were treated with/without 25µM of β-sitosterol and campesterol. The microdomain proteome was characterized by analyzing by LC-MS/MS pooled membrane microdomain fractions searched against the UniProt Mus musculus database using the MaxQuant software in the label-free quantification setup. Sterols are analyzed by an established in-house LC-MS/MS method.

Results:

Upon treatment, PS were enriched in the membrane microdomain preparations to 30% of the total sterol content. Among 2696 identified proteins, 472 proteins were upredulated and 295 downregulated (t-test: FDR=0.01, s0=1). The enrichment analysis performed for differentially expressed proteins (STRING database) evidences the activation of microglial proliferative and migratory functions, amyloid clearance and metabolic processes. We also demonstrate that enrichment with PS in microglia leads to positive regulation of amyloid-beta clearance. The highest fold change (10.3, p-value=0.005) was achieved by the cholesterol transporter Abcg1 which confirms previously demonstrated PS-driven cholesterol efflux. The cholesterol synthesis protein Hmgcs1 exhibited the most significant decrease (fold change=19.7, p-value=0.0009). This aligns with previous findings describing the reduction of cholesterol synthesis by PS.

Conclusion:

Our research reveals that incorporation of plant sterols in microglial cell membranes influences proteins, involved in inflammatory and neurological processes. Further proteomic investigations with lower membrane PS content have to follow for dose dependency and membrane receptor plant sterol interactions.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

Application of a pseudotargeted high-resolution mass spectrometry approach for metabolomics in a mouse hippocampus sample

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Introduction. High resolution mass spectrometry (HRMS) is a key technology in the field of untargeted metabolomics. However, identification of low abundant metabolites in complex sample matrices by HRMS is still a challenge. The application of a novel pseudotargeted analytical workflow designed for acquiring fragmentation data of less intense metabolites in a complex sample was the aim of this work.

Methods. Hippocampus samples from mice fed standard chow and a high fat diet were prepared by an adapted protocol from Reinicke et al., 2020. Chromatographic separation was achieved on a C-18 Kinetex Core-Shell column (Phenomenex Torrance, CA, USA) and HRMS data acquisition was performed using an Orbitrap Exploris 480 following two acquisition strategies. The data-dependent and the novel pseudotargeted AcquireX workflow (Thermo Fisher Scientific) were then compared toward their capabilities of biomarker discovery and identification.

Results. The number of biomarkers associated with differences in the mouse brain tissue due to the various diets increased six fold with the AcquireX workflow compared to a data-dependent (DDA) workflow. The biomarker set extracted from the AcquireX data includes less intense compounds with lower abundancy compared to the DDA dataset biomarkers. The DDA biomarkers reached annotation certainty levels 3 and 4, while two AcquireX biomarkers were annotated with level 2 (according to Schrimpe-Rutledge et al., 2016).

Conclusion. Significant differences in the brain tissue of mice associated with diet could be established. The data acquisition strategy of untargeted HRMS-based metabolomics influences the quality of component identification. The AcquireX pseudotargeted strategy is suited for identification of low abundant biomarkers.

DGKL: 15. Mass Spectrometry, Proteomics, Metabolomics

A simple LC-MS/MS method for the quantification of favipiravir in the treatment of Borna disease virus 1 (BoDV-1) encephalitis

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Introduction: The Borna disease virus 1 (BoDV-1) is a zoonotic RNA virus endemic in parts of Germany, causing rare but typically fatal encephalitis in humans. Despite ongoing research, no established antiviral treatment exists for human BoDV-1 encephalitis. In vitro studies have indicated the potential efficacy of the virostatic favipiravir, exceptionally used in influenza treatment. However, it remains uncertain whether standard oral dosage achieves adequate favipiravir concentrations against BoDV-1 within the central nervous system. In light of this uncertainty, we established an LC-MS/MS assay for the therapeutic drug monitoring (TDM) of favipiravir in BoDV-1 encephalitis.

Methods: Following protein precipitation from 25 µL of sample, favipiravir was chromatographically separated on a Raptor Biphenyl Column (2.1x 100 mm, 2.7 µm, Restek) with mobile phases water-formic acid and methanol-formic acid delivered as gradient. Stable isotope-labelled [13C1-15N1]-favipiravir served as internal standard, and favipiravir was quantified in the range of 1 – 100 mg/L. The LC-MS/MS method was comprehensively validated according to the European Medicines Agency

bioanalytical method validation protocol. Matrix mixing experiments were conducted to assess whether serum could be used as surrogate matrix for favipiravir quantification in cerebrospinal fluid (CSF).

Results: The method provided validation results with inaccuracies between -6.0 % and 0.38 % and imprecision coefficient of variation values ≤ 11.4 % for all quality controls. The internal standard consistently compensated for matrix effects in serum and CSF specimens and serum calibrators were applicable for the TDM of both specimen types. The assay was successfully applied to samples obtained from a patient with confirmed BoDV-1 encephalitis where favipiravir concentrations exceeded the half-maximal effective concentration (EC50) of 50 mg/L.

Conclusion: We hereby present a robust isotope dilution LC-MS/MS method that is suitable for the TDM of favipiravir in human serum and CSF in order to maximize treatment efficacy of BoDV-1 encephalitis and establish dose-response relationships. Due to the simple sample preparation and chromatography setup, the method can easily be implemented in clinical laboratories.

DGKL: 16. Neurology, Cerebrospinal Fluid Markers, Dementia Diagnostics

Consequences of GMPPB deficiency for neuromuscular development and maintenance

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Guanosine diphosphate-mannose pyrophosphorylase B (GMPPB) catalyzes the conversion of mannose-1-phosphate and GTP to GDP-mannose, which is required as a mannose donor for the biosynthesis of glycan structures necessary for proper cellular functions. Mutations in GMPPB have been associated with various neuromuscular disorders such as muscular dystrophy and myasthenic syndromes. Here, we report that GMPPB protein abundance increases during brain and skeletal muscle development, which is accompanied by an increase in overall protein mannosylation. To model the human disorder in mice, we generated heterozygous GMPPB KO mice using CIRSPR/Cas9. While we were able to obtain homozygous KO mice from heterozygous matings at the blastocyst stage, homozygous KO embryos were absent beyond embryonic day E8.5, suggesting that the homozygous loss of GMPPB results in early embryonic lethality. Since patients with GMPPB loss-of-function manifest with neuromuscular disorders, we investigated the role of GMPPB in vitro. Thereby, we found that the siRNA-mediated knockdown of Gmppb in either primary myoblasts or the myoblast cell line C2C12 impaired myoblast differentiation and resulted in myotube degeneration. siRNA-mediated knockdown of Gmppb also impaired the neuron-like differentiation of N2A cells. Taken together, our data highlight the essential role of GMPPB during development and differentiation, especially in myogenic and neuronal cell types.

DGKL: 16. Neurology, Cerebrospinal Fluid Markers, Dementia Diagnostics

Hereditary Spastic Paraplegias: deciphering molecular targets for diagnostic and therapeutic interventions

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Introduction: Hereditary Spastic Paraplegias (HSP), a group of rare monogenic movement disorders, are caused by the degeneration of upper motor neurons in the central nervous system. More than 60 genes and more than 80 genetic loci (spastic paraplegia gene loci; SPG1-80, plus other) have been found to be associated with HSP. Bi-allelic mutations in the genes associated with HSP type SPG11 (KIAA1840), SPG15 (Zfyve26) and SPG48 (AP5Z1) lead to the functional loss of the proteins Spatacsin (SPG11), Spastizin (SPG15) and AP5 (SPG48) resulting in an impaired subcellular homeostasis primarily concerning endo-/lyso-/autophagosomal formation and subcellular dynamics. Currently, there are no common molecular targets established for diagnostic, prognostic or therapeutic interventions in HSP.

Aims and Methods: Our study aims to dissect the functional background for 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 immunocytochemistry quantitative PCR (qPCR), Western Blotting and OMICs based characterization of various organ systems such as murine brain and immortalized embryonic fibroblasts.

Results: By immunofluorescence, we confirmed the subcellular alterations along the endo-/lyso-/autophagosomal axis. Applying the protein immunoblot and qPCR based approach we assume subcellular compensatory mechanism in murine SPG15 model for functionally related genes/proteins outside the HSP spectrum. Current transcriptomic analysis reveals specific gene expression patterns characterizing pre- and post-symptomatic SPG11, SPG15 and SPG48 murine brain tissue, confirming our immunoblot and qPCR results, and suggesting some potentially relevant molecular targets and signaling pathways.

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

DGKL: 16. Neurology, Cerebrospinal Fluid Markers, Dementia Diagnostics

Plasma based biomarkers for early assignment to Alzheimer treatment

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Neurodegenerative disorders, esp. Alzheimer's disease, are a growing problem in a population, which is characterized by increasing average lifespan and urn-shaped age pyramid. So far, all therapeutic approaches revealed only limited benefits for the patients. Both the well-established NMDA antagonists (i.e. memantine) and acetylcholine esterase inhibitors (i.e. rivastigmine, galantamine, donepezil), but also the new antibody derived therapies capable to reduce intracerebral amyloid plaques (i.e. lecanemab, donanemab) show best efficacies when installed early in the progress of disease. This stresses the urgent need for diagnostic tools capable to identify those patients who will profit most from therapeutic measures, which are very expensive and hampered by adverse side effects. In recent studies plasma based biomarkers turned out as promising items to support favorably the classical functional tests, PET scans and CSF based biomarker assays, but high precision, sensitivity and specificity of the assays are mandatory. More over the best biomarker profile (i.e. ß-amyloid 1-40 and 1-42, phospho-Tau 181 and 217) and reliable interpreting rules for the results, based on the respective reference values, are just being established. Based on our long term knowledge in CSF based dementia diagnostics, we here describe the establishment and experiences with the chemiluminescence enzyme immuno assays (CLEIA) on the automated LumipulseTM platform (Fujirebio) for the detection of plasma based biomarkers for Alzheimer's disease in a routine laboratory setting.

DGKL: 16. Neurology, Cerebrospinal Fluid Markers, Dementia Diagnostics

Serotonin1A-Receptor-mediated signaling in Astrocytes and its influence on Major Depressive Disorder

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Introduction

Major Depressive Disorder (MDD) is one of the most common psychiatric disorders, affecting approximately 280 million people worldwide (WHO, 2023). Symptoms can range from anhedonia, sleep disturbances, and feelings of hopelessness to suicide. The mechanism that leads to MDD is not fully understood. Most research on MDD has focused only on neurons and neural circuits. Recent studies suggest that non-neuronal cells, such as astrocytes, play a larger role in the nervous system than previously thought. To date, there is considerable evidence for bidirectional communication between astrocytes and neurons, particularly at their synapses. Astrocytes are capable of responding to neurotransmitter release from nearby neurons with internal calcium elevation and subsequent release of a neuroactive transmitter such as ATP, D-serine, and GABA, which in turn can modulate neurons at the synaptic cleft.

Although the involvement of serotonin (5-HT) in the development and manifestation of depression has been hypothesized for many decades, the exact mechanisms leading to depression are poorly understood. Several studies have implicated astrocytic activity in depression and other affective disorders. Since astrocytes express different 5-HT receptors (5-HT-R), such as the 5-HT1A-R, which is associated with depression, our study focuses on the influence of 5-HT1A receptor-mediated signaling in astrocytes within the medial prefrontal cortex on depressive-like symptoms in mice.

Methods

Our study examines the influence of 5-HT1A receptor-mediated signaling in astrocytes on depressive-like symptoms. In neurons, the 5-HT1A receptor is coupled to the Gi signaling pathway. Interestingly, in astrocytes, G-coupled receptors can induce internal calcium elevation. To test whether this is also true for the 5-HT1A receptor, we first performed calcium imaging in acute brain slices of the mouse medial prefrontal cortex. Pharmacological activation of the 5-HT1A receptor resulted in a significant increase in calcium events in astrocytes.

To selectively manipulate the 5-HT1A-R pathway in astrocytes, we used an optogenetic approach with our previously published light-activatable 5-HT1A receptor chimera (Masseck et al. 2014). We used two established models of depression. To study the social component of depression, we performed the chronic social defeat test (cSDS) and activated the 5-HT1A receptor chimera during stress exposure for 10 consecutive days. A second model, the chronic mild stress test (CMS), was used to study anhedonia.

Results

Our behavioral data from both models show an antidepressant effect due to activation of 5-HT1A-R pathways in prefrontal astrocytes.

Our data support the hypothesis that 5-HT1A-R mediated signaling in astrocytes influences depression and has an antidepressant effect.

DGKL: 16. Neurology, Cerebrospinal Fluid Markers, Dementia Diagnostics

Heterodimerization and Interaction of the Serotonin-Receptors 5-HT1A and 5-HT2C

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Introduction:

G-Protein coupled receptors (GPCRs) are among the most prominent receptors in the central nervous system. Their malfunction is implicated in various neurological and neuropsychiatric disorders, making them a common target for medical treatment. Thus, it is crucial to gain a comprehensive understanding of GPCR functions and mechanisms to develop more effective and targeted medical treatments with fewer side effects.

This study examines the potential interplay between the serotonin receptors 5-HT1A and 5-HT2C, which play a significant role in the pathology of depression.

Methods:

Experiments were performed in transfected HEK-293 cells expressing both receptors. The potential heterodimerization was investigated by Acceptor Photobleaching and FLIM measurements. Further analysis involves Co-Immunoprecipitation/Western-Blot techniques.

Moreover, the interaction between both receptors was analyzed by Calcium Imaging and will be further investgated by Patch Clamp measurements.

Results:

Our findings provide initial evidence of heterodimerization between the 5-HT1A and 5-HT2C receptors, as indicated by FRET in Acceptor Photobleaching measurements. A significant increase in fluorescence intensity of the CFP-tagged 5-HT2C receptor of approximately 10% was observed after bleaching of the YFP-tagged 5-HT1A receptor.

Additionally, Calcium Imaging experiments demonstrated significant signals in HEK-293 cells transfected with both receptors and GCaMP8 when exposed to serotonin.

DGKL: 16. Neurology, Cerebrospinal Fluid Markers, Dementia Diagnostics

Evaluierung von Ferritin im Liquor anhand von 298 Lumbalpunktionen

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Zielsetzung

Eisen spielt im cerebralen Stoffwechsel eine wichtige Rolle. Zahlreiche Prozesse, wie die Produktion von Myelin oder die Synthese von Neurotransmittern, sind auf Eisen angewiesen. Eine Störung der Eisenhomöostase im Gehirn ist mit neurodegenerativen Krankheiten wie Morbus Parkinson oder der Alzheimer-Krankheit assoziiert. Die Mechanismen des Eisentransports aus dem systemischen Kreislauf ins Gehirn über die Blut-Hirn-Schranke und die Blut-Liquor-Schranke sind jedoch noch nicht vollständig geklärt. Insbesondere ist nur wenig über eine mögliche Beteiligung des Eisenspeicherproteins Ferritin bekannt. Ziel dieser Studie war die Beurteilung der Konzentrationen von Ferritin im Liquor in Bezug auf die jeweiligen Serum-Ferritin-Spiegel.

Methoden

Insgesamt wurden 298 Patienten, die eine routinemäßige Lumbalpunktion durchliefen, in die Studie eingeschlossen. Ferritin und Albumin wurden jeweils im Serum und im Liquor mittels Standard-Laborverfahren (Elektrochemilumineszenz-Immunoassay und Bromkresolgrün-Methode) gemessen. Die Verhältnisse zwischen den Liquor- und Serumwerten für Ferritin und Albumin wurden berechnet (QFerr und QAlb)

Ergebnisse

QFerr zeigte einen Median von 0,06 (Interquartilbereich 0,03-0,12). Es zeigte sich eine statistisch signifikante Korrelation zwischen QFerr und QAlb (Spearman's rho -0,135, p = 0,02). Statistisch signifikante Korrelationen zwischen Ferritin im Serum und im Liquor (Spearman's rho 0,415, p < 0,001) und auch zwischen QFerr und Serum-Ferritin (Spearman's rho -0,899, p < 0,001) wurden festgestellt. Besonders im Bereich eines Serum-Ferritins von unter 100 ng/mL steigt das QFerr deutlich an.

Diskussion und Schlussfolgerung

Die festgestellten Zusammenhänge deuten auf einen möglichen konzentrationsabhängigen Transportmechanismus für Ferritin aus dem systemischen Kreislauf ins Gehirn hin. Bei niedrigen Serum-Ferritin-Konzentrationen könnte die zerebrale Eisenversorgung durch einen hochregulierten Ferritin-Transport über die Schranken aufrechterhalten werden.

DGKL: 16. Neurology, Cerebrospinal Fluid Markers, Dementia Diagnostics

Altersabhängige Referenzwerte für Neurofilament light gemessen and zwei unterschiedliche Testsysteme (Lumipulse und Simoa)

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Zielsetzung: Neurofilament light (NfL), ein strukturelles Protein des Zytoskeletts, ist ein vielversprechender, serumbasierter Biomarker für neuroaxonale Schädigung und wird derzeit bei verschiedenen neurologischen Erkrankungen wie z.B der Amyotrophen Lateralsklerose und der Multiplen Sklerose untersucht 1. Da NfL-Werte altersabhängig sind, ist es dringend erforderlich, altersspezifische Referenzwerte zu definieren. Die Komplexität dieses Themas wird durch die Verfügbarkeit verschiedener Testsysteme zur Messung von sNfL erhöht. In unserer Arbeit wurde die sNfL-Konzentration in gesunden Proben mit zwei Testsystemen von FujiRebio (Lumipulse) und Quanterix (Simoa) zur Bestimmung von Referenzintervallen gemessen und die Werte miteinander verglichen.

Methoden: In die Studie wurden 303 Proben von augenscheinlich gesunden Blutspendern im Instituts für Transfusionsmedizin eingeschlossen. Alle Proben wurden in Serum-Monovetten der Firma Sarstedt gesammelt, zentrifugiert und innerhalb von 24 Stunden nach der Entnahme bei -80°C eingefroren, nachdem sie zuvor bei 4°C gelagert worden waren. Alle Proben wurden mit dem Chemilumineszenz-Immunoassay (CLIA) (Lumipulse G Blood NfL, FujiRebio) und dem Single Molecule Array (Simoa) (Neurofilament Light Advantage V2, Quanterix) parallel gemessen.

Ergebnisse: 40% der Proband*innen waren weiblich. Bei allen getesteten Proben konnten mit beiden Plattformen Ergebnisse innerhalb des Messbereichs erzielt werden. Insgesamt 7 Messergebnisse lagen außerhalb des 95% Bereichs des linearen Regressionsmodells (2 mit höheren sNfL-Konzentrationen am Lumipulse und 5 mit niedrigeren sNfL-Konzentrationen am Simoa). Aufgund des fehlenden Goldstandards für die sNfL-Bestimmung wurden diese von der Auswertung ausgeschlossen. Die Ergebnisse der beiden Testsysteme korrelierten gut miteinander (Pearson r=0,89, p < 0,001). Dabei konnte ein konstanter Abstand von ~6 pg/mL zwischen den Methoden beobachtet werden (NfL-Lumipulse = 6.13+0.99*NfL-Simoa). Altersintervalle für die Bestimmung der Referenzwerte mit statistisch signifikanter Unterscheidung für beide Systeme wurden mit Hilfe der k-Means-Methode bestimmt: 18-35 Jahre, 35-55 Jahre und 56-70 Jahre. Für die Berechnung der altersabhängigen Referenzwerte wurden 95%-Konfidenzintervalle gewählt: 18-35 Jahre Lumipulse: 6,6-14,9 pg/ml Simoa: 2,5-7,4 pg/ml, 35-55 Jahre Lumipulse: 7,9.18,9 pg/ml, Simoa: 3,4-13,1 pg/ml und 56-70 Jahre Lumipulse 8,6-26,6 pg/ml, Simoa 5,3-20,2 pg/ml.

Zusammenfassung: Derzeit gibt es keine CE-IVD-zertifizierten Testsysteme für die Messung von sNfL, jedoch häufen sich klinische Hinweise auf einen diagnostischen und prognostischen Mehrwert dieses Biomarkers. Beide betrachteten Testsysteme lieferten vergleichbare Ergebnisse und Referenzintervalle. Aufgrund des fehlenden Goldstandards für die Bestimmung der sNfL-Konzentration wird dringend empfohlen, plattformspezifische Referenzintervalle sowie krankheitsspezifische Grenzwerte zu berechnen.

DGKL: 17. One Health, Migration Medicine, Environmental Medicine, Health Risks of Climate Change, Green Labs

A green laboratory approach to medical sample transportation between two medical laboratories: assessing the carbon dioxide (CO2) footprint of medical sample transportation by drone, combustion car, and electric car

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Background and Aim

This study delves into the ecological implications of medical sample transportation methods, explicitly focusing on drones, combustion cars, and electric cars within two laboratories in two central European countries. With the primary objective of evaluating and comparing CO2 emission, transport distances, and delivery times, the project aims to provide scientific evidence for establishing sustainable practices in healthcare logistics.

Methods

Medical samples were transported between two Laboratories in two different central European countries (Principality of Liechtenstein, Switzerland) and possible future routes representative for different terrains in central Europe. Aerial and road routes were authorized for sample transport by the respective public authorities. Sample transports with vehicles (8 combustion car types, two electric car types, and one drone model) occurred under various weather conditions and distances and terrains including different traffic conditions. Energy consumption was recorded, and CO2 footprint was calculated according to the Swiss Federal Office for the Environment (FOEN) data for imported and renewable electricity. Comparative analyses were conducted to quantify the environmental impact of each transportation method.

Results

Combustion cars exhibited an average CO2 release of 159.5 grams (g) per kilometer (km), the respective amount being lower in electric cars (3.43g/km; 2.15% of combustion cars). Drones demonstrated a CO2 emission of 0.09g/km (0.07% of combustion car; 2.6% of electric car). In addition, relative distance traveled by drone was significantly shorter namely, 16.7% on flat terrain and 25% on mountainous terrain. The efficiency of drone transport was also characterized by its ability to avoid traffic jams and associated detours in car transports, resulting in relative time savings ranging from 36% (regular hour) to 60% (rush hour) per delivery when comparing the transport times in the chosen setting. Also, in mountainous terrain to reach more remote villages, the time saving was 50%.

Conclusion

Integrating drone technology may represent a pivotal strategy for establishing a “green laboratory.” Drone transport was characterized by the lowest CO2 emission per kilometer, shorter transport distances and faster delivery times. The findings underscore the potential for transformative change in healthcare logistics, aligning with the global shift towards sustainability and green practices.

DGKL: 17. One Health, Migration Medicine, Environmental Medicine, Health Risks of Climate Change, Green Labs

Hepatitis-C-Virus Seroprävalenz und Abhängigkeit vom Herkunftsland bei Geflüchteten

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Zielsetzung

Die durch das Hepatitis-C Virus (HCV) übertragene Lebererkrankung Hepatitis C kann in Leberzirrhose und hepatozellulärem Karzinom münden. Nach Schätzungen aus dem Jahr 2022 waren 2020 weltweit ca. 56.8 Mio. Menschen chronisch mit HCV infiziert, das entspricht 0.7 % der Weltbevölkerung. Durch die weltweiten Migrationsbewegungen kommt es zur Zuwanderung aus HCV-Hochprävalenzländern in Niedrigprävalenzländer. Doch über die HCV-Seroprävalenz und den Zusammenhang mit dem Herkunftsland in aktuellen unselektierten Populationen Geflüchteter (> 1000 Personen), die nach Europa/Deutschland eingereist sind, gab es bis zum Jahr 2015 noch keine Daten. Ziel dieser Arbeit war die Dokumentation und Bewertung der Seroprävalenz von Hepatitis C und Untersuchung, ob ein Zusammenhang mit dem Herkunftsland der Geflüchteten besteht. Die Basis hierfür bildet der bislang größte veröffentlichte Datensatz in Deutschland.

Methoden

Bei 12880 Geflüchteten in Rheinland-Pfalz wurde im Jahr 2015 in der Routinediagnostik bei der Erstaufnahmeuntersuchung die HCV-Serologie bestimmt. Die Daten wurden retrospektiv und anonymisiert ausgewertet.

Ergebnisse

Das Geflüchteten-Kollektiv aus 12880 Personen weist eine HCV-Seroprävalenz von 1,5 % auf. Diese ist höher als die HCV-Prävalenz der deutschen Allgemeinbevölkerung (etwa 0,5 %). Insbesondere konnte gezeigt werden, dass ein Zusammenhang zwischen der HCV-Seroprävalenz und dem Herkunftsland besteht. Seit 2015 bis heute ist in Deutschland kein vergleichbarer Datensatz ausgewertet und publiziert worden.

Diskussion und Schlussfolgerung

Um das HCV-Eliminationsziel der WHO bis 2030 zu erreichen, können nationale und internationale Empfehlungen Geflüchtete und Migranten aus Hochprävalenzländern auf HCV zu screenen, bestärkt werden. Chronisch Infizierte sollten zeitnah leitliniengerecht behandelt werden. Nationale, leicht zugängliche Informationen zu HCV-Hochprävalenzländern für die behandelnden Ärzte sind unterstützenswert.

DGKL: 18. Preventive Medicine, Screening Programs, Prenatal Diagnostics, Rare Diseases

Use of laboratories big data to optimize and personalize laboratory test interpretation with applications

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Laboratory medicine has always played a crucial role in the diagnosis, treatment and monitoring of patients. Every day, millions of laboratory tests are performed around the world, requiring interpretation for clinical decision-making. The predominant method for this interpretation remains the use of reference intervals, a concept established almost 75 years ago. Reference intervals for most biological parameters have been established by analyzing a reference population of individuals, encompassing 95% of their values. When the distribution is Gaussian, the lower and upper limits are determined by calculating the mean ± 2 standard deviations from a sample of at least 120 so-called healthy subjects.

We propose a stepwise analysis of variance (ANOVA) approach to optimize and personalize the so-called reference populations. To do this, we have 37,677,310 laboratory test results for 996,975 individuals. Firstly, personalizing parameters (age, sex or blood group) and laboratory parameters are used to build meaningful linear models for different laboratory tests. This allows us to identify which set of parameters are conjointly correlated to a given laboratory test. These models are built step wise. Then, for each model, we derive reference populations by selecting individuals with normal results for the parameters of the model and for each personalizing parameter level. These optimized and/or personalized reference populations of at least 120 can be used to obtain thinner and personalized lower and upper reference values.

In this abstract, we present the results obtained by comparing populations optimized and personalized with sex for serum phosphate and beta 2 globulin with the overall populations for these 2 parameters, which include all the patients available for each laboratory test. Effect size comparisons using Cohen's D and Hedges' G revealed a more pronounced separation by sex in the optimized and personalized populations, with a Cohen's D of 0.4 (small size effect) for the overall population versus 0.85 (large size effect) for the optimized/personalized population.

Similar results were observed for beta 2 globulin, with Cohen's D increasing from 0.15 (no size effect) for the overall population to 0.48 (small/medium size effect) for the optimized/personalized population.

In addition, a reduction in inter-individual variability was observed, as the reference intervals of the optimized/personalized population were often tighter than those of the global population and the reference intervals used today.

In conclusion, we demonstrate that this innovative method for optimizing and personalizing reference populations enables new patterns to be found in the distribution of biological data. The personalization highlighted presently is based on sex, but in practice, other personalization such as sex or blood group can be used. This could enable to facilitate, in a personalized manner, the early detection of certain diseases.

DGKL: 18. Preventive Medicine, Screening Programs, Prenatal Diagnostics, Rare Diseases

Prospektives Hepatitis-D-Screening bei HBsAg-positiven Patienten im „Check-Up 35+“: Daten zur Prävalenz aus der Primärversorgung in Deutschland

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Einleitung: Ein anti-HDV-Screening bei allen neu entdeckten HBsAg-positiven Fällen wird gemäß Leitlinien empfohlen. Aus der Primärversorgung liegen in Deutschland bisher keine prospektiven Daten zur anti-HDV Prävalenz vor. Ein Hepatitis B und C Screening im Rahmen der hausärztlichen Gesundheitsuntersuchung wurde als „Check-Up 35+" im Jahr 2021 eingeführt. Wir untersuchten prospektiv die anti-HDV Prävalenz bei HBsAg positiven Patienten in diesem „Check-Up 35+" in der Primärversorgung.

Methode: Bei HBsAg positiven Blutproben wurde zwischen Oktober 2022 und September 2023 prospektiv ein anti-HDV Screening durchgeführt., Die Identifikation der Proben erfolgte über die Abrechnungsziffer GOP 01865 an 11 LADR-Facharztlaborstandorten. Anti-HDV positive Patienten wurden erneut kontaktiert für eine HDV RNA Testung sowie zur Erfassung von klinischen Angaben bezüglich der Lebererkrankung.

Ergebnisse: Bei den „Check-Up 35+" Patienten (56% weiblich, mittleres Alter 60±14 Jahre) lag die HBsAg Prävalenz bei 1.159/225.901 (0,51%). Eine Reflextestung auf anti-HDV erfolgte bei 700 der 1.159 HBsAg-positiven Patienten (60,4%) und ergab eine anti-HDV Prävalenz von 1,6% (n=18/700; 33% weiblich, mittleres Alter 51 ± 10 Jahre). Bei 4 (Männer mit einem Alter von 42, 47, 53 und 58 Jahren) der 18 anti-HDV positiven Patienten waren klinische Parameter erfassbar und eine HDV RNA (PCR) Analyse möglich. Keiner dieser Fälle wies einen FIB-4 Score > 2.67 auf. Die HDV-RNA war in einem der 4 Fälle positiv (53 Jahre, Herkunft Moldawien, ALT 2,2 x ULN, FIB-4=2; klinisch keine Zirrhose). Vor dem Screening war bei den vier Fällen weder der HBsAg positive noch der anti-HDV positive Befund bekannt.

Als Ursache der nicht durchgeführten anti-HDV Reflextestungen bei HBsAg-Positivität wurden bei 68% (n=310/459) eine für die Analyse nicht ausreichende Serummenge und bei 149 Fällen logistische Gründe nachgewiesen.

Unter der Annahme, dass der Anteil der Proben mit zu geringer Blutmenge bei nicht durchgeführten anti-HDV Tests repräsentativ ist, würde dies für die Implementierung einer anti-HDV Reflextestung in der Routine bedeuten, dass 30,7 % aller Testungen aufgrund von Materialmangel nicht durchgeführt werden könnten. Die anti-HDV Prävalenz bei HBsAg positiven Patienten läge bei 1,8% (18/(1.159-149)).

Schlussfolgerung: Die anti-HDV-Prävalenz im „Check-Up 35+" liegt in unserer Studienpopulation bei 1,6-1,8%. Für eine anti-HDV/HDV-RNA Reflextestung im Alltag sollte auf ausreichendes Probenmaterial geachtet werden. Die vorliegenden Daten können eine Grundlage für eine Erweiterung des Hepatitis Screenings im „Check-Up 35+" sein.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Time as a significant factor in the release of potassium from lithium heparin plasma and serum

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Introduction: In most countries the majority of patients are in outpatient care. In difference to hospitalized patients, their blood samples often take several hours after collection to arrive at the laboratory. Against this background, the study investigates the release of potassium and the development of pseudohyperkalemia in lithium heparin (Li-Hep) and serum blood collection tubes over time.

Methods: From 201 donors (111 female, 90 male; median age 54 y, range 20-88 y) 1 EDTA (for CBC), 4 serum and 4 Li-Hep blood collection tubes were taken. After 0.5, 4, 6 and 8 h, potassium was determined from serum and Li-Hep. To simulate the transport conditions and storage in doctors' office, the samples with a storage time >0.5 h were shaken on a standard shaker for 1h and stored at 4-8°C for the remaining time.

Results: According to the different reference ranges, a significantly higher potassium concentration was found in the serum compared to the plasma after 30 minutes. Over the entire test period, more potassium was released from the Li-Hep plasma than from serum. After 6 h, the two groups were no longer statistically significantly different (p>0.001). Therefore, in the Li-Hep group more donors (176) developed a pseudohyperkalemia after 8 h, compared to 76 in the serum group. The other factors examined (creatinine concentration, number of thrombocytes, leukocytes, erythrocytes and hemolysis), did not appear to have a significant influence on the increase in potassium concentration in both materials over time.

Conclusion: The decision as to which material is best suited for the determination of potassium should not only be based on which value comes closest to the physiological situation immediately after the blood sample is taken. The subsequent circumstances (like transportation conditions and centrifugation) must also be considered. Therefore, serum tubes appear to be at least as suitable for potassium determination as Li-Hep tubes and offers in terms of patient blood management the possibility of performing a wider range of analyses in the outpatient setting, including serum electrophoresis or lithium determination as well as fT3/fT4 reflex testing.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Thrombopoietin external quality assessment - laboratory results and method precision

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Introduction

The aim of the study was to investigate the measurement quality of medical laboratories with regard to thrombopoietin (TPO) determinations within the framework of an external quality assessment (EQA) scheme. Since the results of the past EQA runs since the introduction of the TPO EQA in 2017 were not entirely conclusive, due to the consistently low number of participants, a much more detailed investigation was carried out in this study design with a higher number of samples.

Methods

A total of six laboratories took part in the EQA and were asked to generate two pooled serum samples. The total of 12 different samples were then distributed to the participating laboratories and measured using enzyme-linked immunosorbent assay (ELISA) at least in duplicates.

Results

At the time of submission, data collection and analysis are still ongoing. Preliminary results indicate variability in the measurements between participating laboratories. The average deviation from the mean value of the calculated TPO concentrations in the samples so far is 31.0 ± 11.2 % (n = 4). The average intra-assay variation based on the previous results of the multiple measurements of the optical density values is 6.1 ± 5.0 %. Final results and detailed analysis will be available at the time of the congress presentation.

Conclusion

This study emphasizes the importance of EQA, especially in the context of TPO determinations. The observed variations emphasize the need for continuous objective evaluation of measurement quality to ensure reliable TPO test results and

patient safety. Further investigations into the causes of the variations and the implementation of corrective interventions are currently ongoing and will be presented at the congress.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Robotik in der Labormedizin: ein Erfahrungsbericht zur Implementierung in der stationären Routineversorgung

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Zielsetzung

In vielen Krankenhauslaboren ist die Besetzung der Spät-, Nacht- und Wochenend-Dienste durch MTL auf Grund des hohen Fachkräftebedarfs schwierig zu gewährleisten. Zunehmend wird daher über die Implementierung von Laborrobotik nachgedacht.

Primärziel des vorliegenden Projekts war die Etablierung einer Laborrobotik in einem Krankenhauslabor, dessen Einsatzspektrum die Probenprozessierung mit den im Routinebetrieb eingesetzten Laborgeräten kombiniert und automatisiert. Ziele waren:

a. Vollständig autonomer Nachtbetrieb

b. Hybrider, MTL-unterstützender Tagesbetrieb mit Erhöhung der Walk-Away-Zeit für die angebundenen Laborgeräte/-prozessschritte

c. Etablierung von MTL-vergleichbaren Turn-Around-Zeiten und Zuverlässigkeit

Methoden

Nach Evaluation der vorhanden Laborgeräte, der räumlichen Gegebenheiten und der aktuellen Laborprozesse wurde eine Laborrobotik-Lösung für die Probenannahme, Probenaktivierung im Laborinformationssystem, Zentrifugation, Decapping, Probenrackplatzierung und Übergabe von verschiedenen Probentypen an die Laborgeräte konzeptualisiert. In einer mehrmonatigen Umsetzungsphase erfolgte die Installation, Programmierung/Teaching und kontinuierliche Verbesserung des Laborroboters.

Ergebnisse

Der Laborroboter automatisiert mit hoher Zuverlässigkeit die wichtigsten und häufigsten Laborprozessschritte im Krankenhauslabor. Probenanalyseprozesse werden selbstständig durch den Laborroboter gestartet. In der Zeit von 10 – 18 Uhr arbeitet der Laborroboter im Tages-Hybrid-Modus die Routineproben vollständig autonom ab. Notfallproben, Nachforderungen und Massenprobenaufkommen können durch Pausierung des Roboters jederzeit und ohne Zeitverlust durch Umrüstzeit durch MTL manuell/priorisiert auf den Geräten abgearbeitet werden. Von 6 – 10 Uhr übersteigt das Probenaufkommen die Leistungsfähigkeit des Laborroboters. Es werden in dieser Zeit Wartungen, Gerätevorbereitungen, Kalibrations- und Kontrollmessungen durchgeführt. Von 18 – 6 Uhr arbeitet der Laborroboter im autonomen Nachtbetrieb ohne MTL-Präsenz. Die Validation der Messergebnisse erfolgt telemedizinisch im Schwesterlaborstandort der Klinik, das 24/7h MTL-besetzt ist und als Notfall-Backup-Labor dient.

Diskussion

Standortindividuelle Laborrobotik-Lösungen sind in einem Krankenhauslabor zur autonomen Übernahme von standardisierten, repetitiven Laborprozessen einsetzbar. Sie erhöhen die Walk-Away-Zeit und schaffen Personalentlastung. Die Gewährleistung einer 24/7h-Laboranalytik im Krankenhauslabor kann durch Robotik sichergestellt werden.

Herausforderungen bestehen in fehlenden Geräte-/Industrie-Standards beim Roboter-Einsatz für die Laborautomation. Aktuell sind Implementationen von Laborrobotik-Lösungen im Krankenhauslabor durch ein hohes Maß an Projektindividualität gekennzeichnet und die Realisierung ist mit einer hohen Komplexität verbunden. Standardisierung und zunehmender Routineeinsatz sind für die Zukunft zu erwarten.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Comparison of the SalivetteTMCortisol with the QuantisalTM saliva collection device and a drooling method for the analysis of salivary cortisol with UPLC-MS/MS

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Introduction

Salivary cortisol (C) reflects unbound (free) serum C concentration and has therefore become a valuable diagnostic tool in endocrinology. In this study, we compared the oral fluid (OF) collection with the established SalivetteTMCortisol (Sarstedt) to the QuantisalTM (Immunalysis/Abbott) and a drooling method with a polypropylene straw for the analysis of C with an accredited UPLC-MS/MS in house method.

Methods

OF collection devices SalivetteTMCortisol and QuantisalTM were used as described by the manufacturer. The drooling method worked by spitting at least 0.1 mL OF into a 1.5 mL mini vial (Sarstedt) with the help of a 6 cm long polypropylene medical straw (5 mm diameter). OF concentrations in QuantisalTM samples were determined by weighing. OF samples (50 µL) were analysed after adding 10 µL Cortisol-13C3 (2.5 ng/mL, Cerilliant) followed by salting-out assisted liquid/liquid extraction on a Waters Acquity/Xevo TQ-XS (Waters) tandem mass spectrometer. Separation was performed with linear gradient elution (MoP A = 20 mM ammonium formate + 0.1% formic acid at pH 3, MoP B = methanol + 0.1% formic acid) within 6.5 min on a BEH Phenyl 1.7 µm, 2.1 x 150 mm column (Waters) kept at 50 °C with a flow rate of 0.4 mL /min. Six-point calibration in neat OF (Chromsystems) ranged from 0.43 to 55.60 ng/mL and was applied for OF from drooling and SalivetteTMCortisol. The calibration for QuantisalTM samples was prepared with Chromsystems calibrators in QuantisalTM buffer and ranged from 0.11 to 13.90 ng/mL (n = 6). The mass spectrometer with an UniSprayTM (Waters) ion source mounted was operated in ESI+ mode with 3 transitions monitored for C and 2 transitions monitored for the internal standard. We conducted 2 series of OF collection: series A: 10 volunteers collected 3 consecutive OF samples each with the same collection device (24-50 years, 15 females, 15 males, n = 3x 30 samples). Series B: 30 volunteers (24-55 years, 21 females, 9 males) collected 3 consecutive OF samples using 3 different devices in various order (n = 90 samples). In both series total collection time never exceeded 25 min. Samples from series B were analysed in duplicates (n = 180).

Results

Repetitive, consecutive OF sampling with the same collection device (series A) resulted in similar C concentrations for each volunteer with deviations of < 10%. This could be attributed mostly to measurement uncertainty. In series B the mean C concentration (n = 6) of each volunteer revealed CVs from 2.2% to 13.5%. Agreement of mean C values in OF with the results from SalivetteTMCortisol samples was 1.030 for the QuantisalTM device (r2 = 0.974, n = 30) and 1.010 for the drooling method (r2 = 0.978, n = 30).

Conclusion

Consecutive OF sampling within 25 min with the same or different collection devices resulted in similar salivary C concentrations with our UPLC-MS/MS method. The tested collection devices are therefore of equal value and can be applied according to the patients preferences.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Unmanned high speed aerial vehicles in medical logistics: a comparative analysis of blood sample transportation with weather variability

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Background and Aim

Drones represent a cutting-edge technology with transformative potential in the realm of medical logistics, particularly in the transportation of sensitive medical goods such as blood samples. This study aimed to compare the impact of a high-speed drone transportation with traditional car transportation on the integrity and preanalytics of blood samples, considering various blood materials and analytes.

Methods

Blood samples (EDTA whole blood, serum, and plasma anticoagulated with Citrate and Lithium-Heparin transported as whole blood) were transported both by drone and car, with the drone flight tested under diverse weather conditions (ranging between 0 and 20 degrees Celsius) and compared to storage of samples without sample transport. Maximum drone speed exceeded 100 km/h. A total of 28 analytes were tested on Serum, 20 on EDTA, 26 on Lithium-Heparin, and 5 on Citrate blood. Statistical analyses were conducted using Passing Bablok and Bland-Altman. Constant temperature measurements of the sample during flight were performed at all altitudes and weather conditions. Vibrations were measured using an accelerometer.

Results

For Serum samples, the correlation coefficient (r) ranged from 0.830 to 1.000, and the slopes varied from 0.913 to 1.111. Five analytes (total bilirubin, Calcium, Ferritin, Kalium, and Sodium) showed discrepancies (r lower than 0.800, slope not between 0.8 and 1.2 and mean (%) not between +/- 10%) between the negative control and the transported sample. However, no significant differences were observed between drone and car transportation. For EDTA, Lithium-Heparin, and Citrate samples, similar patterns emerged. R ranged from 0.829 to 0.997, 0.939 to 0.998, and 0.830 to 1.000, respectively and slopes ranged from 0.956 to 1.051, 0.938 to 1.085 and 0.913 to 1.111. Analyzing specific analytes, a few discrepancies were identified, but no significant differences between transportation methods. Accelerometer measurements showed higher vibrations with the drone but without noticeable impact on sample integrity. Furthermore, sample temperature decreased 4.3 °C (From 20.0 to 15.7 °C) at 0°C outside temperature at an altitude of 1800 meter above sea level (AMSL) and 31.8 km flown distance and 30 minutes of flight.

Conclusion

In conclusion, drone transportation of blood samples does not significantly alter analyte concentrations when compared to car transportation. Any observed discrepancies were attributed to the transportation process itself rather than the mode of transportation. Thus, drones offer a comparable, if not superior, alternative to traditional car transportation methods for maintaining sample integrity and preanalytics. This highlights the potential of drone technology to enhance the efficiency and reliability of medical sample transport, particularly in scenarios requiring rapid and reliable delivery for timely diagnosis and treatment.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Commutability as a qualitative requirement for an accuracy-based evaluation of EQA schemes in clinical chemistry

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Introduction

High quality of external quality assessment materials (EQAM) is a necessity to allow conclusive statements on the quality and homogeneity of medical laboratory analysis. EQAM often require artificial processing e.g., to ensure stability of the sample material. The samples of the INSTAND EQA scheme ‘Clinical Chemistry - Conventional Analysis’ are lyophilized pooled sera with spiked analyte concentrations. Such processing might hamper the analysis of individual measurement procedures (MP) which is why the commutability of those EQAM should be verified.

Methods

To determine whether MP-specific differences observed in previous EQA results were due to artificial processing of the samples or if they originate from the various MPs, four native single-donor sera will be tested for γ-glutamyltranspeptidase (γ-GT), creatinine, cholesterol, urea, and sodium in an EQA survey, alongside the two regularly processed EQA samples. Stability testing will be performed for the fresh sera.

As reference MPs are available for the analytes, the MP-specific results can be normalized to the reference measurement values (RMV), to allow comparison of the bias of the two sample types.

Results

In surveys from 2021 to 2023, the median MP-specific bias for the different EQAM batches varied up to 3.4% for sodium, 15.2% for cholesterol, 10.6% for urea and 18.9% for γ-GT. For creatinine the variation in the median MP-specific bias was up to 139%, but decreased to max. 30.5%, when excluding three EQAM batches with creatinine of < 0.75 µmol/L.

For the native EQAM, up to 450 results are expected for each analyte. Based on previous experiences with EQAs, it is expected that the compared bias of the fresh and processed samples will show lower variations than the acceptance limits in the EQA. The limits are set at ±5% for sodium, ±13% for cholesterol, ±21% for γ-GT, ±20% for urea and creatinine according to the guideline of the German Medical Association for quality assurance of medical laboratory analyses.

Conclusion

If the variability in bias comparisons between fresh and processed samples is constant, this would indicate that there are no problems with commutability for individual manufacturer collectives. Fairly broad passing criteria in the EQA scheme consider that minor scatter in MP-specific results could occur, among other things, due to sample effects. If the material processing has major effects on individual MPs, the EQAM would not be suitable for evaluating the EQA based on the RMV. An acceptable evaluation of EQA results can then only be carried out by forming collectives for the respective manufacturers.

Ultimately, commutable EQAM are beneficial to improve quality assurance in medical laboratories at the level of measurement accuracy. The knowledge of specimen characteristics is critical to evaluate the causes of observed non-permissible deviations in medical analysis and thus provide the scientific background for initiating appropriate corrective action.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Einfluss suboptimaler Präanalytik auf die labormedizinische Ergebnisqualität: erweitertes Analytspektrum

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Zielsetzung: In bevölkerungsbezogenen Studien mit Bioproben sind die präanalytischen Möglichkeiten oft eingeschränkt und können die Genauigkeit von Labormessungen beeinflussen. Häufig bleibt bei der Interpretation von Studienergebnissen unklar, welcher Anteil der beobachteten Unterschiede auf Defizite in der Präanalytik zurückzuführen ist. Daher wurde der Einfluss suboptimaler Präanalytik auf die labormedizinische Ergebnisqualität in einer Studie an insgesamt 30 ausgewählten Analyten systematisch untersucht.

Methoden: Von geschulten Untersuchungsteams wurden Ende 2022 an zufällig aus der Bevölkerung ausgewählten Freiwilligen (Alter 16-74 Jahre) durch venöse Blutentnahme jeweils 4 Serumröhrchen abgenommen und ins Epidemiologische Zentrallabor (Epi-Lab) des RKI gebracht. Zwei der vier Serumröhrchen wurde nach 30-45 Minuten Standzeit bei Raumtemperatur 12 min mit 2500 x g zentrifugiert. Ein Röhrchen davon wurde anschließend bei 4 °C gekühlt, das andere bei Raumtemperatur gelagert. Auch von den zwei nicht zentrifugierten Serumröhrchen wurde eines gekühlt und eines ungekühlt gelagert. Die Proben wurden einmal wöchentlich ins Epi-Lab gebracht. Nach dem Eintreffen wurden die nicht zentrifugierten Serumproben zentrifugiert und alle Proben bis zur Analyse bei 4 °C gekühlt. Aus den Serumröhrchen wurden weitere Analyte bestimmt, und zwar Gesamt-IgE, SX1-Screeningtest auf 8 spezifische IgE (ThermoScientific Phadia 1000), ALT, AST, Albumin, Alkalische Phosphatase, C-Peptid, hs-CRP, Kalzium, Kreatinin, LDL, Ferritin, fT3, fT4, GGT, Eisen, Kalium, Magnesium, Natrium, anorganisches Phosphat, Gesamt-Protein, TSH, Transferrin, Triglyzeride und Harnsäure (Abbott Alinity). Die Messwerte der präanalytischen Varianten wurden vergleichend zur Referenzprobe mit optimaler Präanalytik statistisch ausgewertet (Stata/SE 17.0).

Ergebnisse: In die vergleichenden Auswertungen konnten jeweils 116 Probenpaare einbezogen werden. Die Zeitspanne zwischen Blutabnahme und Eintreffen der Proben im Epi-Lab betrug im Mittel 4,5 Tage (Spannbreite 1 - 7 Tage). Zwischen gekühlten und ungekühlten zentrifugierten Proben fielen die Abweichungen der Messwerte insgesamt am geringsten aus (QMDM-Durchschnittswert 6,8%; Spannbreite 0,6% [Na] – 49,4% [SX1]). Zwischen zentrifugierten und nicht zentrifugierten gekühlten Proben lagen die Messwerte deutlich weiter auseinander (QMDM-Durchschnittswert 14,0%; Spannbreite 1,7% [Ca] – 119,5% [Kalium]). Ohne Kühlung und ohne Zentrifugation waren die Abweichungen am größten (QMDM-Durchschnittswert 29,0%; Spannbreite 3,1% [Alkalische Phosphatase] – 303,9% [anorganisches Phosphat]). Die Ergebnisse werden im Detail dargestellt und bewertet.

Diskussion und Schlussfolgerung: Für epidemiologische Studien unter erschwerten Feldbedingungen können – je nach Fragestellung – bei einzelnen Analyten Kompromisse in der Präanalytik akzeptabel sein, insbesondere bei der Kühlkette.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Central laboratory measurements in the multicentric German National Cohort (GNC) – feasibility, quality assurance and baseline results

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Introduction: The German National Cohort (GNC) is the largest multi-centric, population-based cohort study in Germany. Baseline examinations were performed between 2014 and 2019 including altogether more than 205,000 adult participants from 18 study centres located throughout Germany. Clearly defined and selected laboratory analyses in fresh blood samples were performed either in the central study laboratory (University Medicine Greifswald) or in local medical laboratories. We report on the pilot phase assessing the suitability of a central study laboratory with prolonged sample transportation. Further, we report on the methods and results of quality assurance in the central study laboratory.

Methods: The suitability of a central study laboratory was tested at the beginning of the ongoing baseline examination in a pilot phase. During this pilot phase, the measurand stability according to sample age, storage temperature, and laboratory methods was evaluated. For this, 33 measurands were quantified from venous blood samples obtained from 10 healthy volunteers directly after centrifugation and at 24-hour intervals up to 72 hours at different storage conditions. Moreover, 18 measurands were quantified from blood samples obtained from 267 GNC participants of 10 study centres in the cooperating local and central study laboratory.

Results: The experiments in the pilot phase showed that the GNC measurands in the central study laboratory are stable over at least 72 h. The mean percentage changes after 24-72 h compared to the immediate measurement were low under both temperature conditions and within the acceptable ranges of measurement uncertainty as defined by the Rili-BAEK or the reference change value. In addition, the comparison of the results for GNC samples from local and central measurement demonstrated strong laboratory effects, that clearly exceeded the effects of sample aging.

Conclusion: In the multicentric GNC study the unifying positive effects of a central laboratory, i.e. use of designated laboratory methods and platforms as well as standardized sample handling and documentation, outweigh the effects of longer sample transportation time. The centrally implemented quality control procedures during and after data collection, including a comparison of measurement results with data from medical care, confirmed that the centrally measured data fulfills highest standards regarding analytic quality and can be applied in medical research.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

A standardized approach for quality assurance in NMR metabolomics: The NMR Alliance

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Background

Ensuring the quality of scientific data goes beyond the individual user and encompasses the entire process from sampling to data reporting. A standardized procedure promotes transparency of the entire analytical workflow, ensures method repeatability and validates the plausibility of the reported data. For establishing high analytical standards in a multi-center setting, data beyond the measurement results of the quality controls were taken into account. This approach allows insights into the behavior of the highly complex devices and lay the foundations for forward-looking interventions. To address challenges of highly complex NMR spectra data measured at multiple sites, we established an analytical approach based on the main principles established in the guidelines of the

Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations – Rili-BAEK. This approach supports the daily exchange of relevant quality control data between participants in the research network, facilitating easy access and standardization.

Material and Methods

Extended data for quality controls and several other parameters were collected daily for each NMR device in the participating laboratories of the NMR alliance and shared on a common server, located in Oldenburg. R Software for Statistical Computing in conjunction with the Shiny framework was used for on-demand data analysis, visualization and reporting. Levey-Jennings Control charts were used to visualize the QC data and descriptive statistics were calculated for each instrument and parameter. The parameters included physical parameters like temperature and line width as well as measurement results from biological pool samples. Furthermore, the actual instrumental status based on predefined limits for each quality control parameter was given, i.e., if the limits are passed, not passed or QC parameters were not determined. Finally, unsuccessful quality controls can be documented and downtimes, e.g. due to maintenance or repairs, can be labelled.

Results and Discussion

We developed a multicenter QC monitoring system for research instruments according to standards existing in medical laboratory information systems and beyond. By hosting the data on a web server, we create the possibility to exchange QC data and the highest possible level of transparency in real time, because all participations of the network has access to all data very time.

Conclusion

In this study, we build up an infrastructure and create a streamlined workflow to standardize the quality control procedures over multiple laboratories. This includes the development of standard operation procedures for data management and analysis.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Wie genau ist die quantitative Analyse von β2-Transferrin mittels Hydrasis 2 Scan Focusing (Sebia) zur Detektion von CSF Leckagen?

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Zielsetzung

β2-Transferrin, die desialisierte Form des Eisentransporters Tranferrin wird fast ausschließlich im Liquorraum synthetisiert, was es ermöglicht β2-Transferrin als Marker einer Liquorrhoe einzusetzen. Die quantitative Analyse von β2-Transferrin mittels Hydrasis 2 Scan Focusing ist vom Hersteller (Sebia) als "Hilfsmittel" zur Detektion einer CSF Leckage deklariert, ohne jedoch weitere Leistungsdaten der Methode oder Spezifikationen zu deren Verlässlichkeit anzugeben. Ziel dieser Studie ist es, eine erste Einschätzung der Zuverlässigkeit und Eignung dieser quantitativen β2-Transferrin Bestimmung zur Detektion einer Liquorrhoe zu erbringen.

Methoden

Berechnung von Variationkoeffizienten (VKs) der mittels Hydrasis 2 Scan Focusing gemessenen asialo- und disialo-Fraktionen des Transferrins für Liquor, Sekret und Blutproben, sowie die Abschätzung der Auswirkung der ermittelten VKs auf die für die quantitative Analyse benötigten asialo/disialo-Fraktion Quotienten. Abschätzen einer für die Differenzierung von negativen und positiven Proben benötigten "Minimal Distance" der berechneten asialo/disialo-Fraktion Quotienten bzw. der damit verbundenen cut-off Werte.

Ergebnisse

Abhängig vom absoluten Anteil der asialo bzw. disialo-Transferrin Fraktionen können die VKs der einzelnen Transferrin Fraktionen >20% sein. Ausgehend von einem VK von 20% für schwach vorhandene Transferrin Fraktionen, ergibt sich somit für den vom Hersteller angegebenen cut-off von >1 (asialo/disialo Quotient) für eine positive Bewertung von Sekretproben ein "Graubereich" von 0.7 bis 1.5 bzw. eine "Minimal Distance" von >40% für die ermittelten asialo- und diasialo-Fraktionen.

Diskussion und Schlussfolgerung

Bei qualitativ (d.h. optisch) schwierig zu bewertenden Fällen mit niedriger Konzentration von asialo/disialo Transferrin in den jeweiligen Proben, kommt es bei der quantitativen Analyse zu hohen VKs und daraus resultierend zu starken Unsicherheiten bei der quantitativen Bewertung von β2-Transferrin. Die vom Hersteller angegebenen cut-off Werte zu den quantitativ ermittelten asialo/disialo Quotienten vermitteln dem Anwender letztlich eine falsche Sicherheit, da hier die teilweise erhebliche Messunsicherheit nicht berücksichtigt wurde.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

IVDCheckR - Simplifying documentation for laboratory developed tests according to IVDR requirements by introducing a digital tool

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Introduction: A recent challenge confronting clinical laboratories is the lack of clear guidelines for handling significant modifications to CE-marked assays. The modifications may involve for example, extending measurement intervals, changing dilution procedures or using non-validated sample materials. The challenge arises due to the amended Regulation EU 2017/746 on in vitro diagnostic medical devices (IVDR) which is now poised for implementation, despite the extended transition period. The IVDR application imposes challenges not only for diagnostic companies but also for clinical laboratories when using laboratory developed tests (LDT), often referred to as in-house assays. In this context, a coherent and meticulously structured LDT documentation is highly beneficial. While laboratories are obliged to meet the IVDR requirements, the absence of a streamlined framework or guideline hampers the ability to gain a comprehensive overview on the requirements and possible options for their fulfilment.

Methods: To address this issue, we introduce a web based digital tool powered by an R Shiny web application. This tool will facilitate the seamless implementation of IVDR requirements for LDTs across diverse laboratory environments in terms of their transparency and validity. Furthermore, our approach focuses on adequate handling of significant modifications of CE-marked in vitro diagnostic medical devices (IVD).

Results: We developed an open-source tool that is easily accessible and free from system dependencies. The tool promotes a seamless process and a guide to enhance transparency, reliability, and validity of laboratory examination results based on LDTs. Additionally, the tool further provides a convenient and quick solution for routine clinical laboratory calculations such as method comparison and quality control assessment. These solutions can also be extended in the future to address significant modifications in CE-marked assays effectively.

Conclusion: Our Shiny web application-based platform is a digitised, user-friendly tool that incorporates the IVDR requirements to enhance transparency, reliability, and credibility of clinical laboratory examinations based on LDTs.

DGKL: 19. Quality Assurance, Accreditation, Preanalytics, Laboratory Management

Establishing hemolysis index thresholds for eighteen selected routine biochemistry assays in accordance with current IVDR guidelines

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Instroduction: Hemolysis in laboratory samples is one of the most frequent preanalytical errors and bears the risk of clinical misinterpretation of test results and consequently false patient treatment. To prevent impaired results, manufacturers provide hemolysis index (HI) acceptance limits. In some cases, these thresholds do not meet the clinical requirements. However, when laboratories deviate from the manufacturers' instructions, a comprehensive validation as a laboratory developed test (LDT) is necessary to comply with current IVDR guidelines. In this study, we evaluated 18 routine biochemistry assays to verify and improve HI threshold values.

Methods: Blood samples were collected from apparently healthy voluntary participants (n=77). By using lithium heparin plasma and sedimented erythrocytes from each respective sample hemolyzed by freezing at -80°C, dilution series were prepared with defined degrees of hemolysis between 4 mg/dl to 167 mg/dl. In each dilution eighteen common clinical measurands namely sodium, potassium, chloride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), haptoglobin, total bilirubin, direct bilirubin, glutamate dehydrogenase (GLDH), creatine kinase (CK), iron, amylase, phosphate, total protein, enzymatic creatinine and high-sensitive troponin T were measured on the COBASÒ PRO SYSTEM (Roche Diagnostics, Mannheim, Germany). For each measurand distribution of results was depicted as box plots and regression plots. The interference over increasing HI indices was analyzed by Passing and Bablok regression. Across the dilution series cut-off HI thresholds were determined for deviation of measurement results over increasing hemolysis.

Results: Starting from the undiluted sample, HI thresholds for maximum errors of 5, 10, and 20% were determined. Furthermore, cut-off HI values were calculated as median and 5th percentile of the HI thresholds per subject/patient. Using potassium as an example we found HI thresholds at 67 (5th percentile) and 75 (median) with a corresponding error of 5% while the manufacturer HI threshold is 20. Therefore, a higher HI threshold might be tolerated. Overall, for some parameters there was a good agreement between the thresholds from the manufacturer and our findings. However, in some cases a clinically relevant deviation was found.

Conclusion: We established HI thresholds for 18 selected biochemistry assays. Some of our HI values deviated considerably from the manufacturer's recommendations. Optimizing thresholds can help laboratories and patients in both, reducing the risk of unnecessary holding back of results and preventing wrong results from being released. Thus, our study assists in finding clinically useful cutoffs and validate them according to IVDR guidelines.

DVTA

Evaluating the response to oxidative stress and the generation of reactive oxygen species in ABCC6-deficient human mesenchymal stem cells

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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. Furthermore, signs of oxidative stress have been reported in the serum of PXE patients and in PXE fibroblasts. Due to the possible role of p21 in the aging process of stem cells, followed by a loss of regenerative potential, human mesenchymal stem cells (hMSCs) were chosen for these investigations. An altered balance of ROS formation and degradation may contribute to the SASP of fibroblasts derived from PXE patients.

To induce cellular aging, wildtype and knockout-hMSCs were incubated with 1 mM H2O2 for different periods of time 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. In addition, the mRNA expression of ABCC6 was determined by qRT-PCR. Moreover, staining of different types of reactive oxygen species and nitic oxide was performed using various molecular probes and the influence of an ABCC6-deficiency on senescence was evaluated.

The β-galactosidase activity of the wildtype hMSCs increased by 50% following treatment with H2O2 for 1 h with elevated mRNA expression of p21 and senescence-associated cytokines IL1β and IL8 further confirming the senescent phenotype. The immunostaining of p21 revealed an increase in the portion of p21-positive cells to 100%. The mRNA expression of ABCC6 significantly increased up to 10-fold. An RNP-based CRISPR/Cas9-approach was used to introduce an ABCC6-deficiency in hMSCs. Elevated β-galactosidase-activity was detected in knockout-hMSCs. In addition, ABCC6-deficient hMSCs required a longer incubation with 1 mM H2O2 in order to show increased β-galactosidase-activity. Our preliminary data show an influence of ABCC6-deficiency on ROS production in hMSCs whereas an altered senescence-associated phenotype in ABCC6-deficient hMSCs still needs to be elucidated.

The elevated mRNA expression of ABCC6 in hMSCs following treatment with H2O2 indicates an association of ABCC6 with the process of cellular senescence and oxidative stress. Knockout of ABCC6 confirms this hypothesis by showing signs of an induced senescent phenotype along with a partial insensitivity to H2O2.The pathomechanistic link between ABCC6 and senescence will be investigated further by evaluation of markers of senescence and oxidative stress.

DVTA

Can microplastic particles be identified in different medical matrices?

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Background: Interest in microplastics has grown dramatically with the first discovery in 2018 in human stool. More and more research groups are working on microplastics. As these plastic particles continue to decompose, they can penetrate the cell membrane and accumulate, triggering reactions in the body. In this study different medical matrices were qualitatively analyzed for microplastic.

Methods: First, samples were analyzed by a FACS device (Co. PARTEC, Germany) and fluorescence microscopy (Co. Keyence, Germany). Subsequently for Raman spectroscopy (Co. HORIBA, Japan), the proteins underwent KOH digestion and Tween20 treatment. After filtration by silicon membranes the liquid samples were analyzed for microplastic particles using the software particle finder.

Results: In samples from synovial fluid of artificial joints microplastic particles (diameter < 10 µm) could be detected and identified as polyethylene and polystyrene by Raman spectroscopy. In addition, in samples from aquarium water particles (diameter < 10 µm) could be identified as silicone derivates.

Conclusion: By the applied detection procedure of microplastic it was possible to identify microplastic particles with a diameter < 10 µm and determine the chemical composition in different medical matrices.

DVTA

The role of macrophages in pathogenesis of Pseudoxanthoma elasticum

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Background: Macrophages are pivotal players in initiating immunity and driving inflammation.

With increasing age, a shift in macrophage population towards inflammatory M1-phenotype can be seen contributing to low-grade inflammation. Low-grade inflammation is seen age-related diseases such as pseudoxanthoma elasticum (PXE), an autosomal-recessive disorder, caused by mutations in the gene encoding the ATP-binding cassette subfamily C member 6 (ABCC6). Clinically, PXE patients show characteristics of the elderly, like arteriosclerosis, loss of skin elasticity and retinal degeneration. Inflammatory processes in PXE are already described by high expression of inflammatory markers as interleukin-6 (IL-6) in dermal fibroblasts (PXEF) and in sera of PXE patients. The precise role of immune cells in PXE pathogenesis, particularly macrophages, remains incompletely understood. Thus, this study was designed to evaluate the role of macrophages in inflammatory processes in PXE pathogenesis.

Methods: Human monocytes from healthy donors (n = 3) were isolated from buffy coats and cultivated in presence of macrophage colony stimulating factor. After five days, the differentiated inactive M0-macrophages were treated with small interfering RNA targeting ABCC6 (siABCC6M) or with non-targeting siRNA (siKonM). On the sixth day, the siABCC6M and the siKonM were polarized by adding interferon-γ and lipopolysaccharide (M1-phenotype) or IL-4 (M2-phenotype) to the culture medium. Furthermore, we investigated the impact of the PXEF secretome on the phenotype of macrophages derived from healthy donors through co-cultivation experiments. To assess macrophage phenotype, we analyzed a specific set of M1- and M2-markers using quantitative real-time polymerase chain reaction and enzyme-linked immunosorbent assay.

Results: We observed a significant upregulation of M1-markers in M0-siABCC6M compared to the M0-siKonM, accompanied by a decrease of M2-markers at the mRNA level. Following inflammatory polarization towards M1-phenotype, an ABCC6-knockdown results in significant upregulation of M1-marker at both gene expression level and on protein level. Additionally, co-cultivation with PXEF resulted in a significant increase in M1-markers and a decrease in M2-markers at mRNA-level and protein level.

Conclusions: Our findings suggest that an ABCC6-knockdown induce a shift in the M1/M2 macrophage balance towards the inflammatory M1-phenotype. Additionally, we observed an upregulation of M1-marker and a downregulation of M2-markers in macrophages co-cultivated with PXEF. These results indicates that PXEF secretome activates macrophages towards an inflammatory M1-phenotype. Therefore, communication between macrophages and fibroblasts could drive local inflammation. In conclusion, macrophages seem to play a role in PXE pathogenesis but further investigations on the molecular level are necessary for better understanding their role in PXE.

DVTA

The influence of a ABCC6 deficiency on inflammatory processes in human dermal fibroblasts

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Background: Pseudoxanthoma elasticum (PXE, OMIM #264800) is an autosomal recessive disorder which is mainly caused by mutations in the gene encoding the ATP-binding cassette sub-family C member 6 (ABCC6). Recent studies show the development of senescence in human dermal fibroblasts derived from PXE patients. This is accompanied by a senescence-associated phenotype (SASP), which is characterized by increased expression of proinflammatory cytokines. These findings suggest a chronic, sterile inflammation process in PXE. The aim of this study was to analyze the regulation of different inflammatory processes in dermal fibroblasts and the effects of a treatment with the glucocorticoid prednisolone (PSL).

Methods: Normal human dermal fibroblasts (NHDF, n=4) and fibroblasts from PXE patients (PXEF, n=4) were seeded with a final density of 177 cells/mm2. Medium was changed after 24 h to medium with lipoprotein-deficient serum and 10 nM PSL, the medium was renewed every 3 to 4 days. After 72 h or 21 d of cultivation time, respectively, the effects of the treatment on senescence, the SASP, different factors of the complement system and the NF-κB and JAK-STAT3 signaling pathway were analyzed.

Results: Our experiments confirmed an overexpression of proinflammatory cytokines, like interleukin-6 (IL6) and monocyte chemoattractant protein-1, on gene expression and protein level in PXEF, which could be reduced by PSL treatment. Treatment with PSL decreased senescence-associated β-galactosidase activity in PXEF by 40 % after 21 d. However, treatment with PSL showed no clear effect on the expression of complement factors, which are overexpressed in PXEF. Furthermore, it was confirmed that the JAK-STAT3 and the NF-κB signaling pathway are basally active in PXEF and can be inhibited by PSL. The basal fluorescence intensity of RelA and pSTAT3, active dimers of NF-κB/JAK-STAT3, in the nucleus of PXEF were 2.0-fold and 4.3-fold increased in PXEF compared to NHDF. PSL treatment resulted in a significant reduction of the RelA and pSTAT3 fluorescence signals about 38 % and 51.5 % in PXEF.

Conclusion: Our data indicate high expression of inflammatory factors and high activity of the complement system in PXEF. In general, overexpression of C3 can activate inflammatory signaling pathways such as JAK-STAT3. We show that this pathway is basally active in PXEF and can be inhibited by PSL. Since PSL treatment inhibits the NF-κB pathway and thus inhibits, among other things, the IL6 synthesis, this could lead to a reduced activation of JAK-STAT3.

As a result of the basal activation of NF-κB shown here, cellular senescence, SASP and inflammation are promoted. Thus, an association between PXE and the NF-κB pathway can be suspected. In summary, the results of this work support the presence of a chronic, sterile inflammation in PXE pathogenesis. Further investigations on the inflammatory processes are necessary for understanding the role of inflammation in PXE.

DVTA

Drogensituation in Deutschland - Nutzen einer systematischen Auswertung von Labordaten in einem toxikologischen Labor

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Drogenmissbrauch ist ein Problem in Deutschland. Der Drogenkonsum der Deutschen Bevölkerung wird aktuell über Befragungen (ESA Befragung, PREMOS Studie) und Abwasseruntersuchen dargestellt. Aktuelle Trends und neue Drogen lassen sich so nur mit einer zeitlichen Verzögerung feststellen. In dieser Arbeit soll geklärt werden, ob eine systematische Auswertung von Labordaten aus einem toxikologischen Labor eine sinnvolle Ergänzung zu den etablierten Methoden sein kann.

Es wurden die Daten von 3.809 Kapillarblutproben auf folgende Basisparameter ausgewertet: Geschlecht, Alter, Bundesland, Art der Substitution. Im Anschluss wurden alle untersuchten Substanzen entsprechend ihrer Wirkung jeweils einem Toxidrom zugeordnet und die untersuchten Datensätze dementsprechend zugeordnet.

Als letzten Schritt wurden die Basisparameter und die Daten aus der Toxidromanalyse zusammengeführt und im Hinblick aufeinander ausgewertet.

77 % der Proben waren von männlichen und 23 % von weiblichen Patienten. Männer konsumierten häufiger Substanzen aus dem Alkohol und dem Cannabinoid Syndrom (statistisch signifikant) und Frauen häufiger Substanzen aus dem Anticholinergen Syndrom (z.B Triciyclische Antidepressiva). 70 %der untersuchten Patienten waren jünger als 50 Jahre. Jüngere Patienten konsumierten statistisch signifikant häufiger Substanzen aus dem Sympathomimetischen Syndrom und dem Sedativen Syndrom. In Proben von älteren Patienten wurden signifikant häufiger Substanzen aus dem Anticholinergen Syndrom gefunden.

In 61 % der Proben konnte Methadon nachgewiesen werden und in 24 % Buprenorphin. In den Proben in denen Buprenorphin nachgewiesen wurde, konnten statistisch signifikant weniger Beigebrauch nachgewiesen werden.

In 47 % der Proben waren mindestens zwei Substanzen aus unterschiedlichen Toxidromgruppen positiv. Substanzen aus dem Alkohol und Cannabinoid Syndrom waren signifikant am häufigsten ohne weiteren Beikonsum. Substanzen aus dem Sedativen Syndrom waren signifikant häufiger in Proben mit auch Substanzen aus anderen Toxidromgruppen nachweisbar. Auffällig war auch ein statistisch signifikant häufigeres gemeinsames Auftreten von Substanzen aus dem Sympathomimetischen Syndrom und dem Opioid Syndrom.

Im direkten Vergleich mit der ESA-Befragung und der PREMOS Studie, sowie der Europäischen Abwasseruntersuchung konnte gezeigt werden, dass in der vorliegenden Arbeit viele Ergebnisse wiedergespiegelt werden konnte.

Zusammengefasst lässt sich sagen, dass eine standardisierte Auswertung von Daten aus toxikologischen Laboren als Instrument genutzt werden kann, um die aktuelle Drogensituation in Deutschland widerzuspiegeln. Weitere positive Punkte sind zum einen, dass keine Befragungen oder Untersuchungen anberaumt werden müssen und dadurch sowohl Zeit, als auch Kosten gespart werden und zum anderen sind die Daten immer hoch aktuell. Dadurch können sie als Frühwarnsystem dienen und Änderungen auf dem Drogenmarkt oder im Konsumverhalten schnell aufdecken.

DVTA

Characterization of Cardiac Fibroblast Extracellular Matrix Metabolism in the Context of Dilated Cardiomyopathy

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Introduction

Dilated cardiomyopathy (DCM) represents a multifactorial disorder characterized by progressive enlargement of the ventricles and impaired systolic function, leading to heart failure and an increased risk of sudden cardiac death. Despite advancements in medical science, the etiology of DCM remains elusive, involving a combination of various risk factors. Understanding the pathophysiological mechanisms underlying DCM is crucial for developing effective diagnostic and therapeutic strategies. Growing evidence implicates a decisive role of inflammation and immune dysregulation in the pathogenesis of DCM. In this context, damage-associated molecular patterns (DAMPs) are significant in initiating and perpetuating inflammation in response to myocardial injury. The role of DAMPs in cardiac remodeling has almost exclusively been explored within the murine system. Human data is lacking. For this reason, we aim to develop a human in vitro model for cardiac fibroblast (HCF) action in cardiac fibrosis using isolated primary cardiac fibroblasts to assess relevant signal transduction pathways.

Methods

HCFs were isolated from digested tissue through selective adherence to collagen-coated cell culture flasks. Purity was assessed using a variety of positive and negative markers identified by in silico analysis. Maker expression was tracked over four consecutive population dumplings using immunostaining and flow cytometry. In silico analysis of single-cell mRNA-sequencing data initially published by Litviňuková et al. was used to assess initial targets of interest. Effects of treatment with human recombinant DAMPs were assessed using quantitative real-time polymerase chain reaction.

Results

After isolation, initial contamination with 15% endothelial cells decreased to below 1% in the 3rd passage of cultivation. Isolated HCFs were collagen type-I-, vimentin-, CD90-, and platelet-derived growth factor alpha positive. In silico analysis revealed the participation of the DAMPs tenascin-C and fibronectin-extra domain A in developing cardiac fibrosis. Treatment of HCFs with recombinant human DAMPs induces a profibrotic myofibroblast-like phenotype showing increased expression of alpha-smooth muscle actin, collagen, and various proteoglycans.

Conclusion

This implies endogenous DAMP signaling via Toll-like receptors contributes to developing cardiac fibrosis. Analysis revealed dysregulation of the proteoglycan metabolism and collagen biosynthesis, proving the direct involvement of DAMP-mediated signaling in the pathogenesis of human cardiac fibrosis. The model established in this project will be used further to investigate possible pharmacological agents for inhibiting DAMP signaling in the context of cardiac fibrosis. Moreover, this model will be used to identify pathological changes in the glycobiom of cardiac fibroblasts during cardiac fibrosis to elucidate the role of proteoglycans in the pathogenesis of cardiac fibrosis.

DVTA

Automatisierte Diagnostik hereditärer Thrombozytopathien auf Basis phänotypischer Eigenschaften am Beispiel der Thrombasthenie Glanzmann

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Einleitung:

Die Thrombasthenie Glanzmann (Th.G.) gehört zu den autosomal-rezessiven Thrombozytopathien, welche sich durch einen qualitativen oder quantitativen Rezeptordefekt des Glykoproteinkomplex IIb/IIIa auszeichnet und sich klinisch durch eine Blutungsneigung präsentiert (Selleng und Greinacher, 2010).

Aufgrund der Seltenheit und der erforderlichen speziellen labordiagnostischen Verfahren stellt die Diagnostik der hereditären Thrombozytopathien eine Herausforderung in der klinischen Praxis dar (Althaus et al. 2019).

Zur Diagnostik eignen sich u.a. immunfluoreszenzgefärbte Blutausstriche (Greinacher et al. 2017). Ziel der Arbeit ist es, ein optimiertes Standardverfahren zur automatisierten Mikroskopie immunfluoreszenzgefärbter Thrombozyten anhand von Blutausstrichen zu etablieren, welches mit der Durchflusszytometrie vergleichbare Ergebnisse liefert.

Material, Methoden:

Blutausstriche werden fixiert, Antikörper aufgetragen, gefolgt von Waschschritten.

Zur sicheren Thrombozytendetektion werden Doppelfärbungen mit verschiedenen Fluorophoren angewendet. Als Detektionsantikörper dienen u.a. Anti-NMMHCIIa und CD63. Die mikroskopische Analyse findet am Lionheart LX statt.

Ergebnisse:

Die Auswertung erfolgt automatisiert durch speziell entwickelte Protokolle im Softwareprogramm Gen5. Es wird eine festgelegte Anzahl an Gesichtsfeldern, an definierten Stellen mikroskopiert und aufgezeichnet. Durch Subpopulationsanalyse werden nur im definierten Größenbereich liegenden Ereignisse mit einer Doppelfluoreszenz größer gleich dem Threshold im Hinblick auf Signalintensität und Größe ausgewertet. Die Auswertung erfolgt im Vergleich zur Kontrolle. Referenzwerte wurden anhand einer Kontrollgruppe ermittelt und bildeten die Basis der Cut-Off-Wert- Bestimmung.

Für die Th.G.-Diagnostik wurden Blutausstriche zweier Patienten ausgewertet. Dabei wiesen die durch CD63 detektierten Thrombozyten der Patienten unter Verwendung von CD41 und CD61 keine spezifischen Signale auf der thrombozytären Oberfläche auf. Somit kann das Fehlen des GPIIb/IIIa-Rezeptors angenommen werden. Granula und Thrombozytengröße entsprachen der Norm.

Diskussion:

Die Ergebnisse deckten sich mit denen der Durchflusszytometrie und Molekulargenetik. Die Diagnostik einer homozygoten Th.G. anhand der Immunfluoreszenzmikroskopie von Blutausstrichen wurde durch die automatisierte Auswertung optimiert. Die quantitativen Ergebnisse dieses Verfahren sind der Durchflusszytometrie ebenbürtig. Mit Ausnahme der morphologischen Befundung der Granula ist diese Methode nutzerunabhängig und erfüllt die Gütekriterien: Reliabilität, Objektivität und Validität.

Take-home-message:

Die Immunfluoreszenzfärbung von Thrombozyten anhand von Blutausstrichen ermöglicht eine zuverlässige Diagnose der Thrombasthenie Glanzmann. Durch Protokolloptimierung und die automatisierte Analyse, bietet das Verfahren eine objektive, nutzerunabhängige Befundung und stellt dadurch eine Referenzmethode zur Durchflusszytometrie dar.