Biochemia Medica

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ABSTRACT BOOK

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Contents

11th Congress of the Croatian Society of Medical Biochemistry and Laboratory Medicine with international participation

Vodice, Croatia, 9-12 October 2024

Organizer
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s međunarodnim sudjelovanjem

9.-12. listopada 2024.
Hotel OLYMPIA, Vodice

Pre-congress symposium

Patient results: possibilities and potentials in contemporary laboratory settings

PCS-1

Optimization of the continuous control model using patients results – The experience of a large laboratory

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The limitations of traditional internal quality controls, which rely on stabilized control materials, are becoming more evident. These materials often do not behave like actual patient samples, leading to issues with commutability that hinder error detection and may trigger false alarms. Internal quality controls already account for a significant portion of laboratory costs in many countries, yet the frequency of these controls remains insufficient to reliably catch many errors, such as lot-to-lot shifts, temporary assay failures, or the rapid emergence of critical errors.

As a result, patient-based quality control methods are gaining attention as a complement to traditional approaches. These methods typically calculate a mean or median from patient results, with values exceeding predefined control limits indicating a potential out-of-control situation. Because they are directly based on patient results, they avoid commutability issues and reduce the need for expensive control materials. Additionally, these methods can operate in real-time, earning the name patient-based real-time quality control (PBRTQC).

Optimizing PBRTQC involves several parameters, such as block size, truncation limits (TL), and control limits (CL). The block size defines the number of patient results used for each PBRTQC calculation, and extreme values are often excluded or minimized by applying TLs. Patient-based real-time quality control can operate in different modes; in batch-mode testing, metrics are recalculated once enough samples are available to meet the block size, with each patient result influencing only one calculation.

Patient-based real-time quality control optimization can be aided by computer simulations. Calculating control limits using "percentiles of daily extremes" has been shown to yield effective limits, controlling the rate of false alarms. However, changes in measurement distribution can increase the frequency of false alarms. Box-Cox transformations, rather than logarithmic ones, improve error detection, and Winsorization of outliers tends to perform better than simple outlier removal. A wide variation in patient measurements can hinder error detection.

More sophisticated PBRTQC frameworks can also mathematically remove seasonality, minor shifts due to reagent lot changes, calibration, and other subtle changes in the analytical process, focusing on clinically significant errors.

Looking forward, PBRTQC can also play a role in External Quality Control. Programs like the Noklus Patient Median (NOPAM) monitor analyte- and instrument-specific trends to detect errors. Other programs combine frequent, metrological traceable ring trials with PBRTQC.

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PCS-2

Application of moving average procedures for continuous quality control in small laboratories

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Quality control (QC) is one of the fundamental principles of analytical work in medical laboratories, ensuring the issuance of accurate patient results. Traditionally, QC is carried out by analyzing commercially available control samples at specific intervals. The drawbacks of this approach include intermittency and non-commutability. Therefore, in modern laboratory practice, additional tools are sought to compensate for the shortcomings of traditional QC. To this end, the concept of continuous patient-based real-time quality control (PBRTQC) has been introduced. One of the methods for implementing PBRTQC is the moving average (MA).
Moving average involves calculating the average value of an analyte from a series of patient results and using this value for control purposes. Data is continuously updated as patient samples are analyzed, making the average value "moving." The main advantages of MA control over traditional control are its continuity and the elimination of the non-commutability problem. However, there are challenges associated with MA control. Firstly, defining optimal MA procedures for each analyte in each laboratory is a complex task. Additionally, MA quality control imposes significantly higher demands on the laboratory information system (LIS) compared to traditional control, as it requires complex real-time manipulation of patient results. Finally, there is the question of MA procedures' ability to detect bias in less frequently requested tests and in laboratories with a small daily number of samples.
Given these challenges, in the laboratory of the Railway Healthcare Institute, we conducted a study aimed at applying MA procedures as an additional QC tool for analytical work on clinical chemistry analytes in a medical laboratory with a small daily testing volume. In this context, it was necessary to: select, optimize, and validate MA procedures for ten clinical chemistry analytes, implement MA procedures in the LIS and apply them in real-time in the laboratory's routine work, and develop an algorithm for action if MA control is outside the expected limits. The study was designed as a retrospective analysis of data from the LIS and internal and external QC data for ten clinical chemistry analytes: albumin, AST, calcium, chlorides, cholesterol, creatinine, HDL-cholesterol, potassium, sodium, and total protein. The selection and validation of MA procedures were carried out using a bias detection simulation method. Simple MA procedures were chosen as optimal for albumin, AST, calcium, chlorides, cholesterol, HDL-cholesterol, and sodium, while exponentially weighted moving average (EWMA) procedures were selected for creatinine, potassium, and total protein. The optimized MA procedures were then implemented in the LIS, which initially did not support PBRTQC. At our request, certain software adjustments were made, allowing the user to enter all MA procedure parameters into the LIS for each laboratory test. The performance of MA procedures in routine work was monitored over six months by analyzing MA alarms generated by the LIS when control limits were exceeded. An algorithm was designed and successfully applied for handling MA alarms, which included reviewing patient results by a biochemist, re-analyzing patient samples from a stable period, analyzing internal QC samples, and checking analyzer malfunctions and maintenance records. This study demonstrated that even in a medical laboratory with a small daily testing volume, it is possible to successfully implement patient-based control procedures in the laboratory's routine work, thereby strengthening the QC plan and further improving the quality of issued laboratory results.

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PCS-3

Patient-based external quality assessment in medical laboratories

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The comparability of the results of laboratory measurements, independent of the place and measurement system is a necessary prerequisite in providing adequate health care for each patient. The mobility of patients and the need to be diagnostically processed and treated in institutions of different levels of health care (primary, secondary and tertiary) indicate the need to ensure and verify the comparability of measurement results, expecting an unambiguous clinical response. The accuracy and comparability can be assumed by laboratories using CE-marked systems (calibrator, reagent and device from the same manufacturer), as the manufacturers of in vitro diagnostic medical devices are responsible for the traceability of the analytical measurement methods used. The laboratories, in turn, must verify the manufacturers' alignment process and monitor the declared performance claims of the measurement procedures during routine operations.
Continuous monitoring of internal quality control (IQC) procedures and participation in external quality assessment (EQA) schemes provide laboratories with objective information on the quality of the results released. However, the processed materials used in EQA schemes, which require large quantities of control material with challenging concentrations of many analytes, present a major obstacle to assessing accuracy and harmonisation because of commutability issues. Under these circumstances, EQA organisers pool results from laboratories using the same equipment and the evaluation is limited to a peer group assessment. This evaluation gives laboratories feedback on how they perform compared to others using the same equipment and reagents. It also informs manufacturers on how well their tests are performing globally. However, it does not provide information on the standardisation or interchangeability of test results between different laboratories.
With the common goal of assessing the comparability of assays used in medical laboratories, the bottom-up approach was proposed by Thienpont and Stockl's team in the Percentiler program, part of the "Empower" project. The program focused on comparing patient results between laboratories using daily laboratory test medians for comparison to avoid the impact of the commutability of processed materials on the comparability of laboratory results. Laboratories were able to compare their moving medians over time, providing information on both the stability of individual instruments and the comparability of patient results across the group of laboratories. In 2024, the Noklus Patient Median (NOPAM) was launched, replacing the Percentiler program and providing a free, patient-based EQA program that enables data gathering from different laboratories and measurement procedures. The underlying concept remains the same: patient medians are typically stable over time, and any change is usually due to pre-analytical or analytical instability or error. The information provided to participating laboratories can be used to monitor the stability of results over time and the comparability of individual instruments in the respective instrument peer groups and overall. Participation in the programs can be seen as an integrated tool for modern quality management, providing information on the comparability and performance stability of measurement procedures used in medical laboratories.

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PCS-4

Challenges in establishing indirect reference intervals

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Reference intervals (RIs) are the most common decision support tool used to interpret numerical laboratory test results, ideally allowing distinction of healthy and unhealthy individuals. In general, laboratories are responsible for either verifying RIs established by an external source or determining their own. The traditional direct approach for establishing RIs is well defined in CLSI EP28-A3c guideline Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. It is based on collecting samples from the selected reference population (a priori or posteriori), followed by analysis and calculation. The main obstacle lies in selecting a sufficient number (> 120) of healthy subjects for reference population for each subgroup (for example by age or sex). Alternatively, indirect methods of establishing RIs are using routine data stored in the laboratory information system. Indirect methods are faster, cheaper, more convenient and utilize large numbers of data (hundreds, thousands or even millions per group). Key disadvantages of indirect approach can be found in sample filtering and method of calculation. When selecting subjects, the main premise is not to include only healthy subjects, rather to minimize the effect of diseased subjects. Multiple statistical techniques are available for calculating the interval, such as standard statistics (parametric and non-parametric as described by CLSI), traditional methods (Bhattacharya and Hoffman) and modern algorithms (kosmic, refineR). There is no consensus what is optimal approach to minimise effect of diseased subjects nor the best statistical tool to perform calculation. Efforts in this field are continuous and hopefully, the new CLSI guideline EP45 Implementation of Reference Intervals in the Medical Laboratory should define minimal standards for indirect RIs.
Androgens have significant importance in women's reproductive health as they play a key role in normal ovarian function and fertility. Assessment of androgen production in women usually includes testosterone, dehydroepiandrostenedione sulphate (DHEAS) and androstenedione. As production declines from reproductive age towards menopause, there is a need for age specific RIs for more precise interpretation of androgen excess or insufficiency. Initially, our laboratory adopted manufacturer-defined RIs for androgens in reproductive aged adult women. Subsequent verification on 20 healthy subjects showed RIs are not suitable for some age group for DHEAS and androstenedione, but are applicable for testosterone. There was lack of other literature data for DHEAS and androstenedione for our method. In our case, direct approach for establishing new RIs would require large number of women in whom we excluded at least ovary and adrenal gland diseases. We opted for indirect calculation, as it did not require such extensive work. After data purification, form initially 12,156 extracted records, number of samples was reduced to 3500 for DHEAS and 520 for androstenedione from which total and age-stratified RIs were calculated. Results shows significant differences in calculated vs manufacturer-reported RIs. Differences were especially noticeable for androstenedione. Median for our androstenedione total RI was higher than the manufacturer-declared upper reference limit, meaning that more than half of our subjects had elevated androstenedione concentration. Our calculated upper reference limit was almost twice as high. Although sample number used in this study was very small, calculated RIs were significantly more suitable for our population. We adopted new DHEAS and androstenedione RIs in routine practice, but continue to search for other method-specific RIs calculated on similar population and larger number of samples.

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PCS-5

Big data, machine learning and artificial intelligence

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Even a medium-throughput clinical laboratory produces millions of test results annually: this amount of data, by definition, represents the big data (BD). Together with facts that laboratory data is mostly of numerical type and, thanks to laboratory information systems (LIS), structured, BD makes the laboratory medicine very appealing source of information for medical inferences. Structured data sources enable easy data search and filtering, the interpretation of the numerical data is very intuitive and huge amounts of data make inferences reliable.

Huge data sets are not suitable for the visual inspection and computational aid is necessary. Structured query programming languages are needed for structured data search and filtering while natural language models are required for the unstructured data analysis. However, for the recognition of more complex patterns in BD, the machine learning (ML) should be applied, also: BD and ML, together, mimic the human thinking, hence the term "artificial intelligence" (AI). Reliable inference using AI and laboratory BD also requires contexts: LIS alone lacks clinical contexts i.e. other patient-specific data and laboratory contexts i.e. preanalytical and analytical test characteristics. Any BD analysis would be unreliable without information on analytical instruments, on test quality measures, on accurate patient diagnosis, comorbidities, demographics etc. In order to properly include the contexts i.e. detailed description of BD and AI analysis, it has been proposed application of FAIR principles (2).

Laboratory BD and AI may be used for different purposes. Straightforward use of BD is in retrospective clinical studies. Artificial intelligence has been successfully applied for diagnostics and prognostic purposes, for the therapeutic monitoring, in epidemiology etc. The most recent applications include personalized medicine. Also, BD, with, or without help of ML, can be used for improvement of laboratory practices. Different quality assurance measures can be derived from it. It can be used for sample quality assessment and detection of outliers. Even more insightful applications in analyses of turn-around-time or autovalidation and analysis of clinical utility of laboratory tests were described in literature.

However, there are many other obstacles, besides the lack of contexts, for AI application in laboratory medicine to achieve full potential. First, there is a question of BD and AI availability: due to the General Data Protection Regulation or due to the lack of computational resources. Even the BD contained in LIS, ownership questions have been raised. Furthermore, lack of experts in clinical laboratory diagnostics who are proficient in bioinformatics leads to useless AI applications in laboratory medicine. Besides the textual data, image and -omics analyses, also, represent special cases in terms of data format and in terms of computational analysis: integration of all different data formats and data analysis approaches is quite challenging even for the IT experts. Finally, the regulations for medical devices that also apply to commercial AI devices should be improved.

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PCS-6

Information technologies in the service of the medical biochemist

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The rapid development of information technology (IT) has profoundly impacted the field of clinical chemistry, offering new tools and methods to enhance the efficiency, accuracy, and reliability of laboratory processes. This presentation aims to explore the integration of IT solutions in clinical chemistry laboratories, with a particular focus on practical applications that can improve laboratory operations and support or stimulate scientific development.

The first part of the presentation provides an overview of the historical evolution of IT in clinical chemistry, highlighting its role in transforming manual processes into highly automated systems. The discussion emphasizes the importance of IT in modern laboratories, where it not only increases efficiency but also ensures the accuracy and reproducibility of test results. Practical examples illustrate how laboratories have successfully implemented IT solutions, such as Laboratory Information Management Systems (LIMS), to optimize workflow and reduce errors.

The main section of the presentation addresses the various levels of laboratory automation. It categorizes laboratories according to their degree of automation, ranging from minimally automated to fully automated and robotic systems. The discussion covers automation in different phases of laboratory work: preanalytical, analytical, and postanalytical, and the role of IT in supporting these processes. Pre-analytical automation includes sample registration and barcode usage, analytical automation involves the use of automated analyzers integrated with LIMS, while post-analytical automation encompasses automatic result validation, quality control, and reporting. The advantages of automation, such as increased efficiency and reduced human error, are analyzed in relation to challenges like high implementation costs and the need for specialized training.

The presentation also addresses the implementation of autovalidation and quality control (QC) monitoring within laboratories. Autovalidation, where systems automatically validate test results according to predefined criteria, reduces the need for manual review, enabling laboratories to process a larger number of samples in less time. Advanced QC modules integrated into LIMS allow for continuous performance monitoring and ensure compliance with regulatory standards, thereby maintaining the integrity of laboratory results.

Furthermore, the presentation explores the advanced capabilities of widely accessible tools (MS Office: Access, Excel, Power BI; Google: Sheets, Docs, Slides) for data analysis and reporting. These tools are presented as essential for managing laboratory databases, supporting accreditation, conducting statistical analyses, and creating reports and visualizations. The potential of integrating these tools with MSSQL databases is also discussed, enabling complex data analyses that support better decision-making in laboratory management.

A significant portion of the presentation is dedicated to the role of SQL in data mining and processing. SQL is presented as a powerful tool for retrieving and analyzing data stored in laboratory information systems. Practical examples demonstrate how SQL can optimize laboratory operations, such as by identifying trends and generating reports that highlight results outside of reference ranges, enabling prompt intervention and correction.

The integration of artificial intelligence (AI) into clinical chemistry laboratories is another key topic. The presentation reviews AI tools, such as ChatGPT (OpenAI, GPT-4), Gemini (Google, LaMDA), for their ability to automate data processing and assist in structuring and solving specific problems. The potential of AI in handling IT tasks is also emphasized, from simpler computer-related issues to more complex programming solutions for intricate or time-consuming tasks, such as automation with Python scripts, AppScript, Excel formulas, and Visual Basic. This frees up time for more complex analyses. The presentation also covers the potential of AI in extracting data from unstructured sources such as images (DALL-E, Midjourney) and PDFs, using technologies like Optical Character Recognition (OCR) and Natural Language Processing (NLP).

Finally, the presentation addresses data protection and security, which is a critical topic in laboratory informatics today. The discussion covers the implementation of security policies, such as data encryption and security protocols, along with the importance of regular staff training and security audits. Risks associated with data breaches and cyberattacks are analyzed, with recommendations for protecting sensitive data through measures such as multi-factor authentication and regular data backups. The importance of compliance with data protection regulations, such as General Data Protection Regulation, and the significance of educating staff on security practices will also be highlighted.
In conclusion, the presentation emphasizes the crucial role of IT in advancing our profession. By adopting IT solutions, laboratories can significantly improve their operational efficiency, data accuracy, and overall quality of results. The session will conclude with a Q&A segment, encouraging participants to discuss their experiences and further explore the application of IT in their own laboratories.

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Plennary lecture

PL-1

Management of a modern laboratory

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Laboratory profession is undergoing constant changes. Mainly the changes within the laboratory reflect the ongoing changes in medical practice, which is nowadays driven by three p: personalisation, prevention and precision. These three words sound very nice and promising, but in the real laboratory life they often correspond with not only the need to introduce new technologies and methodologies, but also with steeply increasing test demands. Implementing new tests and methods combined with constantly growing test numbers in turn translate into another three p, which have to be dealt with by managers and leaders of a modern laboratory: people, processes and programs (IT). Hereby I would like to stress the distinction between a laboratory leader and manager – they are both neccessary, but they fulfill fundamentaly different roles. A leader deals with vision and mission of a particular laboratory and steers it accordingly. On the other hand, a manager's role is to optimize processes, which bring laboratory towards the desired goals defined by the vision and mission. In a small laboratory, these two roles are by definiton covered by a single person. In a big clinical laboratory, the managerial role translates mostly onto department heads. They need to be aware of all the goals and obstacles in their professional field in order to propose solutions and optimise the way forward. However, this nice theory of laboratory leadership and management often falls apart in daily life. The main reason is of course the same one as ever – a human factor. Therefore, managers (as well as leaders) should be chosen carefuly. My experience in leading the biggest clinical laboratory in Croatia for almost 12 years has steered me toward firm belief that the most important personality trait for any kind of leading position is emotional intelligence (EI). It is by definition an ability to quickly understand and manage both your own emotions and understand (and maybe also manage to some extent) the emotions of other people. Since people's behaviour is mostly driven by emotions, it is of utter importance for both leaders and managers to be aware of their own as well as their collaborators' emotional states. Without it, it is very hard to apply the appropriate management style (either autocratic, burocratic, escapist or democratic). Of course, a good leader and manager should never stick to just one. The hardest trick of all is choosing the right one for the specific situation – this is where EI plays the crucial role. Only by grasping the atmosphere, the „vibe" of any working environment the problems can be pinpointed and the solutions sought in most appropriate manner. This forms the core of situational management, which I will try to elucidate further by real life examples, emphasizing the unavoidable truth that all of us are far from perfect and that it is therefore impossible not to make mistakes in the process. However, the EI is needed not only for leadership skills within the laboratory - the same goes for the grasp on the general medical community needs. It is needed to ensure that laboratory services align with broader patient care goals and contribute to multidisciplinary care teams. Laboratory is not an isolated ivory tower, it is an essential medical service and laboratory directors should always be acutely aware of this position. It is not enough to be recognized as essential when something goes fundamentaly wrong which disrupts the service, as was a case during the recent cyberattack on our hospital. It is of utter importance for laboratory professionals to be widely recognised as cooperative and understanding part of multidiciplinaray teams, hearing out the clinicians' (or patients') needs and trying to accomodate them if possible. We are not on the clinical care frontline; however, we firmly occupy the basis for virtually all kinds of medical procedures and algorithms, so we should be aware of them and act accordingly. Playing hard to get and mystifying our profession does not make us more important in healthcare, it has just the opposite effect. Therefore, laboratory leaders and all people occupying decision-making positions within the profession should be not only equiped with competence and knowledge, but also with the ability to see and feel the wider picture. Unfortunately, contrary to some theories, I am not sure emotional intelligence is fundamentaly something that can be drasticaly improved through experience, willpower or learning attempts. However, even acknowledging its importance in leadership is a good starting point for improvement.

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PL-2

Decision-making

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Decision-making is a crucial cognitive process in medicine and healthcare, impacting both professional and personal aspects of our lives. Despite its significance, many individuals overlook the mechanisms behind decision-making and the factors that influence their choices. Decisions can be classified as intuitive or argumentative. Intuitive decisions do not require rational justification; they rely on innate feelings or various aspects of tacit knowledge. Tacit knowledge is acquired through experience but often eludes verbal articulation. It is also shaped by psychological factors such as mood, temperament, and emotional state at the time of decision-making.

Conversely, argumentative decisions can be explicitly supported by rational arguments based on facts, data, and established knowledge. Both intuitive and argumentative decision-making, however, can be subject to bias. Bias may stem from situational influences, cultural contexts, societal norms, and individual beliefs and prejudices. Even when making rational decisions, one may unconsciously seek out information that justifies their choice, disregarding contradictory evidence.

In medical practice, most decisions, such as diagnosing a condition, rely on established medical knowledge, disease prevalence, and data gathered from various assessments, including laboratory tests and imaging studies. In this context, it is essential to acknowledge that measurement uncertainties and errors are inherent, posing a risk that even well reasoned decisions may be inaccurate.

It is key to understand that while a correct decision typically leads to favourable outcomes and a poor decision may result in adverse consequences, negative outcomes are not always the result of bad decision-making. There are limits to medical knowledge, and biological variability can lead to unfavourable results despite making the best clinical choices.

Considering these factors, it is crucial to understand the foundation and phases of the decision-making process. Each medical professional carries the responsibility for decisions made in both professional and personal contexts. Decision-makers should be rational, well informed, and capable of thinking critically to ensure their conclusions are as precise as possible. The formalization of the decision-making process encompasses six concepts: Goal, Choice, Consequence, Criterion, Limitations, and Circumstances.

Evidence-based medical practice emphasizes the patient's involvement in the decision-making process, necessitating that patients be fully informed.

While decision-making can be challenging, it is indispensable and fundamental. The most detrimental scenario is the fail to make a decision at all. As trained professionals, we are responsible for our choices and must endeavour to ensure that our decisions are both rational and unbiased.

Being aware of the decision-making process allows us to strive for optimal outcomes. However, even this awareness does not guarantee the correctness of our decisions.

In recent decades, the advancement of decision-support informatics tools has rapidly transformed decision-making processes in medicine. These tools utilise knowledge databases, rules, neural networks, and artificial intelligence to expedite access to knowledge, analyse extensive data sets, provide the latest guidelines and recommendations, and evaluate the risk-benefit ratios of various choices in specific situations. While these tools can significantly aid in decision-making, it is vital to remember that they are not replacements for human judgment. The ultimate decision – and the responsibility for it – rests with the individual.

Humans must make the best use of available support, especially when decisions have the potential to change lives, because ultimately, it is the individual's responsibility to decide and bear the consequences.

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PL-3

Artificial intelligence in scientific publishing

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In 2023, artificial intelligence (AI) emerged as a groundbreaking tool with the potential of dramatically changing many fields of science and technology. Despite its many benefits, more and more concerns have been raised, especially in the field of scientific publishing.

When discussing AI in scientific publishing, people generally assume only the usage of tools for generating new text, such as ChatGPT. However, AI tools have been integrated into scientific publishing for several years now assisting in journal selection, keyword suggestion, reviewer suggestions, paper summarization, finding similar articles, language quality evaluation, plagiarism detection, ethical issues detection, document (especially references) formatting, translation, image generation, assessment of study design and statistical analysis. Both, authors and journal editors, have extensively used many of these features without even realizing that they are using AI assisted technology. In most cases, there are no research integrity issues with such practices. Unfortunately, not all scientists are using AI tools responsibly with the intention of getting assistance in some parts of the scientific writing process. AI tools for generating new text might produce fake papers, which presents one of the most serious infringements of research integrity. Paper mills are profit-oriented, unofficial, and potentially illegal organizations that produce and sell fabricated or manipulated manuscripts that appear to be genuine research. Fabrication might occur partially or completely in data collection and presentation or in statistical analysis. In many cases, completely fabricated non-existent references have been observed. By citing these articles, some researchers might unwillingly participate in fraudulent behavior. Despite the efforts of journal editors to detect these papers, many of them have been published and made available to the scientific community. A transparent, honest and high-quality peer review process should prevent their publication.

Detecting the usage of AI tools is still a major problem for scientific journals. Even though the development of generative AI is followed by the rapid expansion of models for detection of AI-generated text, these tools can become obsolete and rather useless very quickly. Some AI-generated papers should be easily recognized if they contain certain phrases. However, somehow, in 2024 the Surfaces and Interfaces journal managed to publish a paper starting with: "certainly, here is a possible introduction for your topic" (the paper has since been retracted). According to the results of research conducted by Scientific American, another evident AI-generated phrase "as of my last knowledge update" appeared only once in the most relevant scientific databases (Google Scholar, Scopus, PubMed, OpenAlex and Internet Archive Scholar) in 2020, while a significant rise was recorded in 2022 (136 times). In many other cases recognition of AI generated text is much harder. However, several researchers have identified some words and phrases that appear more often in AI-generated text (intricate, meticulous, commendable, undoubtedly, scholarly, innovatively, aptly, delve). By analyzing the frequency of these words in recently published papers, Grey A. aimed to explore the prevalence of AI-generated text among papers published in 2023. His results revealed that at least 60,000 papers (just over 1 %) used ChatGPT or some other Large Language Model (LLM) tool for text generation.

Nowadays it is practically impossible to eradicate the use of AI in scientific communities. Publishers therefore need to adapt. Policies among them vary; however, most of them allow the usage to some extent, provided it is clearly noted during the submission process. However, according to the Committee on Publication Ethics (COPE) position statement, AI tools cannot be listed as authors since they cannot take responsibility for the submitted paper, which is one of the main requirements of authorship. Authors of the papers are therefore fully responsible for all AI-generated or assisted data.

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Symposium lectures

S1 Clinical and diagnostic monitoring of lymphoproliferative diseases

S1-1

Lymphoproliferative diseases - etiology, epidemiology, pathophysiology, diagnosis and therapy

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Lymphoproliferative disorders (LPD) comprise a heterogeneous group of diseases characterized by uncontrolled production of lymphocytes that cause monoclonal lymphocytosis, lymphadenopathy and bone marrow infiltration. These diseases often occur in immunocompromised individuals. There are two subsets of lymphocytes: T and B cells that regenerate uncontrollably to produce immunoproliferative disorders, which are prone to immunodeficiency, a dysfunctional immune system, and lymphocyte dysregulation. Several gene mutations have been described as causes of LPD that can be iatrogenic or acquired.
Chronic lymphoproliferative disorders are immuno-morphologically and clinically heterogeneous. Common features of these processes include various immunophenotypes (T, B, and NK cells) and terminal deoxynucleotidyl transferase negativity. Lymphoproliferative disorders encompass a wide range of subtypes, each with distinct characteristics and clinical manifestations.
Infections with Human immunodeficiency virus (HIV), human T-cell lymphotrophic virus type 1 (HTLV-1), human herpes virus-8 (HHV8) and Epstein-Barr virus are all associated with an increased risk of certain types of lymphoproliferative disease, most probably by causing immunosuppression. Infection with HTLV-1, especially in early childhood, is strongly related to the development of adult T-cell leukaemia/lymphoma with an estimated cumulative lifetime risk of approximately 5%.
Patients receiving long-term immunosuppressive drug therapy such as transplant recipients and patients with autoimmune diseases have a higher risk of developing chronic lymphoproliferative disorders. An increased risk is also seen in patients suffering from a variety of autoimmune conditions, for example, rheumatoid arthritis, Sjögren's syndrome and coeliac disease, independent of immunosuppressive treatments. In addition, occupations related to agriculture have been associated with an excess risk of lymphoproliferative disease leading to suggestions that contact with herbicides, pesticides and solvents could be important determinants of risk.
Lymphoproliferative disorders originate when physiological mechanisms of control of proliferation of both T and B cells break down, resulting in the uncontrolled and autonomous increase of immune cells leading to lymphocytosis and lymphadenopathy, and often involvement of extranodal sites, e.g., bone marrow. In immunocompromised patients, EBV can cause a mild disease. However, in immune-suppressed transplant patients, immunosurveillance may be compromised by the lack of T cells, leading to a proliferation of EBV-infected B-lymphocytes and post-transplant lymphoproliferative disorder (PTLD). Polyclonal PTLD can form tumor masses and presents with symptoms of a mass effect. Monoclonal forms of PTLD can manifest as a disseminated malignant lymphoma.
The diagnostic criteria for LPD are established by the World Health Organization (WHO), International workshop on chronic lymphocytic leukemia, the National Comprehensive Cancer Network (NCCN), and the European Society for Medical Oncology (ESMO). In the group of lymphoproliferative diseases, morphology remains one of the bases of classification, but the immunophenotype and genetic characteristics of the tumor contribute to the objectivity of the diagnosis, increase the reproducibility of the classification and sometimes provide significant prognostic information. Flow cytometry (FCM) has become a method of choice for immunologic characterization of chronic lymphoproliferative disease .The diagnosis of the different lymphoma entities is based on a combination of morphology, immunophenotype, EBER in situ hybridization, FISH analysis, and

B-cell clonality analysis. Advances in the understanding of difuse large B cell lymoma herald a transition to a molecular genetic classification. This genetic classification is based on mutational profile, somatic copy number alterations, and structural variants.
In the last decade, there has been a considerable change in the treatment of LPD and an increase in overall survival. Biological therapies or targeted therapies have been added to conventional chemotherapy, which, selectively, try to stop or, in some cases, prevent the progression of tumour cells. These therapies include a series of monoclonal antibodies, checkpoint inhibitors, bispecific antibodies, and chimeric antigen receptor T (CAR-T) cell therapies, receptor analogues and small molecules designed to bind to different molecular targets. Cellular therapies, particularly CAR-T, emerge as a potential revolution for the R/R of several types of NHL, considered incurable until a few years ago. Nonetheless, these advancements call for further investigations, especially in optimizing combination strategies, managing resistance mechanisms, and refining administration timing. These drugs have advantages over chemotherapy in terms of potency and specificity and, theoretically, produce less adverse effects. However, if these drugs are correlated with a greater number of infections it is a difficult fact to establish, since there is a large number of factors that lead to confusion.

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S1-2

Laboratory diagnostics of lymphoproliferative diseases

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Lymphoproliferative diseases (LPDs) are heterogeneous group of lymphoid malignancies derived from uncontrolled production of NK cells and B or T lymphocytes in the later stages of maturation causing monoclonal lymphocytosis, lymphadenopathy and bone marrow infiltration. They comprise a wide spectrum of polyclonal lymphoid proliferations including benign lymphoid lesions of unknown etiology and pathogenesis and monoclonal lymphoid proliferations classified as either leukemias (increased number of malignant cells in the bone marrow circulating in the blood) or lymphomas (increased number of malignant cells in the tissues of lymphatic organs or organs containing lymphatic tissue) depending on the location and the place of origin.
The classifications of lymphoid neoplasms are still based on morphological criteria including clinical and immunophenotype features, but today the importance of genetic, either cytogenetic or molecular gene level changes, prevails. During the differentiation stage of B and T cells, mutations in the deoxyribonucleic acid (DNA) that builds immunoglobulins (Ig) or T cell receptors (TCRs) cause diversity in their structure and help them in effective fight against infections. The consequence of the incorrect construction of immunoglobulins or TCRs due to DNA mutation will be the inability to operate the usual control mechanisms of B and T cells and the loss of an effective immune response, which can cause lymphoma. According to the latest World Health Organization (WHO) classification of hematolymphoid tissue tumors (WHO-HAEM5, 5th edition, 2022) and International Consensus Classification of lymphoid and myeloid neoplasms (ICC, 2022), two basic types of lymphomas caused by malignant, clonal transformation of both mature and immature B or T/NK cells are: Hodgkin's lymphoma (HL) and non- Hodgkin's lymphomas (NHLs) with more than 60 subtypes that differ according to the degree of malignancy and the type of cells. The clinical course varies from indolent (lymphomas originating from B cells) to aggressive (lymphomas originating from either B or T cells). Among B cell NHLs in Western countries, the most common aggresive subtypes are diffuse large B cell lymphoma (DLBCL; 31%), mantle cell lymphoma (MCL; 6%) and Burkitt lymphoma (BL; 2%) and most common indolent subtypes are folicular lymphoma (FL; 22%), marginal zone lymphoma (MZL; 8%), chronic lymphocytic leukemia/small- cell lymphocytic lymphoma (CLL/SLL; 6%) and lymphoplasmacytic lymphoma (LPL; 1%). Among T cell NHLs, common subtypes (10- 15%) include peripheral T cell lymphoma (PTCL; 6%) and cutaneous T cell lymphoma (CTCL; 4%).
After clinical findings of local or distant lymphadenopathy and hepatosplenomegaly either in certain conditions the gastrointestinal tract or lung tissue affection, various specimens for routine laboratory diagnostics can be used including lymph nodes, bone marrow aspirates, peripheral blood, trephine biopsy cores and other body fluids (cerebrospinal or ascitic fluid, pleural aspirates). General tests in cases of LPD are serologic tests for cytomegalovirus (CMV) and Epstein- Barr virus (EBV). Routine laboratory diagnostic tests comprise of hematological tests (complete blood cell count with differential as essential and erythrocyte sedimentation rate) following by biochemical tests (electrolytes, blood urea and creatinine, phosphate, calcium, and uric acid measurement for ruling out tumor lysis syndrome, lactate dehydrogenase measurement to assess the neoplastic burden). Laboratory screening procedure for monoclonal (M) proteins detection and immunoglobulins (Ig G, A, M) measurement is serum protein electrophoresis (SPEP). If a localized band suggestive of a M protein at SPEP is present, immunofixation electrophoresis (IFE) is necessary for confirmation and determination of the heavy and light chain class of the M protein. More specific laboratory tests - flow cytometry immunophenotyping (FCM) and genetic testing based on molecular diagnostic techniques - have become an additional part of the routine diagnosis of lymphomas and thus also of NHLs. Flow cytometry immunophenotyping is used for lineage assignment and classification of LPDs and for the expression of light chains of Ig kappa and lambda reveals the clonal origin of B lymphocytes. The detection of monotypic TCR expression also can be done using flow cytometry for TCR molecules in assessment of T cell clonality. In cases of enlarged lymph nodes due

to the expansion of the monomorphic lymphocyte population, it is difficult to differentiate between reactive and a neoplastic lymphocytosis. Flow cytometry immunophenotyping can provide evidence of light chain restriction indicating the presence of a monoclonal ymphocyte population. Thereby FCM supports a diagnosis of neoplastic rather than reactive lymphoproliferation. Today, it is completely clear that there is no diagnosis of lymphoma without genetic testing based on molecular diagnostic techniques because it provides evidence of abnormal gene rearrangements for the heavy and light chains of Ig B cells and TCRs, chromosomal translocations and impaired gene expression that characterize most B cell NHLs and only a small number of T cell NHLs.

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S1-3

Molecular diagnostics of lymphoproliferative disorders: current state and perspectives of inclusion in clinical practice

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With the development of new therapies, it becomes essential to have developed molecular diagnostics that give us information about the genomic picture of lymphoproliferative disorders. Several techniques, such as flow cytometry, fluorescence in situ hybridization, and various molecular techniques like gene sequencing (Sanger and next generation sequencing - NGS), can be used to obtain a picture of the disease at the molecular level. The molecular parameters currently in use in clinical practice are the sequences of B and T receptors (clonality), the mutational status of immunoglobulin genes (IGHV), deletions, translocations, and other chromosomal aberrations, the mutational status of the TP53 gene, and the like. This information has an essential place in the diagnosis and classification of lymphoproliferative diseases, the choice of treatment, and later in the monitoring of the course of the disease and the response to treatment. The use of targeted drugs can lead to new genetic changes that can affect their effectiveness. Thus, for example, in chronic lymphocytic leukemia (CLL), using BTK inhibitors can lead to changes in the genome that lead to an altered BTK protein that no longer binds the BTK inhibitor to itself or leads to a reduced affinity of the inhibitor. With a different BTK inhibitor that does not bind to the same site, we can block the BTK signaling pathway again. The same situation occurs with BCL2 inhibitors. Another important molecule is the PLCG2 protein, which is located immediately downstream of the BTK and in which the so-called "gain of function" mutations lead to the activation of the BCR signaling pathway independently of the activation of the BTK protein, thus nullifying the influence of BTK inhibitors. For this reason, it is important to monitor the mutational status ("profile") of genes such as BTK, BCL2, and PLCG2, thus guiding the treatment with the targeted agents. Another molecular parameter that may become an essential part of the diagnostics of lymphoproliferative disorders is circulating tumor DNA (ctDNA) (tumor specific cell-free (cf) DNA). It can be instrumental in disease monitoring and evaluation of response, supplementing the radiological methods. In conclusion, molecular diagnostics of lymphoproliferative disorders is a rapidly evolving field that already holds an essential place in routine clinical practice and will become even more crucial in the future. It is closely related to the rapid development of targeted agents and other effective treatment modalities.

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S1-4

Next generation sequencing - diagnostic tool for detecting genetic alterations or a new perspective towards targeted therapy of lymphoproliferative diseases and personalized medicine

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Next generation sequencing (NGS) is widely used in diagnostics, including clinical laboratories for hematooncology. The application of NGS in this field has been especially encouraged by the latest leukemia and lymphoma classifications issued in 2022. The World Health Organization (WHO) revised the previous classification and published its 5th edition, while in parallel the International Consensus Classification (ICC) published its classification of lymphoid and myeloid neoplasms. Both classifications are based on molecular markers of neoplastic cells, which replaced the morphological criteria on which the previous classification has been based.
Diagnostics of lymphoid neoplasm starts with the choice of the appropriate sample – peripheral blood or bone marrow for circulating lymphoproliferation or lymph node biopsy for lymphoma. It is well known that flow cytometry immunophenotyping is essential for diagnosis of lymphoproliferative disorders; it uses specific neoplastic cell phenotype for differential diagnosis, prognosis, therapy approach and follow-up. The WHO/ICC classification of lymphoid tumors for the first time included molecular markers as an essential or desirable part of the diagnostic criteria for a single entity.
Chronic lymphocytic leukemia (CLL) is managed by classifying patients into risk groups based on molecular markers: immunoglobulin heavy chain variable regions (IGHV) mutational status and TP53 mutational status.
Immunoglobulin heavy chain variable regions mutational status is determined by amplifying the clonotypic IGHV-IGHD-IGHJ gene rearrangement, using Sanger sequencing or NGS and assessing percentage identity to the closest germline IGHV gene. Patients are therefore distributed in the poor-prognostic IGHV-unmutated subgroup and the more favorable-prognostic IGHV-mutated CLL subgroup. Additionally, a distinctive amino acid pattern within the variable heavy complementarity determining region 3 (VH CDR3) of the clonotypic immunoglobulin can be assigned to stereotyped subsets with consistent clinical presentation and outcome and can contribute to refined risk stratification of CLL patients.
TP53 mutational status affects the choice of therapeutic approach at the time of diagnosis and disease relapse. TP53 mutations can be detected along the entire coding sequence and are frequently coincided with del(17p). Their number and frequency increases with each relapse of the disease, so it is necessary to analyse the TP53 mutational status before next therapy application. In the Burton's tyrosine kinase (BTK) and BCL2 inhibitors era determination of mutations in BTK, PLCG2 and BCL2 genes is desirable due to high occurrence of a single nucleotide mutation causing resistance to these drugs.
Lymphoma classification is still mainly based on morphology, immunophenotype, and a few genetic characteristics, a reason why NGS is still not the mandatory diagnostic tool. Nevertheless, capture-based targeted panels enable detection not only of single nucleotide variations (SNVs) and indels (insertions and deletions) but also of copy-number alterations (CNAs) and some structural variants providing better lymphoma subclassification, patient risk stratification, and prediction of treatment response. Recently, the use of circulating tumor DNA (ctDNA) from blood plasma seems to be a valid alternative to tissue biopsy when using high sensitive NGS methods for diagnosis but also in measurable residual disease (MRD) monitoring.
Acute lymphoblastic leukemia/lymphoma (ALL) is the most common childhood malignancy, accounting for 25% of pediatric cancers. The WHO/ICC 2022 classification nomenclature for ALL focuses on the molecular events, rather than cytogenetic alterations, to allow for the application of differing techniques for their detection. B-ALL with BCR::ABL1-like features is now an entity and shows significant benefit from targeted therapies. Therefore, it is necessary to detect the enhanced CRLF2 expression, ABL1-like fusions, JAK2 and EPOR fusions, TYK2 rearrangements, RAS signaling pathway mutations, and unusual fusions of other genes using

transcriptome sequencing. Very rare T-cell ALL mutations have a strong prognostic effect, i.e. simultaneously detected NOTCH1/FBXW7 mutation has favorable early treatment response and long-term outcome.
Clonal rearrangement of immunoglobulin gene and T-cell receptor are highly specific for a patient's neoplastic clone and therefore an excellent tool for MRD monitoring, the most powerful independent predictor of relapse risk and long-term survival. High-throughput next-generation sequencing is a reliable method to evaluate MRD that has been increasingly utilized in clinical practice due to its high sensitivity. The future therapy of lymphoproliferative disorders will increasingly utilize MRD as a criterion to either intensify or modify therapy in order to prevent relapse or de-escalate therapy to reduce treatment-related morbidity and mortality.
In conclusion, NGS seems to be a powerful diagnostic tool for detecting genetic alterations, but usability of the obtained information is still challenging when managing patients in a personalized medicine context.

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S2 Diabetes and insulin resistance; challenges in diagnostics and science

S2-1

Pathogenesis of diabetes - should existing knowledge be revised?

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Latest data released by the International Diabetes Federation (IDF) showed that world prevalence of diabetes is 10.5% of the adult population (20-79 years), with almost half unaware that they are living with the condition. The total number of people living with diabetes is projected to rise to 643 million by 2030 and 783 million by 2045. It is estimated that 240 million people all over the word live with undiagnosed diabetes.
Diabetes is a chronic disease, most common metabolic disorder which hallmark is hyperglycemia. Hyperglycemia results from a relative or absolute deficiency of insulin, the decreased sensitivity of target cells to insulin, and the glycolipid and protein metabolism disorders. Diabetes also has a high mortality rate, each year 1.5 million deaths are directly attributed to diabetes. Pancreatic cells, their function declines slowly before the symptoms of clinical hyperglycemia. Current knowledge and understanding of the mechanisms of the disease is crucial for novel therapies and preventing diabetic complications.
Glucose homeostasis is the maintenance of blood glucose concentration in a relatively stable range through a series of body regulations. It depends on the communication network comprising various hormones and neuropeptides released by the brain, pancreas, liver, and intestinal organs as well as fat and muscle tissues. Therefore, the imbalance of glucose homeostasis leads to metabolic disorders. The pancreas is also an important participant in glucose homeostasis. The interaction between insulin and glucagon is a crucial process that makes it possible for the pancreas to maintain blood glucose concentrations within a quite narrow range. The impairment of any link may lead to a disruption in glucose homeostasis, resulting in insulin resistance and ultimately developing into type 2 diabetes. There are many mechanisms involved in glucose homeostasis and diabetic insulin resistance as the PI3K/Akt signalling pathway, which is one of the major pathways of insulin signalling. The long-chain non-coding RNA (lncRNA) associated with diabetes play an important role in the pathogenesis of diabetic complications especially with diabetic retinopathy. Yan et al. found that 303 lncRNAs were abnormally expressed in the retinas of patients with early diabetic retinopathy, and an enrichment analysis showed that these lncRNAs were mainly involved in the MAPK signalling pathway, complement and coagulation cascade, chemokine signalling pathway, pyruvate metabolic pathway, and axon guidance signalling pathway.
Regarding literature and guidelines, diabetes is classified conventionally into several clinical categories (e.g., type 1 or type 2 diabetes, gestational diabetes mellitus, and other specific types derived from other causes, such as genetic causes, exocrine pancreatic disorders, medications and underlined pathophysiology). Type 1 diabetes mellitus is usually caused by autoimmune destruction of the pancreatic islet b-cells, rendering the pancreas unable to synthesize and secrete insulin. Type 2 diabetes mellitus results from a combination of insulin resistance and inadequate insulin secretion. Gestational diabetes mellitus (GDM), which resembles type 2 diabetes more than type 1, develops during approximately 17% (ranging from 5% to 30%, depending on the screening method, diagnostic criteria used, and maternal age) of pregnancies, usually remits after delivery, and is a major risk factor for the development of type 2 diabetes later in life. Type 2 diabetes is the most common form, accounting for 85% to 95% of diabetes in developed countries. Monogenic subtypes of type 2 diabetes have been identified but are rare. Some individuals cannot be clearly classified as type 1 or type 2 diabetes and an increasing fraction of people with type 1 diabetes may have superimposed metabolic characteristics of type 2 diabetes owing to the increasing prevalence of obesity. Classification is important for determining personalized therapy, but some individuals cannot be clearly classified as having type 1 or type

2 diabetes at the time of diagnosis. The traditional paradigms of type 2 diabetes occurring only in adults and type 1 diabetes only in children are not accurate, as both diseases occur in all age groups.
It is important for healthcare professionals to realize that classification of diabetes type is not always straightforward at presentation and that misdiagnosis is common and can occur in 40% of adults with new type 1 diabetes (e.g., adults with type 1 diabetes misdiagnosed as having type 2 diabetes and individuals with maturity-onset diabetes of the young (MODY) misdiagnosed as having type 1 diabetes). Difficulties in distinguishing diabetes type may occur in all age groups at onset of the disease, so the awareness of that is crucial for an accurate diagnosis.

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S2-2

Obesity, insulin resistance and diabetes - a global epidemic

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The lecture summary is not available.

S2-3

Insulin resistance and diabetes from a laboratory point of view

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Insulin resistance (IR) is one of the primary contributors to the development of type 2 diabetes mellitus (T2DM). It represents an inadequate response of primary tissues, such as the liver, muscles and fat to insulin stimulation. Insulin resistance directly impairs the uptake of glucose into cells, leading to increased insulin production by the pancreatic β-cells. This results in hyperinsulinemia, and over time, hyperglycemia and T2DM. Type 2 diabetes mellitus is a widespread health concern that significantly increases the risk of complications affecting the blood vessels, kidneys and nervous system. Therefore, early diagnosis and treatment are of great importance.

The diagnosis and management of T2DM are standardized and well-established. Accurate diagnosis of T2DM is mainly based on laboratory test results. Recommended diagnostic laboratory tests for T2DM include fasting plasma glucose, 2-hour plasma glucose (2-h PG) following a 75-g oral glucose tolerance test (OGTT), random glucose measurement with typical hyperglycemic symptoms and glycated hemoglobin A1c (HbA1c), each with established standardized metodology and cut-off values. The listed tests can identify both diabetes and the prediabetic stage, prediabetes, in individuals at risk. Prediabetes is a condition where blood glucose concentrations are higher than normal but do not meet the criteria for a diabetes diagnosis. One of the most significant aspects of prediabetes is its reversibility, whereas diabetes is generally a chronic and irreversible condition. Given this understanding, along with the previously mentioned consequences of T2DM, the focus of diabetes management increasingly shifts towards prevention. The main prevention strategies involve early detection, lifestyle improvements and comprehensive medical care. Although we have reliable tests for diagnosing prediabetes and diabetes, the primary challenge is to uncover IR at the earliest stage. Since IR can manifest even before glucose homeostasis is disrupted, timely detection is essential for preventing the advancement to prediabetes and diabetes.

Clinically, IR is identified through a comprehensive evaluation of metabolic abnormalities and associated symptoms that suggest compromised insulin sensitivity. The laboratory evaluations of IR usually involve measuring insulin, c-peptide, glucose or triglycerides. The gold standard for assessing IR is the hyperinsulinemic-euglycemic clamp technique. For routine assessment, the method is too complex and has never become established in practice. As a result, indirect incides of IR have been developed and are better suited for routine practice. The most commonly employed indirect indices of IR are sourced from Homeostatic Model Assessment (HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI) and Matsuda Index. The listed indices provide a reliable evaluation of IR. However, their application in daily practice should be approached with caution, as reference ranges and cut-off values can vary depending on age, sex, population, and methodology.

With the growing rates of insulin resistance, prediabetes and diabetes, laboratories are encouraged to utilize all available tests to thoroughly evaluate and manage these metabolic disorders. By incorporating a broad range of diagnostic tests, laboratories can ensure early detection or even prevention. This proactive approach is crucial, as it allows healthcare providers to identify individuals at risk before the onset of disease. By doing so, timely lifestyle interventions and preventive measures can be implemented, significantly reducing the likelihood of developing diabetes and its associated complications.

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S2-4

N-glycome and genome in understanding type 1 diabetes

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N-glycosylation is a modification of proteins in which oligosaccharide structures are added to protein backbones. Glycans are one of the main regulators of different features of the immune system and they show biomarker potential in many diseases. Individual variation in plasma N-glycosylation has mainly been studied in the context of diabetes complications, and its role in type 1 diabetes (T1D) onset was largely unknown. We analyzed the N-glycome of total plasma proteins and IgG in almost 2000 children with recent-onset type 1 diabetes from the Danish Registry of Childhood and Adolescent Diabetes (DanDiabKids). Same individuals were genotyped at 183,546 genetic markers. A subset of 188 participants also had 244 unaffected siblings included in the study. Our study showed that onset of type 1 diabetes was associated with an increase in the proportion of plasma and IgG high-mannose and bisecting GlcNAc structures, a decrease in monogalactosylation, and an increase in IgG disialylation. Models including age, sex and N-glycans yielded notable discriminative power between children with type 1 diabetes and their healthy siblings, with AUCs of 0.92 and 0.87 for plasma and IgG N-glycans, respectively. To search for genetic variants contributing to these changes, we undertook a genetic association study of the plasma protein and IgG N-glycome in this cohort. Our study confirmed previously known loci associated with plasma protein and IgG N-glycosylation and identified novel associations that were not previously reported for the general European population. Novel genetic associations of IgG-bound glycans were found with SNPs on chromosome 22 residing in two genomic intervals close to candidate gene MGAT3. Second, two SNPs in high linkage disequilibrium, located on chromosome 19 within the protein coding region of the complement C3 gene (C3) showed association with the oligomannose plasma protein N-glycan. Complement component C3 is a central protein of all complement activation pathways. To study the N-glycosylation of C3 we developed the first high-throughput LC-MS based method. We analysed C3 glycopeptides to compare N-glycans between children with T1D and their healthy siblings, search for connections between N-glycans and T1D related complications in adults, as well as to reveal the genetic regulation of its N-glycosylation. C3 glycopeptides were analysed in 61 children newly diagnosed with T1D and 84 unaffected siblings form DanDiabKids, as well as 189 adults with T1D from Croatia and 816 adults from general Croatian population (GWAS on glycans). We showed that C3 has two N-glycosylation sites, with three high-mannose glycans on Asn63 and six on the Asn917. Significant changes were found between children with T1D and healthy siblings, expressed through an increase in glycans with more mannose units, especially the Man9Glc. A regression model showed discriminative power of C3 N-glycome with AUC of 0.88. Significant changes were also seen in severe albuminuria, and in subjects with hypertension, but less with non-proliferative retinopathy. Glycans were associated with HbA1c concentrations. Smoking, eGFR and the disease duration had no effect on C3 N-glycome. We also identified six genome-wide significant loci associated with C3 N-glycosylation. Currently, we are analyzing N-glycosylation of total plasma proteins, IgG and C3 in the at risk new-borns to evaluate the potential of glycans as prognostic markers for the development of autoimmunity. Our results suggest the involvement of N-glycosylation in T1D development and its significant potential for assessment of T1D risk in children. It also has a value in distinguishing subjects with diabetic complications, and potential of monitoring the disease progression, supporting the potential of N-glycans as novel targets in unravelling the pathogenesis of T1D.

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S3 Challenges of laboratory diagnosis of preeclampsia

S3-1

Although we know so much about preeclampsia, why haven't we cured it?

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Convulsions related only to pregnancy were first described by Hippocrates around 400 BC. For a very long time the nature of the disease was unknown. In the late 1800's, a theory of toxins was formed. Then, the only prescribed treatments for preventing convulsions in pregnant women with headaches and edema were bleeding and purging, aiming for the purification of the body of toxins.
The real progress started in the 19th century, when proteinuria in eclamptic patients was described. After the discovery of Scipione Riva-Rocci's mercury manometer, the disease was recognized as a hypertensive disorder. From then until now, the new onset of hypertension and proteinuria have been the major signs used in the classification of preeclampsia. The journey of preeclampsia, "the disease of theories," starts, but not until the 1960s. The father of preeclampsia in the second part of 20th century, Leon Chesley, researched not only renal functions but also related preeclampsia to the hereditary origin of the disease. Since the 1960's, prenatal care and the diagnosis of preeclampsia have been based on the measurement of blood pressure and urin analysis. Treatment options were primarily directed to the management of clinical signs without clear knowledge about an underlying disorder. The only definitive, though imperfect, "cure" is the delivery. Now we know that the incidence of preeclampsia is 5-14% of all pregnancies, while severe preeclampsia accounts for approximately 25% of all cases. It is a pregnancy-specific disorder involving endothelial dysfunction and vasospasm. Usually occurs after 20 weeks of gestation and can present as late as 4-6 weeks postpartum. It is clinically defined by new onset of hypertension and proteinuria, with or without severe symptoms. The disease may lead to liver and renal failure, disseminated intravascular coagulopathy (DIC), and central nervous system (CNS) symptoms, generalized clonic and tonic seizures in cases of eclampsia. Based on the time of onset of the disease, it can be categorized into two types. Type I begins before 34 weeks of gestation and is considered to be of placental origin due to malplacentation in early gestation. Type II starts after 34 weeks of gestation and is considered to be of maternal origin due to abnormal maternal responses to late pregnancy for various reasons. On the molecular level, there are three subclasses of PE: 1) presenting established PE markers and molecular phenotypes; 2) immune response-related; and 3) due to poor maternal responses to the pregnancy. The severity of clinical symptoms corresponds to the severity of maldevelopment of the placenta. Impaired development of extravillous trophoblast (EVT) leads to EVT dysfunction and inadequate villous trophoblast (VT) invasion, resulting with the failure of the physiological transformation of the maternal spiral arteries. All that leads to abnormal blood flow to the placenta, which can clinically be presented not only with maternal symptoms but also with intrauterine fetal growth restriction or placental abruption. Hystology shows changes consistent with vascular malperfusion of the placenta. Due to inadequate VT invasion, the release of anti-angiogenic factors, pro-inflammatory cytokines, and syncytiotrophoblast debris into the maternal circulation causes an anti-angiogenic state and exaggerated maternal systemic inflammation. The severity of maternal response correlates with the severity of preeclampsia. The damage to the maternal endothelium correlates with the the damage to the kidneys, liver, and central nervous system. Various preexisting maternal disorders (obesity, chronic hypertension, diabetes, and metabolic, kidney, and autoimmune diseases) are related to Type II preeclampsia. They can also affect VT dysfunction and induce a maternal pro-inflammatory response. Also, maternal endothelial damage can result from an exaggerated sensitivity to factors released from the placenta due to maternal genetic predisposition for cardiovascular disease. Now it has been determined that preeclampsia has a genetic predisposition with high heritability of both phenotypes, with presence of the same risk alleles for coronary artery disease. Recent studies suggest genetic influence on major cellular processes, inclu-

ding blood pressure (BP) regulation, apoptosis, development, hormone secretion, metabolism, homeostasis, and signaling. Genes predominantly expressed by the placenta have pathologic and diagnostic significance in preeclampsia. In addition, the influence of preeclampsia on the fetus and the risk of developing cardiovascular disease later in life have been determined. The effect is known as fetal programming.

There are known risk factors for developing preeclampsia such as: nulliparity, multifetal gestations, preeclampsia in a previous pregnancy, chronic hypertension, pregestational diabetes, gestational diabetes, thrombophilia, intrauterine growth restriction, or placental abruption in previous pregnancies, systemic lupus erythematosus, prepregnancy body mass index greater than 30, antiphospholipid antibody syndrome, maternal age 35 years or older, kidney disease, assisted reproductive technology, obstructive sleep apnea. Preeclampsia without severe features may be asymptomatic. Many cases are detected through routine prenatal screening, which now is not reduced only to measuring blood pressure and detection of proteinuria. Now we have a few screening tests that can be done from the early stages of pregnancy onwards. The tests include laboratory testing of biomarkers for preeclampsia, ultrasound measurement, and a combination of the two. In addition, there are calculation tables that calculate the risk for preeclampsia for every trimester separately.

The only treatment for preeclampsia is delivery—not a cure, actually, but the first step in the recovery process. Without severe features induction after 37 weeks' gestation. In case of developing worsening preeclampsia or complications of preeclampsia, the immature fetus is treated with expectant management with corticosteroids. Induction of delivery should be considered after 34 weeks' gestation. In these cases, the severity of the disease must be weighed against the risks of infant prematurity. In the emergency setting, control of BP and seizures should be priorities.

In conclusion, preeclampsia is one of "the great obstetrical syndromes" composed of overlapping pathologic processes that activate cell endothel, inflammation, and syncytiotrophoblast stress. Since there are so many possible factors and pathways underlying the onset of the disease, there is an answer to why we know so much about preeclampsia and still could not cure it.

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S3-2

Clinical utility of the sFlt-1/PlGF ratio in the prediction, diagnosis and management of preeclampsia

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The pathophysiology of preeclampsia is complex, characterized by placental and maternal endothelial dysfunction, leading to complications such as fetal growth restriction, placental abruption, preterm delivery and stillbirth. Recent advancements in predicting and diagnosing preeclampsia have been made through the discovery and clinical evaluation of biomarkers, particularly those originating from placenta. The discovery of circulating angiogenic factors has significantly advanced our understanding of preeclampsia, enhancing both diagnosis and prognosis. Key factors include the anti-angiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) and the pro-angiogenic placental growth factor (PlGF). These can be measured in serum and plasma using automated platforms, typically reported as a ratio. Both sFlt-1 and PlGF are primarily produced in the placenta and serve as non-invasive markers of placental health. The sFlt-1/PlGF ratio is highly effective in ruling out the development of preeclampsia within 7 days among women with suspected preeclampsia, as well as predicting adverse maternal outcomes or delivery with preeclampsia within 14 days. Utilizing angiogenic markers to guide care can expedite the diagnosis of preeclampsia and identify women at higher risk of severe maternal morbidity, including postnatal hypertension. Combining these markers with other clinical, routine laboratory, and ultrasonographic data can further enhance the prediction of adverse outcomes. The sFlt-1/PlGF ratio is a valuable tool for predicting and diagnosing preeclampsia from 20 weeks up to 36 weeks and 6 days. It is particularly useful for high-risk women or those with clinical suspicion of preeclampsia. High-risk women include those with a positive Fetal Medicine Foundation (FMF) screening result, a uterine artery pulsatility index above the 95th percentile at 20-22 weeks, or other clinical risk factors. After 37 weeks, the ratio test can be used in all suspected cases of preeclampsia or to evaluate uteroplacental dysfunction. It also helps in personalizing the decision to induce labor or wait for spontaneous onset. This test aids in better management and timely intervention, improving outcomes for both the mother and baby. Women without clinical signs of preeclampsia generally should not be tested, except those at high risk. These asymptomatic women can be monitored monthly with sFlt-1/PlGF ratio tests. For women showing clinical signs of preeclampsia, a sFlt-1/PlGF ratio below 38 can rule out the condition for the next 2-4 weeks. Those with a ratio between 38-85 need enhanced outpatient monitoring and should be retested in 1-2 weeks or sooner if their condition changes. If the ratio is 38-85 and there are clear clinical signs of preeclampsia or suspected fetal compromise, hospitalization is recommended based on local protocols. Women with a sFlt-1/PlGF ratio above 85 are highly likely to have or develop preeclampsia and need intensive monitoring, often as inpatients. Inpatient care is typically for those needing urgent intervention due to severe symptoms, significant concerns about the pregnancy, a sudden rise in the ratio, or inability to follow outpatient protocols. The sFlt-1/PlGF ratio is valuable for ruling out imminent preeclampsia and is particularly useful in differentiating it from conditions with similar symptoms. The rate of change in the sFlt-1/PlGF ratio, or delta, is a crucial indicator of disease severity and progression in preeclampsia. While the ratio can help estimate the expected time to delivery, it should be used alongside other clinical features. Extremely high ratio values are particularly useful for predicting short-term adverse maternal outcomes in preeclampsia. The higher the ratio, the greater the risk of maternal complications requiring hospital intervention, such as acute lung edema, HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome, placental abruption, renal failure, refractory hypertension, or eclampsia. Additionally, the ratio can predict fetal outcomes related to prematurity and growth issues; higher ratios indicate higher risks for both mother and baby. The sFlt-1/PlGF ratio also provides valuable prognostic and diagnostic information in twin pregnancies up to 29 weeks of gestation. In summary, the sFlt-1/PlGF ratio is a valuable laboratory tool for screening, diagnosing, predicting, and monitoring placenta-related disorders in both singleton and twin pregnancies. It helps in early detection, assessing disease severity, and guiding clinical decisions to improve outcomes for both mother and baby.

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S3-3

Biomarkers in first trimester to predict the preeclampsia

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Preeclampsia is a condition that specifically affects pregnant women. In recent years, two screening tests have been developed to predict preeclampsia. The first is performed in the first trimester, aiming to identify high-risk women, and the second is performed after 20 weeks of gestation, with excellent performance to rule out preeclampsia within a week. Shortening the time between testing and symptom onset enhances the reliability of test screening, and the latter has been successfully implemented in routine work while first-trimester screening is not widely available. Low-dose prophylaxis with acetylsalicylic acid initiated before 16 weeks of gestation proved to be helpful in the prevention of preterm preeclampsia. According to US Preventive Services Task Force recommendations, low-dose aspirin between 60 to 150 mg per day can be used as preventive medication after 12 weeks of gestation. The therapeutic aim is to act on the presence of developing angiogenic imbalance, endothelial activation, oxidative stress, inflammation and vasoconstriction. Therefore, ongoing studies are focused on identifying biomarkers in early pregnancy and detecting the disease before the clinical onset of any symptoms, enabling better care and risk assessment. Traditionally, predicting preterm preeclampsia relied on maternal factors such as pregnancy history and maternal characteristics, including previous preeclampsia, obesity, chronic renal disease, chronic hypertension, nulliparity, maternal age (> 35 or 40 yrs), diabetes mellitus, conception by assisted reproductive technology, autoimmune disease, interpregnancy interval more than ten years. All of them are classified as moderate or high-risk factors. Screening solely with maternal factors has several disadvantages. It lacks sensitivity with a detection rate below 50%, it has binary classification without quantifying individual patient-specific risk, and it treats each of the risk factors as separate screening tests. A new first-trimester screening test uses predictive models to identify pregnant women at increased risk for early-onset preeclampsia. The multivariate models combine pre-existing risk based on three diagnostic steps. The first is the assessment of maternal risk factors with physical characteristics (body mass index, mean arterial pressure). The second is performing uterine artery Doppler ultrasonography by measuring the uterine artery pulsatility index, and the third is measuring the serum concentration of placental growth factor (PlGF) with or without pregnancy-associated plasma protein A (PAPP-A) measurement. It offers better sensitivity compared to relying solely on maternal factors, leading to more frequent monitoring of women deemed to be in the high-risk category. Different prediction models have been developed, but only a few have undergone external validation. The algorithm from the Fetal Medicine Foundation and the multivariate Gaussian distribution model are the most widely studied. Both identify 80-90% of pregnant women who will develop early preeclampsia, but they are less reliable for late preeclampsia, with a detection rate of 60-70% at a 10% false positive rate. Although they have shown similar performance in detection, there are substantial differences. The most used model is developed at large and validated in various populations by the Fetal Medicine Foundation; thus, it can be applied to different populations. Based on the Bayes theorem, the model represents the competition between delivery before or after the development of preeclampsia in theoretically infinite-lasting pregnancy. Hence, it enables the assessment of individual risks of preeclampsia that require delivery before a specified gestation. The time window for blood sampling is between 11+0 and 13+6 weeks. Another approach is the multivariate Gaussian distribution model, which consists of estimating risk a priori, month-over month (MoM) calculations based on specific population-derived medians, and posterior risk definition based on comprehensive prospective studies. The time window for blood sampling of PlGF and PAPPA is 8+0 weeks and 13+6 weeks Gaussian algorithm and a priori risk is not derived from the study population but from the more extensive meta-analysis.

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S3-4

Strategy for prediction and prevention of preeclampsia: a research perspective

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Preeclampsia is a multisystem disorder characterized by new-onset hypertension and proteinuria or end-organ dysfunction occurring typically after 20 weeks of gestation. It complicates 2 to 8% of pregnancies and remains one of the leading causes of maternal as well as perinatal morbidity and mortality worldwide. Although the exact mechanisms that lead to development of preeclampsia have not yet been fully elucidated, it is now clear that angiogenic imbalance is central to the pathophysiology of the disease. Angiogenic imbalance often presents weeks to months before overt clinical signs and symptoms of preeclampsia. Two placenta-derived angiogenic biomarkers, soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF), have proved useful for screening and early diagnosis of preeclampsia.
Most professional societies currently recommend offering all pregnant women 1st trimester screening for placenta-associated disorders (such as preeclampsia and fetal growth restriction). This can be based on maternal risk factors and blood pressure measurements alone, but detection rates are significantly higher with addition of uterine artery Doppler examination and maternal PlGF level measurements. Women at high risk for developing preeclampsia should be offered prophylaxis with low-dose acetylsalicylic acid starting at 12 weeks of gestation.
Angiogenic markers are also useful for early diagnosis of preeclampsia. Maternal sFLt-1 and/or PlGF concentrations can support and supplement clinical examination in order to confirm and exclude the diagnosis of preeclampsia or to assess the progression of disease. However, additional benefit of angiogenic markers in guiding clinical decisions remains unclear. We currently lack prospective interventional studies determining predictive value of angiogenic markers for maternal and perinatal outcomes in women with preeclampsia. As a result, at present, angiogenic imbalance (i.e., maternal blood concentrations of sFLt-1 and/or PlGF) in itself should not be considered an indication for delivery in the absence of clinical indications.

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S4 Biological therapy from a clinical and laboratory perspective

S4-1

What have we achieved with the development and application of biological therapy?

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Until the beginning of this century, patients with rheumatoid arthritis (RA) and spondyloarthritis (SpA) were treated exclusively with nonsteroidal anti-inflammatory drugs, glucocorticoids and drugs from the group of conventional synthetic drugs.
A major turnaround in the treatment and prognosis of these patients is the period in the last 20 years with the arrival of biological and targeted synthetic drugs.
According to the mechanism of action, biological drugs currently available in our country for the treatment of RA include tumour necrosis factor alpha (TNF-α) inhibitors, interleukin (IL) 6 receptor inhibitors and monoclonal anti-CD20 antibodies. In addition to TNF-α inhibitors, IL 17 inhibitors and IL 23 inhibitors are also used for the treatment of SpA. Targeted synthetic drugs act on the principle of inhibition of Janus kinase and are used in the treatment of both RA and SpA.
Rheumatoid arthritis and SpA are the most common inflammatory rheumatic diseases. Estimation of RA prevalence is about 1-1.5%, and most often affected are young and middle-aged women with characteristic morning stiffness and symmetrical swelling of the small joints of the hands. In addition to causing a high rate of disability, the disease is also associated with significant comorbidities. Patients with RA have a significantly higher risk of cardiovascular disease, interstitial lung disease and fibrosis, an increased risk of infections, osteoporosis, anaemia and amyloidosis. That causes significantly higher mortality.
The prevalence of SpA is estimated at 0.5-1.9%, and the disease can be axial if it predominantly affects the spine and sacroiliac joints, or peripheral if it predominantly affects peripheral joints or enthesis. The prototype of axial disease is ankylosing spondylitis in which structural damages of the spine and/or sacroiliac joints are visible on X-rays, most often in the form of sacroileitis. Clinically, such a condition is reflected in the presence of inflammatory low back pain, but is also associated with extra-articular manifestations such as inflammatory bowel disease, psoriasis, anterior recurrent uveitis and heart involvement.
The positive effects of biological and targeted synthetic therapy on the joints, axial skeleton, as well on the extra-articular manifestations of RA and SpA have also influenced the raising of awareness of early recognition and diagnosis of these diseases, which is primarily based on medical history and clinical evaluation, in addition with modern laboratory and radiological diagnostics.
Early recognition of RA and SpA and timely initiation of treatment led to a significantly better prognosis of these patients in terms of articular and extra-articular manifestations and associated comorbidities. These are the reasons for the reduced rate of disability, better work productivity, improved quality of life and longer survival of these patients.

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S4-2

Laboratory testing in biological therapy era - are we able to recognize possible interferences?

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As we entered the era of biological therapy, especially the use of monoclonal antibodies (mAb) in clinical treatment, we became more aware of the significance of laboratory testing before and after the treatment to screen for potentially sensitive patients, check the patients' health status as well as to evaluate the response to treatment. Considering the structure and mechanism of action of mAbs, it is justified to raise a concern about the possible interference of mAbs with clinical laboratory tests. Limited literature data is available regarding this issue so this lecture summarizes published data as well as an experience in routine laboratory work.
Because mAbs are widely used for both diagnostics and treatments, there could be cross-reactions for some laboratory tests. So far, some of the detected interferences are ones of Palivazumab with respiratory syncytial virus (RSV) immunological assay, Abatacept with blood glucose testing, Omalizumab with IgE, Abciximab with thrombocyte count, Elotuzumab with serum proteins electrophoresis (SPE) and immunofixation (IFE) techniques, Certolizumab pegol with activated partial thromboplastin time (aPTT) etc. Daratumumab is so far the most influencing mAb that interferes with five different laboratory tests including serological testing, indirect Coombs test, and serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE). Most common mAb interference is direct cross-reactions with the test targets, but also the suppression of the patient's physiological functions and activation of inflammatory processes. Awareness, detecting and resolving the interference is critical to ensure the accuracy of the results and safety of patients. Using an alternative method of testing would be optimal for excluding the interference but that is often not possible so many interferences, especially in automated methods remain unresolved. Detecting mAb interference in SPE and IFE methods is easier because we are able to visualize them. Recently, it even became possible to remove the interferences in IFE using antiserum to Daratumumab. When in doubt of the possible interference, the communication between laboratory staff and clinicians is crucial, so when a laboratory reports show non-conformity with the status or medication history of the patient it is easier to investigate and warn about possible impact on altered or questionable results. New research and publications will allow us to expand knowledge and include new information in interpretation of laboratory results in patients treated with mAbs. This also indicates importance of incorporating data of possible interferences in laboratory and hospital information systems in order to facilitate the alert of laboratory professionals.

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S4-3

The utility of therapeutic drug monitoring of biologics and antibodies to biologics

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Biologic therapy represents one of the most significant advances in the treatment of chronic and autoimmune diseases in gastroenterology, immunology, dermatology and neurology, such as Crohn's disease (CD), ulcerative colitis (UC), rheumatoid arthritis (RA), psoriasis and ankylosing spondylitis.
Biologics, which are large, complex proteins often derived from living cells, have revolutionized the management of these conditions by targeting specific components of the immune system.
Despite the proven effectiveness and success of biological drugs, some patients do not respond adequately to this type of therapy. In almost 30% of patients with inflammatory bowel diseases, the primary response during the induction therapy with TNF antagonists may be absent. The underlying reasons for primary non-response (PNR) may involve altered pharmacokinetics such as insufficient drug exposure and pharmacodynamics associated with inadequately targeted anti-inflammatory therapy.
In patients who showed improvement of clinical symptoms during the induction therapy with TNF antagonists, secondary loss of response (SLR) to therapy and relapse may occur over time. It has been observed that up to 50% of patients with RA and CD develop secondary response failure. The cause of SLR may be a consequence of immunogenicity by development of antibodies to the drug (Anti-Drug Antibody, ADA).
In the beginnings of biologic therapy, the approach to its monitoring was empirical. During PNR and SLR, empirical adjustments were performed in doses and intervals according to clinical parameters.
Over time, the need for better disease control emerged to ensure the maximum clinical benefit from the use of biologic therapy. Therapeutic drug monitoring (TDM), which measures the serum concentration of a biologic drug and ADA, allows clinicians to achieve better clinical therapeutic efficacy with greater precision than the empirical approach.
During TDM, it is optimal to measure the trough concentration of the drug, right before the next administration of the drug.
There are two strategic approaches to the therapeutic drug monitoring, i.e., reactive and proactive. Reactive TDM measures the serum concentration of the drug and ADA in the case of loss of therapeutic response in PNR and SLR. Reactive TDM can be helpful to distinguish between immunogenic, pharmacokinetic, and pharmacodynamic loss of response. Reactive TDM is widely accepted in practice, especially for anti-TNF therapy, e.g., the American Gastroenterological Association recommends reactive TDM to guide treatment changes.
Depending on the results of drug concentrations and ADA, in the clinical context of therapeutic failure, an algorithm of interpretation of laboratory results with four possible scenarios is proposed. In this way, it is possible to optimize the dose by escalation or de-escalation of the therapy or changing the drug administration interval, as well as switching to a different agent within or out of the class.
A proactive TDM approach measures drug and ADA concentrations in predetermined time periods, regardless of disease activity, targeting optimal drug levels to achieve better outcomes and prevent therapy failure. The goal of proactive TDM is to predict the patients' needs, prevent side effects and increase the long-term effectiveness of the therapy, which enables the personalization of the therapy. Proactive monitoring of anti-TNF drugs is recommended in patients with high inflammatory burden, and increased drug clearance, whose risk of inadequate drug exposure, immunogenicity and treatment failure is high.
Treatment with biologics is expensive due to the complex process of production and development. The cost of treating an individual patient with biologic therapy represents a significant burden on the healthcare funds and can limit the availability of treatment. The use of TDM can significantly reduce treatment costs through improved treatment outcomes and reduced drug consumption.

Several large studies have proven that reactive TDM with infliximab compared to the empirical approach reduces costs by more than 50% without affecting treatment outcomes. Proactive TDM for infliximab can have a long-term clinical benefit with a moderate cost-effectiveness compared to a reactive approach.
Monitoring the concentration of biologic drugs and ADA is essential for achieving optimal results in the treatment of patients. This approach enables personalization of therapy, reduces the risk of side effects, optimizes costs and improves the quality of life of patients. The introduction of different approaches to treatment, i.e., reactive and proactive, allows medical doctors to select the strategy that best suits the individual needs of the patient and the specific aspects of the disease. The combination of these approaches, along with regular monitoring and adjustment of therapy, is key to achieving optimal results in the treatment of chronic and autoimmune diseases. By introducing regular monitoring, health systems can better manage resources and achieve better clinical outcomes for their patients and enable long-term disease control.

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S4-4

Clinical perspective in development and implementation of biological therapy

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Clinical perspective in development and implementation of biological therapy should be viewed in two ways. One is the growing need for new therapeutical options and the other is challenges in assessing the effects of such treatment due to lack of reliable biomarkers in individual diseases.

In autoimmune disorders kidney is often the target organ and the disease course usually leads to kidney failure and either death or the need for kidney replacement therapy. Steroids are still the cornerstone of treatment of autoimmune diseases in nephrology. As effective as they are, steroids carry the risk of multiple adverse effects such as increased infection rates, Cushing's syndrome, diabetes, myopathy, psychological changes, osteoporosis and other. Other therapeutical modalities are limited and it has only been in the recent two decades that we have seen progress in treatment options in nephrology.

The main drives for developing new biological drugs are the need for the new therapeutical options, better understanding the pathophysiology of the disease and specific pathways and the fact that current drugs have either low success rate or significant side effects.

One size does not fit all, is perhaps, the main reason we fail to develop new drug class that could be used in most of diseases with similar pathophysiology, except maybe in the case of steroids. The second reason is the difference between the data from clinical drug trials and real-life data mainly due to selection criteria for patient inclusion in clinical trials.

How do we assess the success of the treatment in autoimmune kidney diseases? There are no widely used reliable biomarkers. Even if these are intrinsic to disease pathophysiology such as ANCAs, ANA, anti dsDNA, complement concentrations, CD19/CD20 count, CD4/CD8 count, they were shown not to be reliable. There is a promise on urine soluble CD163 but it has yet to be applied in practice. We still mostly use surrogate markers, i.e. eGFR slope, serum creatinine concentrations and proteinuria.

The challenges in new treatment development and practical implementation can be illustrated through three diseases: ANCA associated vasculitis (AAV), IgA nephropathy (IgAN) and Lupus nephritis.

ANCA vasculitis treatment included biologics only recently, firstly rituximab and recently there were attempts to use tocilizumab, belimumab but with limited success. Avacopan, oral anti C5aR drug, though not an antibody was approved as a first viable substitution for steroids in the induction treatment. There are ongoing trials concerning the use of rituximab combined with telitacicept in induction treatment, telitacicept in the remission maintenance treatment, rituximab with belimumab combined as well as abatacept.

IgA nephropathy is the most common glomerular disease in the world and yet there was little or no progress in drug development up until recently with the approval of budesonide (oral steroid; no absorption in the gut, local effects on Peyers lymphatic tissue).

At the moment there are several ongoing clinical trials in IgA including the use of Felzartamab (anti-CD38). In this IGNAZ study premise is that CD38+ plasma cells are likely the main source of pathogenic Gd-IgA1, and the related autoantibodies in IgAN. Felzartamab is being studied across immune-mediated kidney diseases beyond just IgAN such as primary membranous nephropathy, lupus nephritis, and antibody mediated rejection.

Since recent findings on alternative complement activity in IgN, Iptacopan (LNP023) is an oral, proximal alternative complement inhibitor studied in IgAN.

Molecules such as anti-APRIL, or BAFF/BLYS inhibitors such as blisibimod, humanized IgG4 anti-APRIL, as well as atacicept, a soluble TACI-Immunoglobin fusion protein, that may dually inhibit BAFF- and APRIL-mediated B-cell class switching to reduce antibody concentrations, are all studied in IgA nephropathy and other diseases.

Lupus nephritis is difficult to treat due to varying success of biological drugs. There was not much success with rituximab though it is used, belimumab shows promises but long-term follow up is needed. Cyclophosphamide is still the mainstream therapy alongside mycofenolic acid or calcineurin inhibitors. Currently Felzartamab (anti-CD38) and atacicept as well as some new molecules are being studied in lupus nephritis trials.
I would also like to mention the use of plasma exchange treatment (PLEX) in AAVs and sometimes in lupus patients and its effect not only on disease outcomes but effects on laboratory results. Novel add-on therapy to prevent worsening of kidney function that includes SGLT2 inhibitors, finerenone and GLP1R antagonists affect both clinical and laboratory follow up.
To summarize, development, approval and implementation of novel biological drugs in autoimmune diseases consists of both progress and setbacks with the main aim being achievement of stable disease remission both clinically as well as objectified by quantifiable, reliable biomarkers.

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S5 Challenges of biomarker application in cardiometabolic diseases

S5-1

Cardiometabolic biomarkers: a biochemist's view

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Cardiometabolic diseases (CMD) remain a leading cause of morbidity and mortality globally. Despite advances in healthcare, the decline in CMD-related morbidity and mortality has plateaued in recent years, underscoring the urgent need for novel biomarkers to unravel the underlying pathophysiological mechanisms and improve disease management.

In this session, we review established CMD biomarkers and explore emerging ones, alongside discussing innovative technologies and analytical approaches such as omics technologies.

Well-known cardiac biomarkers such as cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are highlighted. It is widely recognized that cTnI is released earlier and in higher concentrations following myocardial injury, whereas both cTnI and cTnT exhibit high diagnostic accuracy when used in the European Society of Cardiology (ESC) 0/1-hour algorithms for acute myocardial infarction (MI). However, recent studies call for further exploration of sex-specific differences in these biomarkers. Moreover, there are currently no guidelines recommending widespread population screening of cTn for cardiovascular disease (CVD), despite its potential to improve current risk scores in the general population. Another area lacking clarity is how to treat patients with mildly elevated high-sensitivity cTn concentrations, who face an increased long-term risk.

The role of natriuretic peptides in heart failure was thoroughly examined, particularly N-terminal pro B-type natriuretic peptide (NT-proBNP), which is valuable for risk prediction, diagnosis, and treatment monitoring in CMD patients. Emerging biomarkers such as insulin-like growth factor binding protein 7 (IGFBP-7) are also discussed, with recent evidence suggesting its involvement in cardiac senescence and inflammation, marking it as a promising target for personalized therapy in heart failure.

Another exciting development is the identification of SVEP1, an extracellular matrix protein expressed in vascular smooth muscle cells, which plays a role in promoting inflammation and atherosclerosis. Other promising risk stratification biomarkers include growth differentiation factor 15 (GDF-15), bone morphogenetic protein 10 (BMP-10), fibroblast growth factor 23 (FGF23), and angiopoietin 2 (ANGPT2).

Regarding lipid metabolism and its role in CMD and atherosclerosis, well-established lipid biomarkers such as low-density lipoprotein (LDL), non-HDL cholesterol, high-density lipoprotein (HDL), apolipoprotein B (APOB), and lipoprotein (a) (Lp(a)) are discussed. Lipoprotein (a) is a genetically determined variant of LDL and is increasingly recognized as a significant risk factor for CVD. Current guidelines recommend measuring Lp(a) in individuals at high CVD risk, with growing evidence linking elevated Lp(a) concentrations and high-sensitivity C-reactive protein (hsCRP) to an increased risk of CVD.

The session also explores the role of inflammation and obesity in CMD development. C-reactive protein (CRP) is strongly associated with insulin resistance and atherosclerosis. Additional inflammatory mediators such as interleukin-6 (IL-6), soluble vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) are closely linked to CMD incidence. Biomarkers associated with both insulin resistance and obesity, including retinol-binding protein 4 (RBP4), branched-chain amino acids, adiponectin, and leptin, are also discussed.

Looking forward, integrated diagnostics, including biomarker panels and large-scale proteomic, metabolomic, and transcriptomic analyses, represent the future of CMD research. Polygenic risk scores (PRS), which assess millions of genes across the genome, hold potential for CMD prevention and risk stratification, though

their clinical utility remains under investigation. The development of point-of-care testing (POCT) for biomarkers could also revolutionize CMD risk stratification in primary care or even home settings.
In a study presented during the session, the enzyme amylase was investigated as a marker of sympathetic overactivity in patients with myocardial infarction (MI). Excessive sympathetic activation is known to be associated with worse outcomes in MI. The study included 202 patients admitted for acute MI, who underwent comprehensive clinical evaluation, echocardiography, and invasive cardiology work-ups. Patients with adverse outcomes had significantly higher serum amylase activitiy (87 U/L vs. 55 U/L, P<0.001). Multivariate analysis revealed that serum amylase activity independently predicted adverse outcomes, regardless of age, sex, MI type, creatinine and cardiac troponin concentrations. These findings suggest that serum amylase may serve as a simple, non-invasive marker of increased sympathetic activity and adverse outcomes in MI patients, offering a potential new tool for risk stratification.

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S5-2

Thinking outside of the box: The role of CA125 in heart failure

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Congestive heart failure is a multifaceted clinical syndrome marked by impaired myocardial function, elevated intracardiac pressures, and/or insufficient cardiac output at rest and/or during exertion. While the crucial role of congestion in heart failure is well recognized, its link to disease progression remains unclear, frequently leading to suboptimal management strategies. Consequently, despite notable advancements in pharmacological and device-based therapies that have significantly improved mortality rates across nearly all age groups, the prognosis for heart failure remains poor. One of the possible approaches to overcome this issue is the ostensible use of blood biomarkers in the management of these patients.

CA125 is widely recognized as a biomarker of ovarian cancer. Although its clinical application has primarily focused on the management of patients with suspected or confirmed ovarian cancer, elevated plasma CA125 concentrations are not exclusive to neoplastic conditions. CA125 concentrations can also rise during menstruation and early pregnancy. Additionally, CA125 is upregulated in various pathological states, such as peritoneal trauma, liver cirrhosis, pelvic inflammatory disease, ascites, and lung malignancies. Emerging evidence further implicates CA125 in the pathophysiological mechanisms of heart failure, where its serum concentrations have been associated with tissue congestion. The precise mechanisms leading to elevated plasma CA125 in the context of heart failure remain to be fully understood, but current data suggest that at least two partially overlapping pathophysiological processes drive this increase. The first is direct mechanical stress due to volume overload, which results in the shedding of CA125 from the cell surface. The second involves low-grade inflammation, a common feature of heart failure, which enhances CA125 synthesis via the c-Jun N-terminal kinase (JNK) pathway. As inflammation exacerbates volume overload, and vice versa, these processes appear to collectively contribute to the rise in circulating CA125 concentartions.

There are several reasons why it might be beneficial to include CA125 in the management of patients with heart failure. First, CA125 is a very useful parameter in differentiating between patients with true volume overload, in which one would expect elevated CA125 concentrations, and intravascular congestion, in which CA125 is usually not elevated. Namely, the latter group of patients benefits from more intensive diuretic therapy, and since it is not easy to differentiate between the two solely on clinical grounds, the use of CA125 can be beneficial in this setting. Furthermore, CA125 can be used in tailoring diuretic therapy in a way that serial measurements of CA125 at the time of decompensation can inform clinicians on whether the diuretic regimen should be intensified or the dose should be reduced. Finally, CA125 is an independent predictor of poor outcomes in these patients. Conversely, the primary factors that hinder the use of CA125 in managing congestion include: 1) its long half-life, which restricts the ability to infer short-term changes; 2) its lack of specificity, as it can be elevated in conditions such as ovarian tumors, pleural effusions, and cirrhosis; and 3) the need to consider sex differences when interpreting CA125 concentrations, since female patients may exhibit higher concentrations than males in the context of heart failure.

In conclusion, while CA125 shows promising potential as a biomarker for managing heart failure, the current limitations and scarcity of data necessitate further large-scale studies. These studies are essential to fully determine and validate the role, optimal timing of measurement, and impact of CA125-guided management on clinically relevant outcomes in heart failure.

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S5-3

The role of GDF-15 in cardiovascular diseases: from pathophysiology to practice

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This lecture will summarize the importance of growth differentiation factor-15 (GDF-15) as an emerging and important biomarker, as well as potential therapeutic target in cardiovascular disease (CVD). This stress-responsive cytokine, a member of the transforming growth factor-β superfamily, plays a complex role in the pathophysiology of various cardiovascular conditions. GDF-15 is normally expressed at low levels in most tissues but is markedly upregulated in response to cardiovascular injury, inflammation, oxidative stress, and ischemia. Cardiomyocytes, macrophages, vascular smooth muscle cells, endothelial cells, and adipocytes have all been shown to produce GDF-15 under pathological conditions. In atherosclerosis, GDF-15 expression increases in response to oxidized LDL and is elevated in atherosclerotic plaques. While its exact role is still debated, GDF-15 appears to have both pro- and anti-atherogenic effects. It may promote plaque instability but also exhibits anti-inflammatory and antiapoptotic properties that could be protective. Furthermore, GDF-15 has demonstrated cardioprotective effects in animal models of ischemia-reperfusion injury. It activates protective signaling pathways like PI3K/Akt and ERK in cardiomyocytes, reducing infarct size and mitigating damage. However, chronically elevated levels in heart failure and following myocardial infarction are associated with worse outcomes, suggesting a maladaptive role in the long term.

When considering clinical studies, elevated circulating GDF-15 concentrations have been consistently associated with increased cardiovascular and all-cause mortality across a spectrum of CVD, including acute coronary syndromes, stable coronary artery disease, and heart failure. GDF-15 provides prognostic information independent of traditional risk factors and other biomarkers like troponins and natriuretic peptides. However, the relationship between GDF-15 and non-fatal cardiovascular events appears to be less clear, with inconsistent associations reported for outcomes. However, higher GDF-15 concentrations have been linked to more severe coronary artery disease on angiography and increased risk of heart failure hospitalization. GDF-15 may also play a role in endothelial dysfunction, a key early event in atherosclerosis. It promotes endothelial cell proliferation, migration, and nitric oxide production through activation of Akt, ERK, and SMAD2 pathways. Elevated GDF-15 is associated with impaired flow-mediated dilation, suggesting it may be a marker of endothelial dysfunction. In the clinical setting, GDF-15 shows promise as a biomarker for risk stratification in both primary and secondary prevention. It improves risk prediction when added to traditional risk factors and other biomarkers in healthy individuals and those with established CVD. GDF-15 may be particularly useful in identifying high-risk patients who could benefit from more aggressive therapy. However, several challenges remain before GDF-15 can be widely implemented in clinical practice. Its inverse relationship with LDL cholesterol and the impact of renal function on its concentration need further investigation. Standardization of assays and establishment of universal cut-off values are also necessary. While GDF-15 does not appear useful for diagnosing acute myocardial infarction, it retains prognostic value regardless of the etiology of chest pain or baseline cardiovascular risk. This suggests it could be a valuable addition to risk assessment algorithms across the spectrum of CVD.

In conclusion, GDF-15 is a promising biomarker in CVD that reflects multiple underlying pathophysiological processes including inflammation, oxidative stress, and myocardial injury. Its strong association with mortality and potential to improve risk prediction make it an attractive candidate for clinical use. However, further research is needed to fully elucidate its biological roles and optimize its application in cardiovascular medicine. As our understanding of GDF-15 grows, it may not only serve as a biomarker but also point towards new therapeutic strategies in CVD.

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S5-4

Interpretation of cardiometabolic parameters: challenges in the intensive care unit

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Historically, elevated troponin concentrations have suggested the presence of myocardial necrosis, but it is now known that this laboratory parameter is elevated in many other conditions without the presence of myocardial necrosis. The implementation of hs-Troponin assays has shown that they have higher negative predictive value for acute myocardial infection, meaning that a negative test result has a much higher certainty for truly representing the absence of acute myocardial infarction. This, however, poses another challenge in the clinical setting: increased sensitivity of this test has now been shown to detect hs-Troponin in a variety of clinical situations that are not traditionally associated with myocardial infarction. Although the pathophysiological interpretation of elevated hs-Troponin values in noncardiac conditions is not entirely clear, studies suggest that its presence suggests worse outcomes in these patients.
From a clinical point of view, we must differentiate between myocardial injury, myocardial ischemia and myocardial infarction. The term "myocardial injury" refers to elevated hs-Troponin concentrations regardless of the underlying cause. Myocardial injury can be further categorized as acute or chronic. Acute myocardial injury is used for describing a dynamic increase or decrease in hs-Troponin values that exceed typical biological values or analytical variation, while chronic myocardial injury is characterized by permanently elevated hs-Troponin values. Myocardial ischemia is defined as a mismatch between myocardial oxygen supply and metabolic demand. Classically, it is the result of narrowing of the coronary lumen secondary to atherosclerosis. However, myocardial infarction is defined by the presence of acute myocardial injury and evidence of myocardial ischemia.
Many studies in intensive care patients, who mostly have signs of multiorgan failure, indicate that elevated hs-Troponin values are correlated with morbidity and mortality. Reflexively associating hs-Troponin with myocardial infarction should be avoided by clinicians, as well as using terms such as "troponinemia", "troponin leak" or "troponinitis". However, question arises when elevation of hs-Troponin is found in intensive care patients, how to properly interpret the findings and the need for further cardiac diagnostics, or we can always say "it never means anything". In conditions such as acute respiratory distress syndrome (ARDS), septic shock, acute kidney injury, rhabdomyolysis or gastrointestinal bleeding, a rise in hs-Troponin is noted.
Of 1057 patients with ARDS in the FACTT and ALVEOLI studies, 94% had elevated hs-Troponin values without any sing or symptoms of acute myocardial ischemia. As both studies excluded patients with documented signs or symptoms of myocardial ischemia, it suggests that these results relate to the presence of myocardial injury. Cellular changes in myocytes without presence of necrosis are mentioned as the mechanism of hs-Troponin elevation, which includes increased myocyte permeability with a change in the cell membrane and results in the release of troponin. As we observe patients with acute kidney injury, which are admitted to intensive care without the presence of cardiovascular symptoms or conditions, studies have shown that 30% of them have elevated hs-Troponin values. A decrease in kidney clearance is mentioned as the cause od increased hs-Troponin. We know that in patients with acute kidney injury, function of kidneys is initially reduced, which is followed by recovery, therefore initially elevated hs-Troponin with a control decrease in values, often causes confusion in the interpretation of findings. We know that rising and falling hs-Troponin concentrations are more indicative of acute myocardial infarction, while stable hs-Troponin concentrations are more indicative of chronic myocardial injury. A special entity are patients with septic shock. It is estimated that 31-80% of patients with a systemic inflammatory response have elevated hs-Troponin values. The true mechanism in not fully understood. However, in the literature, mismatch of the myocardial need for oxygen and supply,

microvascular dysfunction and the influence of inflammatory mediators on the increased permeability of the myocardial cell membrane, are mentioned as causes. In previous prospective studies, it has been shown that these patients have a higher cardiovascular risk, even those who had no previous cardiovascular disease, unlike patients with septic shock in whom no increase in hs-Troponin is recorded.
Interpretation of abnormal hs-Troponin concentrations is both a science and an art especially in intensive patients. It depends on careful consideration of anamnestic data, patient examination, findings of electrocardiography and with observation of all laboratory data as a part of the whole clinical picture in order to bring validate diagnosis, but also the therapy itself, which greatly affects the outcome of patients.

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S6 New insights into metabolic diseases

S6-1

Lysosome – more then a recycling center

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Since its discovery in 1955 by the Belgian biochemist Christian De Duve, lysosomes are best known as the primary degradative compartment of eukaryotic cells. Lysosomes are single membrane-limited, dynamic, heterogeneous organelles, which vary in their positioning, morphology, size, enzyme content, and substrates. They are intracellular organelles with an acidic interior. Lysosomes are central for degradation and recycling of macromolecules delivered by endocytosis, phagocytosis, and autophagy.
In the recent years, our understanding of lysosomal biology has progressively improved. Lysosomes are no longer viewed as organelles exclusively involved in catabolic pathways, but rather as highly dynamic elements of the autophagic-lysosomal pathway, involved in multiple cellular functions, including signaling, and able to adapt to environmental stimuli.
Nowadays we know that lysosomes are dynamic organelles, which can fuse with a variety of targets and undergo constant regeneration. They can move along microtubules in a retrograde and anterograde fashion by using motor proteins, kinesin and dynein, being main players in extracellular secretion, intracellular components degradation and recycling. Moreover, lysosomes interact with other intracellular organelles to regulate their turnover, such as endoplasmic reticulum, mitochondria and peroxisomes.
This lecture will present new refined vision of lysosome and show how this has impacted our understanding of the lysosomal contribution to diseases, including lysosomal storage disorders, neurodegenerative disorders, cancer, cardiovascular diseases and aging.

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S6-2

A new class of substrates for glucocerebrosidase derived from plant food

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Deficiency of the enzyme glucocerebrosidase (GCase) causes Gaucher disease (GD), a recessively inherited lysosomal storage disorder (LSD) caused by mutations in the GBA1 gene coding for GCase, the lysosomal beta-glucosidase. The primary substrate for GCase is the glycosphingolipid glycosylceramide (GlcCer). Endogenous glycosphingolipids, including GlcCer, normally are fragmented in lysosomes during recycling. Expectedly, in GCase-deficient GD patients the lipid GlcCer is poorly fragmented in lysosomes and consequently massively accumulates there, particularly in lysosomes of tissue macrophages. The characteristic lipid-laden macrophages ('Gaucher cells') of GD patients, have a unique morphology and release specific proteins (chitotriosidase, s-GPNMB, CCL18) and lipids (glucosylsphingosine; GlcSph). These presently serve as biomarkers assisting diagnosis and disease monitoring. Recently the occurrence of glucosylated metabolites like glucosylated cholesterol (GlcChol) has been recognized and abnormalities in GD patients have been observed. The role of GCase and that of a cytosolic beta-glucosidase GBA2 in metabolism of glucosylated metabolites ('glucolites') has been elucidated. GlcChol and other glucosylated metabolites seem potential next-generation biomarkers of GD.

In this brief presentation various GD biomarkers are discussed, and attention is focussed to an exciting discovery in this respect. Plants contain glucosylated phytosterols quite similar in structure to human GlcChol. Prominent is glucosylated sitosterol (Glc-sitosterol). We observed that Glc-sitosterol is a substrate for GCase in the test-tube. Moreover, Glc-sitosterol (and other plant Glc-phytosterols) are, like GlcChol, increased in spleens of type 1 GD patients. It appears that plant food derived Glc-phytosterols are absorbed and need to be degraded by GCase.

This seems the first example of a lysosomal enzyme in viscera being involved in degradation of a food-derived substrate. The potential implications of the new findings are discussed.

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S6-3

Laboratory diagnostics of inherited metabolic disorders in the age of new technologies: possibilities and challenges

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Inherited metabolic diseases (IMDs) are one of the subgroups of rare diseases. Their clinical data are highly heterogeneous, often nonspecific and multisystemic, while symptoms of various intensity may occur at any age of life. Establishing early diagnosis and introducing therapy at presymptomatic stage have become the major challenges in the IMD field. Fast development of the technologies used in laboratory diagnostics also considerably contributes to detection of novel IMDs.

For a number of these disorders, the best diagnostic approach is implementation of newborn screening from a dried blood spot on a filter paper. However, metabolic screening after established diagnosis leaves a large room for diagnostics of more than 1900 rare metabolic disorders. Understanding the underlying pathophysiological basis of an individual IMD enables the choice of adequate diagnostic approach and application of appropriate therapy. In this process, the availability and use of latest technologies in metabolic diagnostic centers have a significant role, as well as multidisciplinary approach to interpretation of obtained results.

Traditional approach to laboratory diagnostics of IMDs ordinarily starts with basic and specific metabolic tests. These tests rely on analysis of certain metabolites that are intermediate and end products of various enzymatic processes. Identification and measurement of concentration of such molecules with the known chemical structure in biological samples have been made possible by methods of targeted metabolomics. Different spectrophometric, fluorimetric, immunochemical and simple chromatographic methods have been utilized in diagnostic centers for analysis of such molecules. However, these methods are being increasingly replaced by more specific and sensitive ones, by combinations of chromatographic methods with mass spectrometry that allow separation of various molecules in the sample based on the mass to charge relationship. The most often used methods of targeted metabolomics in clinical practice are gas chromatography coupled with mass spectrometry and high performance liquid chromatography coupled with tandem mass spectrometry.

Unlike targeted metabolomics, nontargeted metabolomics offers a possibility of detection and measurement of all analytes and related substances in biological samples. Therefore, such analytical approach provides better insight into complex metabolic pathways and allows broader view of the metabolic phenotype of disease, while it also contributes to understanding of the pathophysiogical background of rare diseases and to detection of novel specific markers. Different combinations of instruments based on high-resolution mass spectrometry are in routine use as they allow metabolomic profiling from a small volume of biological samples, i.e. next generation metabolic screening. However, fundamental limitation for a wider application of nontargeted metabolomics in clinical practice is impossibility of method standardization, along with still high costs of laboratory equipment. Additional possibility for better understanding of the pathophysiological background of the IMDs that involve accumulation of metabolites within cells and tissues may be found in using matrix-associated laser desorption ionization time-of-flight. In addition to mass spectrometry, highly specific nuclear magnetic resonace spectroscopy of hydrogen nuclei from body fluids may also be utilized. Still, the ever more frequent use of metabolomic screening technologies results in identification of a number of features of unknown significance. Management of an exceptionally high number of complex data in nontargeted metabolomics is certainly the strongest challenge, for which there is still no standardized approach that could be applied in routine diagnostics. It should be noted that considerable assistance in this process is provided by artificial intelligence tools.

Development and application of next generation sequencing (NGS) have the greatest influence on contemporary IMD diagnostics. Next generation sequencing methods have become acceptable in terms of price whi-

le utilization of bioinformatics has improved data processing speed. The experience gained so far has shown that appropriate and rational application of these methods may significantly shorten the path to diagnosis and enable proper therapy approach and genetic counselling. Fast development and broad application of NGS methods bring along numerous challenges in interpretation of obtained results and gene variants. Transcriptomics plays an increasingly important role in this, as well as RNA sequencing and functional genomics.
Novel technologies also allow implementation of analyses from dried blood spots or urine on standardized filter paper. Such approach has considerably facilitated sample transport to diagnostic centers and sped up the path to diagnosis. Volumetric absorptive microsampling is a new method of sampling small blood volumes that reduces hematocrit influence.
Traditional approach to laboratory diagnostics of IMD is increasingly being replaced by novel technologies and sample types for analyses that, however, also involve new issues. Present-day approach should strive for preventive, predictive, personalized and participatory medicine. Such laboratory medicine requires the use of various omics (metabolomics, lipidomics, proteomics, epigenomics, genomics, transcriptomics, glycomics, metagenomics, etc.), functional analyses, and collaboration of a multidisciplinary team in interpreting a large amount of obtained data. On this way, there are still many obstacles that should be gradually overcome in order to transform the data provided by current technologies into clinically useful information.

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S6-4

Current strategies for the treatment of inherited metabolic diseases

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Inherited metabolic diseases are a growing group heterogenous genetic disorders with around 1900 different entities described to date. The common pathogenesis is the disruption of important biochemical processes that cause an accumulation of toxic molecules or a deficiency of important substrates, leading to disturbed cellular homeostasis and a wide range of disease manifestations. Clinical presentation is very diverse, but the majority of the IEMs are multisystemic, progressive, and very often debilitating with poor prognosis if left untreated. Luckily, today are many inherited metabolic diseases treatable, or at least partially treatable, and we are witnessing new therapies becoming available. With specific treatment, the phenotype of some diseases has been completely changed. One great example is phenylketonuria, the first-ever treated metabolic disease, in which neurodegenerative processes are halted with early-onset dietary treatment. Dietary management is still a cornerstone of the treatment of many disorders, particularly those affecting amino acid metabolism. With the aim to detect patients before irreversibile damage occurs, newborn screening was implemented first for phenylketonuria 60 years ago, and during the years for dozens of other conditions. Today newborn screening is one of the most important tools for early diagnosis and timely treatment of congenital diseases. The important breakthrough in the treatment of inherited metabolic diseases was enzyme replacement therapy, which revolutionized the management of lysosomal storage diseases and dramatically improved the outcome of many patients. Another important progress was organ transplantation, which has been used successfully in a range of conditions. Recent advances in molecular genetics, biotechnology, and personalized medicine significantly changed the therapeutic landscape, with gene therapy, mRNA-based treatments, small molecule therapies, and next-generation enzyme replacement therapies that are becoming the standard of treatment. Early diagnosis through newborn screening and next-generation sequencing coupled with these innovative therapies and multidisciplinary approach have dramatically improved outcomes and quality of life for individuals suffering from inherited metabolic diseases.

Modern treatment approaches in lysosomal storage disorders nicely represent the shift towards precision medicine. Lysosomal storage diseases are a group of 70 different disorders caused by the dysfunction of lysosomal enzymes or transporters, resulting in accumulation of undegraded substrates and consequent organ dysfunction. The first treatment effective for some lysosomal diseases was hematopoietic stem cell transplantation. Since 30 years ago, when the first disease specific therapy for Gaucher disease became available, enzyme replacement treatment become a mainstay for several lysosomal diseases. Unfortunately, there are important limitations of enzyme replacement therapy, such as inability to cross the blood-brain barrier, limited effectiveness across all tissues, and immune responses. Advances in protein engineering and biotechnology have led to the development of new-generation enzymes that are more effective and less immunogenic. There are also new delivery approaches, such as intrathecal administration, and nanotechnology-based drug delivery systems that are being investigated. Another treatment modality is small molecule-based treatment, which, due to the small size of molecules, has the advantages of good biodistribution and peroral administration. Small molecule treatments include pharmacological chaperones, which stabilize and enhance residual enzyme activity, and substrate reduction therapy, which reduces the production of the accumulated substrates. Gene therapy has emerged as promising treatment option for many patients with neurodegenerative lysosomal diseases and it is slowly entering in the clinical practice (now being available for the treatment of metachromatic leukodystrophy). Despite having various treatment modalities accessible, there are still significant unmet needs for optimal treatment and patient outcomes. Research is ongoing to develop more effective therapies, including novel gene-editing techniques and combination therapies.

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Working groups sessions

WG-1 Working group for seminal fluid

Automation of seminal fluid analysis. Revolution and the future?

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Semen analysis is an underrepresented procedure in Croatian medical-biochemistry laboratories (MBLs), facing challenges related to standardization, harmonization, and lack of guidelines. Recognizing the need for improvement, the Croatian Society of Medical Biochemistry and Laboratory Medicine (CSMBLM) established the Working Group for Semen Analysis (WG SA) to develop clinical practice guidelines for MBLs. To gather data, WG SA conducted an online survey between November and December 2023. A total of 19 laboratories participated, answering 17 questions that covered preanalytical, analytical, and postanalytical aspects of semen analysis.

Participants in the survey were primarily from clinical hospital centers and hospitals, accounting for 80% of the responses. The participating laboratories typically handle between 200 and 700 semen samples annually. In the preanalytical phase, it was noted that patients generally receive instructions on sample collection and requirements, with 67% of the laboratories providing these instructions on their websites. However, fewer than half of the laboratories allowed patients to deliver samples from home, highlighting a need for more flexible preanalytical processes.

The analytical phase revealed significant inconsistencies among laboratories. For example, while semen volume is consistently determined across all participating laboratories, other macroscopic parameters lack uniformity in analysis and reporting. There was considerable variability in the types of counting chambers used to evaluate semen concentration, with approximately 30% of laboratories using different chambers such as Makler, Neubauer, and Fuchs-Rosenthal, potentially leading to biases in concentration results. Morphological analysis also showed wide variations, particularly in staining techniques, with only 20% of laboratories adhering to the World Health Organization (WHO) recommendation of using Papanicolaou staining. Most laboratories (73%) assess sperm vitality using microscopic examination of eosin-nigrosine stained samples, while about half report morphology after microscopy with oil immersion. Sperm motility is predominantly assessed with phase-contrast microscopy (69%), and automated semen analysis is rare, with only 12% of laboratories using automated systems.

Quality control, crucial for minimizing errors in both manual and automated analyses, was insufficiently implemented, with fewer than 30% of laboratories performing both internal and external quality control measures. Postanalytical considerations focused on laboratory reporting practices, including interpretative comments. A significant majority (86%) of laboratories align their recommended cutoff values with WHO guidelines, showing some adherence to international standards. Nevertheless, the survey results underscore a clear need for national guidelines, especially for the analytical and postanalytical phases of semen analysis, to improve standardization and quality across laboratories.

Historically, the understanding of male infertility dates back to ancient civilizations, with evidence from ancient Egypt indicating recognition of male ejaculate's role in pregnancy. The development of andrology, a field encompassing aspects of urology, gynecology, endocrinology, and dermatology, saw significant advancements in the 20th century. The first scientific journal dedicated to andrology was established in 1969, and the WHO published its first laboratory manual for semen analysis in 1980, followed by a second edition in 1987. Today, the primary method for assessing male fertility involves evaluating sperm count, motility, and morphology through either traditional manual analysis or Computer-Assisted Semen Analysis (CASA) systems.

Manual semen analysis is widely used in reproductive laboratories due to its simplicity and low cost. However, its accuracy and reliability are often affected by the technician's experience and subjective judgment, as well

as technical factors such as inconsistent sample preparation. For example, the Makler chamber, commonly used in assisted reproduction laboratories, has specific dimensions that affect the spread and distribution of semen samples, potentially leading to inaccuracies in sperm count and motility measurements.
Computer-Assisted Semen Analysis systems, introduced in the 1970s, were developed to reduce subjectivity in semen analysis. With advancements in digital imaging and computer technology, CASA has significantly improved the precision and objectivity of semen analysis, offering more detailed information on sperm kinetics, which is critical for predicting in vitro fertilization success. Despite the benefits, CASA systems are not without challenges; variability in the type of counting chamber and loading methods can affect the accuracy of motility and concentration measurements. Furthermore, errors such as clumping of spermatozoa or misclassification of immotile spermatozoa can impact the accuracy of results.
Both, manual and CASA methods are used, with the choice depending on the laboratory's needs and the purpose of the analysis. Accurate results in semen analysis require well-trained personnel and a thorough understanding of the sources of errors and limitations inherent to each method. The development of national guidelines in Croatia, as driven by the WG SA's survey, aims to address these issues by promoting standardized procedures, enhancing quality, and ensuring harmonization across MBLs.

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WG-2 Working group for post-analytics

How to implement autoverification procedures and quality indicators in routine laboratory work - steps suggested by the Working group for post-analytics

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In 2019, the Working Group for post-analytics of the Croatian Society of Medical Biochemistry and Laboratory Medicine (WG POST CSMBLM) published National recommendations for the post-analytical phase of laboratory work. This document covered all phases of the post-analytical work process, and in the continuation of its activities, the emphasis is on: (1) the autoverification procedure, (2) the introduction and monitoring of quality indicators, and (3) the comparison and implementation of the new HRN EN ISO 15198:2022 in the existing recommendations of the post-analytical phase of laboratory work. New recommendations for Autoverification are in the publication process, while recommendations for Quality Indicators are being developed.

Autoverification procedures (AV) is a computer-based postanalytical tool that uses a sequence of procedures to verify laboratory test results without manual intervention. The Working Group for post-analytics has created a procedure for implementation of AV in routine laboratory work, which complements the existing national recommendations for the laboratory process and aims to clarify the AV procedures.

Before implementation, it is necessary to determine the need for AV in routine laboratory work; then appoint the AV team, whose role it is to decide in which area of laboratory work AV will be implemented; then create an algorithm setup and monitor the verification of AV. Autoverification procedures avoids the subjective assessment of laboratory results since it is based on the same rule and is in a way standardized, which further increases the quality of the results. Some of the standard rules in the algorithm are patient data, messages from the analyser, values of serum indices, AV range and delta check. All criteria set in the algorithm should be documented, signed, and approved by the head of the laboratory.

This procedure from the WG POST CSMBLM shows the basic rules of AV that can be applied by every laboratory in the initial phase. The use of AV will be justified by the number and complexity of laboratory tests, the number of employees, and certainly the financial and material resources available.

For decades, planning an error prevention and assessing the quality of laboratory processes has been one of the priorities of laboratory experts. The implementation of technological solutions that automated most of the analytical phase, but also many processes in the pre- and post-analytical phase, enabled a significant reduction in the number of errors and the analysis of a large number of samples in a short period of time. On the other hand, due to the rapid generation of a large amount of data and information, a need to design an effective quality control system for the laboratory processes themselves emerged. Quality indicators (QI) make possible to measure the quality of a laboratory process. QI describe the efficiency of the laboratory process in the form of a numerical value and represent objective evidence of the compliance of the specified process with predefined criteria. Working Group for post-analytics decided to present recommendations that would describe necessary steps in the design and monitoring of QI, with special emphasis on the post-analytical phase. These recommendations should facilitate laboratory professionals to plan implementation of QI, especially in the part of the selection and analysis of the data to be monitored. Proposed criterions for QI of post-analytical phase, which include turnaround time, errors during transcription of results/incorrect laboratory reports and notification of critical results, will enable a standardized and objective assessment of the quality for these processes in laboratories of all levels of health care.

The new standard ISO 15198:2022 cancels and replaces the third edition of the standard ISO 15189:2012, which has been technically revised, and replaces HRN EN ISO 15189 - Point-of-care (POCT) - Requirements for quality and competence (ISO 22870:2006; EN ISO 22870:2006). Considering that the new edition of the HRN EN ISO

15189:2022 norm is available, and the transition period for its implementation of the requirements is 3 years (until December 2025), WG POST CSMBLM is working on intensive comparing the requirements for the post-analytical phase. The post-analytical phase in the new standard includes points 7.4.1.1. - 7.4.1.8 and 7.4.2. (compared to the norm from 2012: 5.7. - 5.9) with no difference in the described procedures, but not in content, which requires an additional comparison. After that, WG POST CSMBLM will plan on supplementing or new version of its recommendations.

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WG-3 Working group for cerebrospinal fluid diagnostics

Cerebrospinal fluid diagnostics - how to reach the final finding

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Laboratory analysis of cerebrospinal fluid (CSF) is challenging for most laboratories due to the specificity of the sample. To improve CSF diagnostics and to encourage participants to take part in a discussion about their problems and doubts, we presented the most common clinical cases encountered in routine laboratory practice: acute inflammation, traumatic lumbar puncture (LP), metastases in the central nervous system (CNS), and intrathecal antibody synthesis. Commonly used analytical methods, limitations of automated cell analysis and different reference ranges were also commented on.

The development of automated body fluid counting modes enabled rapid and accurate enumeration of CSF cells on some hematology analyzers (HA). Although some HA could not achieve perfect alignment in red blood cell (RBC) concentrations with the reference method (Burker Turk counting chamber), especially in low RBC count, Siemens Advia 2120i HA has a declared sensitivity of 0 $x10^6$/L RBCs in CSF. However, the linearity of the method is up to 1500 $x10^6$/L, opening a possibility for a false increased white blood cell count (WBC) if an RBC count exceeds this upper limit. Possibly, the biggest problem for an automated CSF analysis would be CSF samples obtained after traumatic LP that are heavily bloody, with a high RBC concentration. Perhaps the sole solution to this problem would be a combination of an HA and a manual method. Alternatively, the correction factor for WBC counts in traumatic LP can be applied. The case of a traumatic neonatology LP, and the limitations of a HA CSF analysis were presented.

Cerebrospinal fluid analysis is an essential tool for the early diagnosis of CNS infections, particularly acute bacterial meningitis. Bacterial meningitis is a rapid and progressive infection of the CNS that can cause long-term consequences, and if not treated, the disease can become complicated. The case of a 68-year-old patient who was brought to the emergency department with a disturbed state of consciousness, agitated, and tachypneic was presented. On admission, purulent content was observed in the right ear. The patient's neurological condition rapidly deteriorated, and he was admitted to the intensive care unit, where LP was performed. The CSF was sent for laboratory analysis. Cloudy and white (purulent) CSF was associated with typical BM findings: marked pleocytosis (WBC = 7800 $x10^6$/L) with a predominance (85%) of polymorphonuclear neutrophils (PMN), decreased glucose (< 0.6 mmol/L), increased lactate (23.3 mmol/L) and elevated protein (8720 mg/L) concentrations. Serum CRP and procalcitonin concentrations were elevated. In CSF cytological differentiation, extracellularly located bacteria were observed. Streptococcus pneumoniae DNA in CSF was identified by PCR testing. The diagnosis of pneumococcal meningitis with sepsis syndrome resulted in poor outcome for the patient.

Brain metastases are the most common intracranial tumors in adults and account for significantly more than half of all brain tumors. Lumbar puncture and CSF analysis are standard procedures and often the only method to confirm or exclude malignant infiltration into the CNS. Abnormalities in CSF composition can be found in more than 90% of patients with leptomeningeal metastases. They include pleocytosis, the presence of tumor cells, increased protein concentration, and decreased glucose concentration in CSF. The case of a 28-year-old patient with acute myeloid leukemia (AML) whose CSF was analyzed due to suspicion of relapse in the CNS was presented. In the xanthochromic CSF, 650 $x10^6$/L mononuclear cells were found, and the cytomorphological findings (immature blast-type cells, monocytoid cells) indicated a relapse. A reduced concentration of glucose and markedly elevated concentrations of lactate, total proteins, and lactate dehydrogenase confirmed the diagnosis. During the next four months, the patient's CSF was repeatedly analyzed to monitor the response to intrathecal therapy.

A case of multiple sclerosis (MS) in the acute, hyperactive phase is presented. Multiple sclerosis is a chronic, autoimmune inflammatory disease of the CNS. A 37-year-old Caucasian male was admitted to the hospital with facial and right arm numbness, unsteadiness while walking, and memory loss. He is treating type 2 diabetes and has a positive quantiferon test. Lumbar puncture was performed as part of the treatment. The CSF was clear and colorless, and the number of leukocytes was 14 $x10^6$/L (100% mononuclear). The glucose concentration in the CSF was 9.42 mmol/L (serum glucose concentration was 22.9 mmol/L), lactate was 2.33 mmol/L, and proteins were 354 mg/L. Reibergram showed intrathecal synthesis of IgG 32%, IgA 76%, and IgM 86%. According to the international consensus, the finding of oligoclonal bands belongs to Type 3: OIgG in CSF with additional identical bands in CSF and serum (intrathecal synthesis). Magnetic resonance imaging shows numerous lesions in the brain and in the spinal cord, which supports the suspected diagnosis of MS.

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WG-4 Working group for laboratory coagulation

Coagulation screening assays: testing practice and adherence to National recommendations

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Coagulation laboratory assays are essential for diagnosing and treating hypercoagulability and bleeding disorders. To reduce the possibility of errors and ensure accuracy and quality in the performance of coagulation assays, the procedures must be standardized. Research carried out to date has shown that the practice in a particular phase of laboratory work among laboratories that perform coagulation assays is different and therefore the Working group for laboratory coagulation (WGLC) of the Croatian Society for Medical Biochemistry and Laboratory Medicine in 2019 published the National recommendations for performing screening coagulation tests and D-dimers. The recommendations summarize the standardized procedures in the extra analytical and analytical phase of conducting screening coagulation assays: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, fibrinogen and D-dimers. The publication of the guidelines is the first and one of the most important steps in achieving harmonization. However, numerous studies have shown that despite the existence, practical standards and guidelines are commonly not applied in practice. To determine the extent to which laboratories adhere to the National guidelines, the data included in the WGLC research before and after the publication of the guidelines will be presented as well as the „checklist" that will be distributed to laboratories that perform coagulation assays. The basic goal is promoting adherence to the standards and the guidelines.

Besides adherence, for efficient results, it is important to continuously work on the standardization of the procedures where standardization is not sufficient.

Thus, in 2020 WGLC investigated the differences in the reporting of aPTT results, reference intervals (RI) in use, and the method of calculating the aPTT ratio along with its impact on the comparability of the results among Croatian laboratories. In the absence of global standardization, to achieve better comparability, WGLC recommended calculation of the aPTT ratio by dividing the patient's aPTT measured in seconds (aPTTs) with the mean value of reference interval for the reagent/coagulometer system that the laboratory uses. In addition, aPTT ratio should always be reported along with aPTTs. The observed results showed that laboratories using the same reagent/coagulometer system often apply different denominators to calculate the aPTT ratio. Although not clinically significant, differences in the results obtained due to non-uniform calculation of the aPTT ratio can affect the comparability of external quality control results and sometimes lead to unacceptable deviations despite acceptable aPTTs results. Data analysis also revealed that a small number of laboratories stated the use of the manufacturer's RI for reporting aPTTs, but the intervals they specified did not correspond to those assigned for the combination of the reagent/coagulometer they use.

Furthermore, according to a WGLC survey, Croatian laboratories mainly use the RI of 1.8 to 3.5 g/L for fibrinogen which is recommended by the Croatian Chamber of Medical Biochemists, in the document Harmonization of Laboratory Assays. However, the RI is based on the literature data with results obtained in other populations, analysis of samples drawn into 3.8% three sodium citrate containers, and/or a combination of reagent/coagulometer systems that differ from those used today. Therefore, as a primary task in 2024 WGLC postulated the determination of the reference interval for functional activity of fibrinogen in our population by the recommended method and with a reagent/coagulometer system combination mostly used by Croatian laboratories. Cooperation was agreed upon with the Croatian Institute for Transfusion Medicine in Zagreb, as well as the Clinical Institute for Transfusion Medicine, and the Institute for Transfusion Medicine at Clinical Hospital Centres in Osijek and Split, respectively. A questionnaire was created with the purpose of

excluding subjects with pathophysiological conditions that can affect the concentration of fibrinogen. The protocol included analyses of fibrinogen in fresh plasma samples and in addition to fibrinogen, determination of global coagulation assays and C-reactive protein as a further help in appropriate selection of the subjects. It is planned to collect blood samples from 120 voluntary donors in each of the following groups: women aged 18-50 years, women aged 50-65 years, men aged 18-50 years, men aged 50-65 years, men and women aged > 65 years. Upon sample analyses and data collection statistical analyses will be performed and the reference intervals determined will be compared to the currently recommended intervals. The collection of samples from voluntary blood donors has started and preliminary results will be presented.
However, inadequate performance of coagulation assays can significantly affect the treatment outcomes in patients with haemostatic system disorders. Therefore, WGLC's main task is continuous work on the identification of critical phases of the process and improvement of adherence to standardized procedures.

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Poster abstracts

A Hematology

A-01

Deregulated glutamate dehydrogenase activity in lymphocytes of B-cell chronic lymphocytic leukaemia patients

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Introduction: Glutamate dehydrogenase (GLDH) is linked to the Krebs cycle, serving as a key link between carbohydrate and amino acid metabolisms, which are more active during cell proliferation. This study aimed to investigate GLDH activity, and GLUD1 and GLUD2 expression in peripheral blood mononuclear cells (PBMC) of untreated B-chronic lymphocytic leukaemia (CLL) patients, healthy individuals (HI), and patients with infectious mononucleosis (IM), assessing its activity as potential CLL biomarker.
Materials and methods: GLDH activity was measured using a commercial GLDH kit in supernatant from pelleted PBMC. B cells from PBMC were purified using CD19+ monoclonal antibody positive selection. GLUD1 and GLUD2 mRNA expression was assessed by quantitative real-time PCR. Serum thymidine kinase activity (TK) data were available for 38 CLL patients (median 7.13 U/L, range 2.32 to 41.5 U/L).
Results: The highest GLDH activity was in PBMC of the CLL group (N = 62), followed by the HI (N = 60) and IM (N = 21) groups (median 6.59/3.67/2.46 µkat/g protein, P < .001). PBMC GLDH activity was higher in 60% of CLL patients compared to the HI reference interval (2.17-5.70 µkat/g protein). Increased GLDH activity was also observed in CD19+ cell preparations of CLL patients (2 out of 3), but not in HI (N = 3). Median GLUD1 expression was highest in the IM group (N = 11), followed by HI (N = 14) and CLL groups (N = 59) (median 4.69/3.78, P < 0.005; 4.69/2.91, P < 0.001). GLUD2 expression did not significantly differ between groups. A significant correlation between GLUD1 expression and serum TK activities was found in female CLL patients (R = 0.64; P = 0.014, N = 14), but not in males (R = - 0.08; P = 0.73, N = 24).
Conclusions: Increased GLDH activity is specific to B-lymphocytes in CLL patients. GLUD1 expression, but not GLUD2, varies between activated and CLL lymphocytes. In female CLL patients, GLUD1 expression correlates with higher TK activities, indicating a proliferative compartment among CLL cells.

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A-02

The multiple comparison of erythrocyte sedimentation rate tubes

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Introduction: Due to the global trend forward automatization of erythrocyte sedimentation rate, our distributor (Beckton, Dickinson and Company, New Jersey, USA) issued a product discontinuation notification regarding BD Vacutainer erythrocyte sedimentation rate (ESR) tubes for the manual method by Westergreen. To ensure continuous ESR measurement alternative ESR tubes have been compared to the existing BD tubes.
Materials and methods: Three types of alternative ESR tubes were included in the study: open Vacutube ESR 4NC (LT Burnik, Vodice, Slovenia) and two closed Vacuette 4NC ESR sodium citrate 3.2% tubes (1.5 and 2.75 mL) (Greiner Bio-One, Austria). ESR analysis was performed by the Westergreen manual method. An average bias between the samples obtained from different tubes was calculated by employing the Bland-Altman plot. Average bias was expressed as a percentage and compared to the minimum allowed bias according to the CROQALM acceptance criteria (± 25%). Statistical analysis was performed in MedCalc v22.016 statistical software (MedCalc Software Ltd, Ostend, Belgium).
Results: 200 patients were included in the comparison study (100 for Burnik tubes, 100 for Greiner tubes - 50 patients per two different tube volumes). ESR measurements spanned from 1 to a maximum of 140 mm/3.6 ks (median: 10 mm/3.6 ks, IQR: 5-22). Bland-Altman plot revealed a satisfactory comparison between BD and Burnik tubes (average bias 13.1%, 95% confidence interval (CI): 7.7-18.5). However, an unsatisfactory comparison was revealed for both types of Greiner tubes. The average bias was 29.2% (95% CI: 9.4-49.1) and remarkably high 51.6% (95% CI: 29.9-73.3) for 1.5 mL and 2.75 mL tubes, respectively.
Conclusions: The replacement of BD Vacutainer ESR tubes with LT Burnik Vacutube ESR 4NC tubes does not significantly affect testing results, and thus LT Burnik tubes are acceptable for ESR measurement by the Westergreen manual method. Both Greiner closed ESR tubes cannot be used as an alternative to open BD Vacutainer ESR tubes.

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A-03

Neutrophil band assessment accuracy - a Croatian national pilot study

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Introduction: Due to the lack of consistent definition and the ambiguity in morphological characterization, the distinguishment between segmented and band neutrophils is prone to the observer's subjectivity. The national pilot project aimed to assess the degree of variability in classifying band neutrophil granulocytes among Croatian medical biochemistry laboratories.
Materials and methods: The study was conducted as a short survey through CROQALM (Croatian Quality Assurance in Laboratory Medicine), a national quality control provider. Eight different segmented/band neutrophil granulocyte cell pictures were chosen, from two different patients. The cells were chosen in the following manner: two of the chosen cells were indisputably recognizable segmented neutrophil granulocytes; for two cells there should be no doubt that the band neutrophil granulocyte was depicted because of an easily recognizable horseshoe shape. The rest of the four cells can be debated if segmented or neutrophil granulocytes were depicted.
Results: The response rate was 55% (99/180 Croatian laboratories). For two recognizable segmented neutrophil granulocytes, the percentage of correct answers was expectantly high (> 90%). However, surprisingly 17% of participants answered metamylocte for a recognizable band neutrophil granulocyte. The depicted cell did not contain primary azurophil granules, and the indentation of the nucleus was more than 1/2 of the diameter of the perfectly rounded nucleus. The distribution of answers for the remaining four cells where morphological interpretation could go both ways was expected. In two examples more than 25% of participants gave answers of lower/higher ranking than the rest of laboratories. The most stunning result was observed for one depicted cell where the distribution of results between segmented and band neutrophil granulocytes was almost perfectly divided (50/50%).
Conclusions: The observed results confirm the international literature data about high variability in band cell enumeration. Action is needed on a national level to improve harmonization in the field.

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A-04 (Oral presentation)

Can we use vortexing to disaggregate platelet clumps?

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Introduction: The possible disaggregation of platelet clumps by vortexing EDTA samples was investigated in a multicentric study.
Materials and methods: A total of 122 EDTA whole-blood samples with a hematology analyzer (HA) morphology flag for possible platelet clumps (regardless of the total platelet count) were included in the study. Samples were analyzed in three different University Hospitals by Siemens Advia 2120i (Siemens, Marburg, Germany), Sysmex XN-1000 (Sysmex, Kobe, Japan), and Beckman Coulter DxH 900 (Beckman Coulter, Brea, USA) HA. The peripheral blood smear was scanned, and the presence of platelet clumps was noted. Afterward, samples were vortexed for 1 minute, re-analyzed on the HA, and the peripheral blood smear was re-examined for the presence of clumps. If clumps were still present, the sample was vortexed for another 2 minutes, and the whole procedure was repeated. The comparison of CBC parameters was performed by repeated measures ANOVA. Statistical analysis was performed in MedCalc v22.016 statistical software (MedCalc Software Ltd, Ostend, Belgium).
Results: Platelet count gradually increased when the EDTA samples were vortexed. The mean platelet counts were 169, 220, and 239 x10^9/L in the non-vortexed sample and samples vortexed for 1 minute and 2 minutes, respectively. The number of positive samples with identified platelet clumps in the blood smear was 89% but decreased significantly to 54% after 1 minute of vortexing ($P < 0.001$). The decrease was even more pronounced after two minutes of vortexing (38%, $P < 0.001$). However, no significant difference was observed between the number of leukocytes ($P = 0.262$), erythrocytes ($P = 0.328$), and hematocrit ($P = 0.278$) in the three measurements, suggesting that cell integrity was preserved despite vortexing.
Conclusions: The vortex method for disaggregating platelet clumps in EDTA samples for CBC measurement significantly reduced the number of samples with confirmed platelet clumps, thus reducing the need for repetitive venipuncture.

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A-05 (Oral presentation)

Q-values for left-shift and atypical lymphocytes should be adjusted to optimize flagging performance on Sysmex XN-1500 analyser

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Introduction: Sysmex XN-1500 hematology analysers (Sysmex Corporation, Kobe, Japan) flag complete blood count (CBC) results if peripheral blood smear (PBS) is needed, indicating with Q-value (Q) the probability of abnormal cells presence. The Q is ranged from 0 to 300 with default threshold setting at 100. The aim of our study was to optimize flagging performance of the analyser according to Q for left shift and atypical lymphocytes.
Materials and methods: The study included 175 K3EDTA blood samples (Greiner Bio-One, Kremsmünster, Austria) from patients in which CBC was determined on Sysmex within 4 hours of blood collection. PBS was performed by Cellavision DM 1200 (Lund, Sweden) by single laboratory expert. The diagnostic accuracy of each flag was determined by receiver operating characteristic curve analysis, the area under the curve (AUC) was reported with confidence interval of 95% (95% CI) and optimal cutoff for Q selected according to Youden index. Defined criteria were 4, 10 and 20% of band cells for Q-left shift and 3 and 5% of reactive lymphocytes (RE-LYMP) for Q-atypical lymphocytes, selected according to available literature. $P<0.05$ was considered statistically significant. Statistical analysis was performed in Medcalc statistical software, ver.14.8.1 (Ostend, Belgium).
Results: The AUC were 0.83 (95% CI: 0.76 to 0.88) on $Q > 0$, 0.88 (95% CI: 0.82 to 0.92) on $Q > 30$ and 0.90 (95% CI: 0.84 to 0.99) on $Q > 90$ with band cells criteria of 4, 10 and 20%, respectively for the left shift flag. For the atypical lymphocytes flag and RE-LYMP of 3 and 5%, AUC were 0.84 (95% CI: 0.77 to 0.90) and 0.86 (95% CI: 0.79 to 0.91) on $Q > 50$. All P-values were statistically significant ($P < 0.001$). AUC values indicate very good diagnostic accuracy.
Conclusions: To cover the clinically significant presence of band cells and RE-LYMP in PBS, Q threshold should be reduced to 30 and 50 for corresponding flags. This would increase the number of samples with PBS examination need and additionally encumber the laboratory staff.

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A-06 (Oral presentation)

Comparison of immature granulocytes and reactive lymphocytes obtained by Sysmex XN-1500 and Cellavision DM 1200

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Introduction: Hematology analyser Sysmex XN-1500 (Sysmex Corporation, Kobe, Japan) is able to quantify immature granulocytes (IG) and reactive lymphocytes (RE-LYMP). The aim of our study was to compare these parameters obtained by Sysmex analyser and Cellavision DM 1200 (Lund, Sweden) after peripheral blood smear (PBS) examination.
Materials and methods: The study included 202 K3EDTAblood samples (Greiner Bio-One, Kremsmünster, Austria) from patients in which complete blood count was determined on Sysmex. PBS was examinated by single laboratory expert at Cellavision. The difference between hematology parameters on two analysers was obtained by Mann-Whitney test and Bland-Altman analysis. IG as a sum of metamyelocytes, myelocytes and promyelocytes obtained by Cellavison was divided in groups depending whether there are or not IG present in PBS. The diagnostic accuracy for IG on Sysmex was determined by receiver operating characteristic curve analysis, the area under the curve (AUC) was reported and optimal cutoff selected according to Youden index. $P < 0.05$ was considered statistically significant. Statistical analysis was performed in Medcalc statistical software, ver.14.8.1 (Ostend, Belgium).
Results: There is statistically significant difference in IG obtained by two analysers ($P < 0.001$), while there is not difference in RE-LYMP ($P = 0.156$). Mean bias for RE-LYMP between two analysers is 0.11 (95% CI: - 0.26 to 0.48)% in apsolute values and 34.2 (95% CI: 19.8 to 48.5)% in relative values indicating proportional difference. Mean bias for IG between two analysers is 1.08 (95% CI: 0.86 to 1.30)% in absolute values and 144.6 (95% CI: 132.4 to 156.7)% in relative values, indicating both proportional and constant difference. The AUC of 0.88 (95% CI: 0.82 to 0.92) on IG > 2.6% at Sysmex analyser ($P < 0.001$) indicates very good diagnostic accuracy.
Conclusions: Current IG criteria of 0.10 $x10^9$/L for PBS examination is too exacting and new criteria of 2.6% can be applied to minimize the number of PBS in routine practice. RE-LYMP obtained by Sysmex could be used as screening tool for patients with reactive lymphocytosis.

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A-07 (Oral presentation)

Detection of PML::RARA and BCR::ABL1 fusion transcripts at diagnosis using ready-to-use EasyPGX assay

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Introduction: Standardized method for fusion transcripts detection was published as BIOMED-1 protocol and it is still the gold standard in fusion transcript detection in molecular laboratory. This procedure is demanding, time-consuming with a lot of sample manipulation, therefore a ready-to-use commercial tests are developed to enable faster and simpler analysis while providing the same relevant information (transcript type, breackpoint region). Our aim was the comparison of ready-to use EasyPGX tests (Diatech Pharmacogenetics, Italy) for the detection of PML::RARA and BCR::ABL1 fusion transcripts with the standardized method according to the BIOMED-1 protocol.
Materials and methods: Samples used for validation were remains of isolated ribonucleid acid from external quality control (EQC) analysed using standardized BIOMED-1 protocol at the time of trial. Archieved samples were used for 42 analysis of PML::RARA and 44 analysis of BCR::ABL1 fusion transcript with EasyPGX assay (Diatech Pharmacogenetics, Italy). Analysis results were interpretated with Diathech software and compared with known results from EQC report.
Results: PML::RARA fusion transcript was detected in all positive samples (bcr1 N = 17, bcr2 N = 2 and bcr3 N = 9) and was negative in all negative samples (N = 9). BCR::ABL1 fusion transcript was detected in all positive (e13a2 N = 9, e14a2 N = 2, e13a3 N = 2, e1a2 N = 4, e1a2+e13a2 N = 2, e13a2+e13a3 N = 1) and was negative in all negative samples (N = 18). Residual 5 positive PML::RARA and 6 positive BCR::ABL1 samples were diluted with negative sample (1:1000) to check the sensitivity of the assay. There was no reported result for those diluted samples therefore sensitivity of this test is limited to diagnostic samples as was decladed by manufacturer.
Conclusion: This mini validation of EasyPGX PML::RARA and BCR::ABL1 fusion transcript assay showed optimistic result for use in rutine laboratory diagnostics, althouth each laboratory must perform assay validation according to prescribed validation protocol.

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A-08 (Oral presentation)

Stereotyped CLL patients in Croatian population

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Introduction: The mutational status of the immunoglobulin heavy variable gene (IgHV) directly correlates with CLL patient survival. Bioinformatic tools revealed stereotyped B cell receptor immunoglobulin gene (BcR Ig) which share similar IGHV-IGHJ- IGHD gene rearrangement in VH CDR3 region among both mutated and unmutated patients. Patients in certain stereotyped subsets display the same biological characteristics and clinical presentation irrespective of SHM IGHV status and therefore subset defining contribute to more precise risk stratification. To get an overview of BcR Ig gene mutational status and prevalence of stereotyped cases among CLL patients in Croatian population, analysis of data collected from 2020 to 2023 in the Department of Laboratory Diagnostics, University Hospital Centre Zagreb was performed.
Materials and methods: SHM IgHV gene status in comparison with a germline reference gene panel was determined using the IMGT/V-QUEST tool. For identification of stereotyped IGHV-IGHJ-IGHD gene rearrangement ARResT/AssignSubsets tool was used.
Results: All patients had a single productive IGHV-IGHD-IGHJ gene rearrangement. 12.9% of all CLL cases were assigned to stereotyped subsets and the incidence was higher in the group of unmutated CLLs (U-CLL 13.8%, M-CLL 8.0%). The most prevalent IGHV-IGHJ-IGHD gene rearrangements among stereotyped cases were IGHV1-69 and IGHV3-21. IGHV4-34 gene was present in 6/11 mutated stereotyped patients. Atotal of 15 different subsets were identified. Subset #2 was the largest, comprising cases from both M-CLL and U-CLL (13 cases, 2.9% of U-CLL and 2.0% of M-CLL), followed by subsets#1 (3.2% of U-CLL) and #3 (2.0% of U-CLL). The most prevalent subset among M-CLL was #4 (3.4%) with no occurrence in the U-CLL group. There were 3 cases, all among U-CLL patients, of subset #8 encoded by the IGHV4-39.
Conclusion: Although subset identity report is currently recommended only for subsets #2 and #8, as they have the best-established clinical value, growing number of studies and meta-analysis collects evidence for novel candidates.

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A-09

Validation of method for determination of CD3+ cells on flow cytometer BD FACS Canto II

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Introduction: The best-known type of immune effector cell therapy is chimeric antigen receptor T (CAR-T) cells. CD3 antigen is a T-lymphocyte marker whose determination on a flow cytometer is essential for the quality assessment of cell products for CAR-T therapy. The aim of this study was to validate the method for the determination of CD3+ cells in fresh leukapheresis products.
Materials and methods: The study was performed on the flow cytometer BD FACS Canto II using single-platform method. For inter-assay precision commercial control sample BD Multi-Check Control (lot BM0723N) was used and analysed in triplicate for five consecutive days. For intra-assay precision, as well as for linearity, one sample with high (135,180 $x10^6$/L) and one sample with low (123 $x10^6$/L) CD3+ cell count was used. Sample dilutions for linearity assessment were analysed in duplicate according to a predefined protocol. Stability study included 10 samples of fresh peripheral blood haematopoietic stem cell products which were analysed immediately after the end of cell collection. The fresh samples were split into two aliquots which were stored at room temperature (RT) and +4°C. After 24 hours aliquots were again labelled and analysed.
Results: Coefficients of variation (CV) for precision and accuracy were less than 10%, except for intra-assay precision (CV for the sample with low absolute CD3+ cell count was 10.8 %). In linearity study, CV was 16.6%, which meets the set criteria. Sample stability analysis showed a statistically significant difference between the results of samples stored fresh vs. stored at RT (percentage of CD3+ cells P = 0.040, absolute viable CD3+ cell count P = 0.008), without difference between the results of fresh samples *vs*. stored at +4°C.
Conclusion: Validation study for the CD3+ cell determination showed satisfactory precision, accuracy and linearity. Fresh leukapheresis samples stored for 24 hours at + 4°C maintained their stability.

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A-10

Is it safe to use a centrifuged blue top sodium citrate tube for platelet count measurement?

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Introduction: Ablue top sodium citrate coagulation sample was used as an alternative sample to determine platelet count and to resolve EDTA-induced platelet clumps. However, it is controversial whether it is safe to use a centrifuged blue top tube for platelet count measurement. The aim of the study was to compare the platelet counts between an EDTA, a non- centrifuged and a centrifuged sodium citrate whole blood sample.
Materials and methods: Atotal of 54 patients with an available EDTAwhole blood and a blue-top sodium citrate coagulation tube were included in the study. Samples were analysed in two different university hospitals using the Siemens Advia 2120i (Siemens, Marburg, Germany) and the Beckman Coulter DxH 900 (Beckman Coulter, Brea, USA) hematology analyzer (HA). The complete blood count (CBC) was analysed in both the EDTA and non-centrifuged sodium citrate samples. The coagulation tube was then centrifuged, homogenised and re-analysed on an HA. The peripheral blood smear was scanned and the presence of platelet clumps was determined. Comparison of EDTAand recalculated citrate CBC parameters was performed using one-way analysis of variance (ANOVA). The statistical analysis was performed with the statistical software MedCalc v22.016 (MedCalc Software Ltd, Ostend, Belgium).
Results: No platelet clumps were visible in the included EDTAand citrate samples, so the EDTAsample gave a reliable platelet count. Platelet counts ranged from severe thrombocytopenia to normal platelet count (6 to 352 $x10^9$/L). No significant difference was found between white blood cell count (P = 0.516), red blood cell count (P = 0.920), hemoglobin (P = 0.799), platelet count (P = 0.409) and mean platelet volume (MPV) (P = 0.128) in the three types of samples.
Conclusion: The results of our study suggest that the centrifuged blue top sodium citrate tube is safe for alternative platelet count measurement in case of EDTA-induced platelet clumping. It is not necessary to redraw a sodium citrate tube if a centrifuged sample is already available.

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A-11

Comparative evaluation of body fluid cellular analysis by Siemens Advia 2120i and Sysmex XN-1000 hematology analyzer

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Introduction: Body fluid (BF) cellular analysis verification on hematology analyzers imposes a significant challenge due to the instability and unavailability of extravascular BF samples. The aim of this study was a comparative evaluation of BF cellular analysis by Siemens Advia 2120i (Siemens, Marburg, Germany) and Sysmex XN-1000 (Sysmex, Kobe, Japan) hematology analyzer (HA).

Materials and methods: We included remnant patient extravascular BF samples (pleural, synovial, and drain fluids) spanning the reportable range of each assay. While Siemens Advia 2120i reports total nucleolated cells (TNC) and red blood cells (RBC), Sysmex XN-1000 differentiates between white blood cell count (WBC) and TNC and performes a two- class differential as well (mono- and polymorphonuclear cells). Therefore, we compared the performance of TNC and RBC between analyzers by performing Passing-Bablok regression and rank correlation. An acceptance criteria was: coefficient of correlation (r) ≥ 0.9 for TNC, ≥ 0.8 for RBC, slope 0.7-1.3. The additional aim was to verify the trueness of BF differential by comparing the automated method and manual microscopy performed by a cytospin preparation technique. Statistical analysis was performed in MedCalc v22.021 statistical software (MedCalc Software Ltd, Ostend, Belgium).

Results: A total of 30 extravascular BF samples were collected during the verification period. Fourteen samples were below the LoQ of the method for RBC on Siemens Advia 2120i and were excluded from further statistical analysis. An excellent correlation was observed both for TNC (r = 0.99, 95% CI: 0.98 to 0.99) and RBC (r = 0.97, 95% CI: 0.88 to 0.99), with an acceptable slope (TNC = 1.22, 95% CI: 1.17 to 1.29; RBC = 1.08, 95% CI: 0.92 to 1.20). Additionally, there was an excellent agreement in BF differential and manual microscopy (r = 0.97 for mono- and polymorphonuclear cells).

Conclusion: The Sysmex XN-1000 HAis comparable in BF cellular analysis to routine HASiemens Advia 2120i and can be introduced into routine practice.

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A-12

Cell differentiation count in body fluids using automated Sysmex XN-1000 hematology analyser

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Introduction: The cellular composition of body fluids (BF) provides insight into disease pathology and is an important diagnostic parameter. The Sysmex XN-1000 hematology analyser offers automated BF analysis. Although conventional manual microscopy is still considered the gold standard, automated analysis is growing in popularity because of the reduce interobserver variability and faster response time. The aim of the study is to compare the Sysmex XN-1000 system's body fluid mode for cell differentiation parameters with the results of the manual microscopic count.
Materials and methods: Pleural, peritoneal and synovial fluids from routine analysis of the Institute of Clinical Chemistry and Biochemistry where included in the evaluation. Manual microscopic cell differentiation count was performed in cytospin slides after Pappenheim staining. BF samples where analysed with XN-1000 in the body fluid mode. For method comparison where used and calculated Spearman's correlation coefficient and Bland- Altman plot. Statistical analysis was carried out with MedCalc software.
Results: A total of 200 samples where included. Analysis parameters included PMN-BF%, MN-BF% as well as research parameters NE-BF%, LY-BF%, MO-BF% and EO-BF%. In most BF samples EO-BF% were very low or absent so they where excluded from the study. According to Spearmans's correlation coefficients (r) for the all parameters showed very strong correlation PMN-BF%: 0.87(0.84-0.90), MN-BF%: 0.86 (0.82-0.89), NE-BF%: 0.87 (0.84-0.90), LY-BF%: 0.90 (0.86-0.92), MO-BF%: 0.84 (0.79-0.88). The Bland-Altman showed that Sysmex XN compared to the manual method presented slightly higher count for PMN-BF% (for 4.91%), NE-BF% (for 4.25%) and LY-BF% (for 8.67%) and lower for MN-BF% (for 4.76%) and MO-BF% (for 12.53%).
Conclusion: Sysmex XN-1000 BF mode can count and identify cells but its use for cell differentiation is still being explored. Althought method has advantages, due to the great diversity of mononuclear leukocytes, presence of mesothelial and malignant cells in BF samples still need further confirmations.

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A-13

Assessing the efficacy of automated fragmented red cell (FRC) count and "Fragments?" flag for detecting schistocytes in peripheral blood smears

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Introduction: Schistocytes, also known as fragmented red blood cells (FRC), serve as crucial indicators in the diagnosis of hematological and systemic disorders. Sysmex analyzers (Sysmex, Kobe, Japan) use fluorescence flow cytometry to quantify FRC count as a research parameter. By analyzing numerical data and comparing the size of red blood cells (RBCs), the analyzers generate the flag "Fragments?" which indicates the potential presence of FRC. This study aims to evaluate the efficacy of using the FRC count and the flag "Fragments?" to determine the presence of schistocytes in peripheral blood smears (PBSs).
Materials and methods: The retrospective observational study during the year 2024 included the data of 50 patients with elevated FRC count (> 1.0%) measured during the analysis of a complete blood count accredited according to the ISO 15189 standard. FRC count and the flag "Fragments?" were determined on Sysmex analyzers (XN1000 or XN2000). The presence of schistocytes was assessed manually by light microscopy as part of a routine RBCs morphology examination at the Clinical Institute for Pathology and Cytology. More than two schistocytes per high power field was considered a positive finding.
Results: Among 50 patients with elevated FRC count (1.01-12.88%), 15 (30%) had schistocytes in PBS while other 16 (32%) exhibited other abnormalities in PBS (inclusions in RBCs or rouleaux formations). In the remaining 19 patients PBSs (38%) predominantly anisocytosis, poikilocytosis and/or hypochromia were found. The flag "Fragments?" was present in 23 patients, and in 12 of them (52%) schistocytes were detected in PBS.
Conclusion: Combining the automated FRC count with the flag "Fragments?" showed the best results for schistocyte detection. However, every positive result should be confirmed manually by light microscopy examination. The FRC count serves as a valuable parameter for indicating abnormalities in PBSs and identifying which smears warrant manual review.

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A-14

Verification of basophil granulocyte reference interval on the Sysmex XN-550 analyzer

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Introduction: Basophil granulocytes are the least common population of peripheral blood leukocytes. Moderate increase in absolute and relative basophil granulocyte count is a rare condition found in patients with chronic myeloproliferative diseases, infectious diseases (varicella, smallpox), hypersensitivity reactions to food or medication, hypothyroidism and inflammatory bowel diseases. Physiological conditions such as ovulation and pregnancy may be associated with an elevated basophil granulocyte count.
Materials and methods: During general medical examination of apparently healthy individuals, we observed a proportion of patients with relative basophil granulocyte count above the upper reference limit (0-1%). Samples with elevated relative basophil granulocyte counts were examined microscopically after blood smear preparation and Hemacolor rapid staining. The preestablished reference interval was verified by following CLSI EP28-A3c guidelines. Statistical analysis was performed using MedCalc software version 22.023.
Results: Microscopic examination did not confirm the presence of elevated relative basophil granulocyte count. Verification procedure results showed that 3 out of 20 randomly selected patient samples fell outside 95% RI. Dixon-Reed method was applied to detect outliers, confirming that none were present, and all data points could therefore be included in statistical analysis. Subsequently, we proceeded with the development of our own reference interval. The reference interval ranging from 0.1 to 1.3% was calculated from 217 patient samples using a non-parametrical percentile method (which excludes a selected percent of values, usually 2.5%, at either end of the range according to CLSI EP28-A3c guidelines).
Conclusion: Our study reaffirmed previously published data regarding basophil granulocyte reference intervals measured on Sysmex automated hematology analyzers. The results we obtained indicate the necessity of developing new reference intervals for selected parameters in laboratory hematology, such as basophil granulocytes, and integrating the data into the official laboratory analysis harmonization document (CCMB).

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A-15

Analytical verification and comparability of red blood cell count parameters, leukocytes, platelets on the hematology analyser Celltac G

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Introduction: This study aimed to verify the analytical performance of the automated hematology analyser Celltac G, Nihon Kohden and compare it with the Advia 2120i, Siemens for red blood cell count, leukocytes, and platelets.
Materials and methods: According CLSI guidelines EP15-A2, we evaluated within-run precision, between-run precision, and accuracy using three levels of control samples. Coefficients of variation were compared with acceptance criteria based on manufacturer specifications and biological variability (EFLM BV criteria). Method accuracy was assessed by comparing results from the Celltac G with Siemens ADVIA 2120i. The whole blood samples were obtained from 40 individuals in K3-EDTAtubes.
Statistical analysis was performed using MedCalc 20.104, with results presented using Bland-Altman plots and linear regression.
Results: All parameters met the manufacturer's criteria, indicating comparable precision. However, hematocrit did not meet the EuBIVAS criterion (CV 1.9%) for between-run precision in low control samples. MCV did not meet the criterion (CV 0.5%) for repeatability in low level and for between-run precision at both low and high levels. RDW across all control levels, platelets at low levels for between-run precision and MPV for repeatability at low levels did not meet EuBIVAS criteria (CVs were 4.2%, 2.4% for PLT and MPV). Other parameters met desirable and/or minimum EuBIVAS criteria. Correlation coefficiensts for all measured parameters were acceptable ($r > 0.95$) No deviations from linearity ($P > 0.100$) were found. Leukocytes showed a constant difference (95% CI did not include 0) and a proportional deviation (95% CI did not include 1). Erythrocytes and hemoglobin exhibited a constant difference without proportional deviation. No significant differences were found for hematocrit, MCV, platelets, and MPV.
Conclusion: The results confirmed that the Celltac G analyser meets the analytical criteria for precision and accuracy. Thus, Celltac can be used in routine work to determine red blood cell count, leukocytes, and platelets.

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A-16 (Oral presentation)

Examination of platelet parameters in patients with a clinical suspicion of thrombotic microangiopathy

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Introduction: Thrombotic microangiopathies (TMAs) are disorders characterized by the formation of microclots in the peripheral bloodstream, leading to increased platelet consumption and an expected increase of thrombopoiesis. This study aimed to examine the bone marrow thrombopoietic activity in patients with a clinical suspicion of TMA, by measuring platelet parameters on a hematology analyzer and correlating them to a-disintegrin-and-metalloproteinase-with-a-thrombospondin-type-1-motif, member-13 (ADAMTS13) activity, the parameter for distinguishing different TMAs.
Materials and methods: Platelet distribution width (PDW), mean platelet volume (MPV), and immature platelet fraction (IPF%) were measured in K3-EDTAwhole blood samples from 58 patients (37 women, 21 men) on an automated hematology analyzer Sysmex XN-3100 (Kobe, Japan). ADAMTS13 activity was measured in citrated plasma samples on Ceveron-S100 (Technoclone, Austria). Statistical analysis was performed using MedCalc 16.2.0 software (MedCalc Software, Belgium).
Results: Obtained results were: medians (interquartile ranges) for PDW and IPF% 11.9 fL (10.6-14.2 fL) and 4.6% (2.8-8.4%), respectively; means ± 2SD for MPV and ADAMTS13 activity 10.6 ± 2.4 fL and 0.53 ± 0.59 kIU/L, respectively. IPF% results above the reference interval (RI) (> 8.6%) were obtained in 14/58 (24.1%) samples, while MPV and PDW above RIs (> 12.7 fL and > 17.4 fL, respectively) were encountered in 4/58 (6.9%) samples, resulting in a significantly different comparison of proportions (P = 0.021). No significant correlation between ADAMTS13 and IPF% was found neither in all samples (Spearman's rank correlation test ρ = 0.01, P = 0.948) nor in samples with decreased ADAMTS13 activities (ρ = 0.08, P = 0.675). However, a subset analysis of increased IPF% values revealed a significant correlation with ADAMTS13 activities (ρ = -0.79, P = 0.001).
Conclusion: This examination has revealed a statistically significant 3.5-fold higher frequency of increased IPF% than increased MPV and PDW values in patients with a clinical suspicion of TMA. Astrong negative correlation between increased IPF% and ADAMTS13 values was identified. In summary, among all investigated platelet parameters IPF% was revealed as the most sensitive indicator of platelet activation in TMA.

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B Hemostasis

B-01

The comparison of Euglobulin clot lysis time reference intervals in Beckton Dickinson and Kima 3.2% coagulation tubes

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Introduction: Euglobulin clot lysis time (ECLT) varies in a broad range among healthy normal individuals. The study aimed to establish reliable reference intervals for ECLT. Additionally, the influence of different vacuum tubes on ECLT reference ranges was explored.
Materials and methods: The study was performed according to CLSI EP28-A3c guidelines by a posteriori direct sampling technique. Both Kima and Beckton Dickinson (BD) 3.2% sodium citrate coagulation tubes were drawn, and ECLT was measured in duplicate. After employing the predefined exclusion criteria, 120 patients and their fibrinolysis results were enrolled in further statistical analysis. Their health status was examined by an experienced specialist in internal medicine. Neither of them had malignant or hepatobiliary disease, there was no history of DVT/PE, nor were they in the acute inflammatory state at the time of the venipuncture, confirmed by white blood cell count (WBC) (Siemens Advia 2120i), fibrinogen (Siemens BCS XP), and sedimentation rate analysis (Westergreen manual method). Statistical analysis was performed in MedCalc v22.016 statistical software (MedCalc Software Ltd, Ostend, Belgium).
Results: The median age of the participants was 49 years (range 24-75), male/female ratio 39/81. Median values for WBC count and sedimentation rate were 6.4 x10^9/L (IQR: 5.5-7.5) and 5 mm/3.6 ks (IQR: 3-9), respectively. Global coagulation parameters were within normal reference ranges: PT median value 106% (IQR: 98-114), aPTT median value 24.4 s (IQR: 22.7-25.7). Reference interval for ECLT measured in KIMA coagulation tubes ranged from 130 to 297 minutes and did not differ significantly from BD tubes (120 to 292 minutes).
Conclusions: In comparison to previously used cut-off value for ECLT (> 180 minutes), there was a significant difference in the lower limit of reference interval. However, no difference was observed depending on the different manufacturer coagulation tubes used.

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B-02

The exhaustive verification of the BCS XP automated coagulation analyzer

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Introduction: Some studies regarding BCS XP coagulation analyzer (Siemens, Marburg, Germany) verification exist but are constructed mostly around precision and comparability. Therefore, we aimed to perform an exhaustive verification of the BCS XP automated coagulation analyzer, including the alternative measurement wavelength at 570 nm.
Materials and methods: The verification protocol was created according to the CLSI H57-AProtocol for the evaluation, validation, and implementation of coagulometers (2008) and Recommendations for the evaluation of coagulation analyzers by Gardiner et al. (2006). The verification protocol included: precision testing (both repeatability and total precision) performed on quality control materials, as well as on pooled patient plasma, expressed as coefficient of variation (CV, %), comparability with the current routine coagulometer (Siemens BCS XP) on remnant patient samples spanning the reportable range of each assay, sample and reagent carryover, as well as on-instrument reagent stability. A performance assessment included the coagulation screening tests: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), the Clauss fibrinogen assay, and immunotubidimetric D-dimer assay. All reagents and control materials used in the study were manufactured by Siemens. Statistical analysis was performed in MedCalc v22.016 statistical software (MedCalc Software Ltd, Ostend, Belgium).
Results: All imprecision CVs (%), both on control materials as well as on patient-pooled plasma, were within the manufacturer's acceptability criteria (< 5/10/15%, depending on the assay). Higher CVs (4.8%) were observed for aPTT measurements on a pooled plasma due to analyte instability during the storage time at - 20°C. Both BCS XP were comparable in the reportable range, as expected because they used the same technology and reagents. There were no sample nor reagent carryover ($P > 0.05$). We confirmed the claimed manufacturer's on-board reagent stability (48h).
Conclusion: The BCS XP automated coagulation analyzer excelled in performance and can be safely used and introduced into everyday laboratory routine.

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B-03

Analytical verification and comparability of coagulation assays on the analyser CA-600 series

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Introduction: The aim of the paper was to carry out an analytical verification of the automated coagulation analyzer CA-600 (Sysmex, Japan) and a comparison with the different analyzer for measurement of the parameters in a routine coagulation testing.
Materials and methods: The verification was carried out in accordance with the EP15-A2 procedure (CLSI), and included the following tests: within- run precision, between-run precision, and accuracy by using commercial control samples at two levels; the comparison of results of patient samples with coagulation analyzer BCS XP (Siemens). All 40 blood samples for method comparison were collected from hospitalized and ambulatory patients in tubes containing sodium citrate.
Results: Evaluation of the performance of CA-600 in the analysis of prothrombin time (PT), International Normalized Ratio (INR), activated partial thromboplastin time (APTT) and fibrinogen (Fbg) showed good performance for all parameters. The reproducibility (CV%) was stated in the analyzer specifications (CV < 5% for PT %, PT-INR, APTT and < 7% for Fbg). By examining repeatability, the lowest CV was obtained for the APTT (0.36%) (s) and PT-INR (0.80%) (ratio), whereas the highest CV was obtained for the Fbg (5.18% and 2.06%). The smallest deviation from the target value (bias) was found for APTT (1.30%) (s), while the highest deviation was found for the Fbg (13.26%). With method comparison the value of the correlation coefficients obtained was r > 0.95, except for the APTT. There is no statistically significant difference (95% CI of the mean difference contains 0) for PT/INR and APTT results.
Conclusion: The obtained results confirm that the tested automated blood coagulation analyzer CA-600 meets the set analytical criteria for precision and accuracy. Hence, it can be used in routine coagulation testing.

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C Cardiovascular Diseases

C-01 (Oral presentation)

Screening for familial hypercholesterolemia in preschool children - cholesterol results from eight laboratories in Croatia

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Introduction: Familial hypercholesterolemia (FH) is the most common hereditary disorder of lipid metabolism that leads to the early onset of cardiovascular diseases. National program for screening and early detection of FH, introduced in Croatia in 2023, includes measurement of total cholesterol (TC) in children during a systematic examination for enrollment in elementary school. The aim of this work was to present TC results in preschool children obtained from eight laboratories in Croatia.
Materials and methods: Data were retrospectively extracted from laboratory information system for period from January to October 2023 from laboratories in Bjelovar, Dubrovnik, Metković, Koprivnica, Šibenik, Pula, Zadar and included children with diagnosis Z02.0 Examination for enrollment in an educational institution. Cholesterol was determined using the photometric method with cholesterol oxidase on the Beckman Coulter (Bjelovar, Dubrovnik1, Dubrovnik2, Metković), Abbott (Koprivnica, Šibenik) and Roche (Pula, Zadar) platforms according to the manufacturers's specifications and using proprietary reagent. According to the national program strategy, TC ≥ 6.1 or ≥ 5.1 mmol/L with a positive family anamnesis is considered as a positive screening result.
Results: The value of TC ≥ 6.1 mmol/L was found in 39 of 3902 children (1.00%); Bjelovar 9/560 = 1.61%, Dubrovnik1 7/349 = 2.01%, Dubrovnik2 3/301 = 1.00%, Metković 2/278 = 0.72%, Koprivnica 3/471 = 0.64%, Šibenik 3/365 = 0.82%, Pula 2/514 = 0.39%, Zadar 10/1064 = 0.94%. TC ≥ 5.1 and ≤ 6.0 mmol/L was found in 320 of 3902 children (8.20%); Bjelovar 54/560 = 9.64%, Dubrovnik1 45/349 = 12.89%, Dubrovnik2 42/301 = 13.95%, Metković 21/278 = 7.55%, Koprivnica 41/471 = 8.70%, Šibenik 28/365 = 7.67%, Pula 27/514 = 5.25%, Zadar 62/1064 = 5.83%.
Conclusion: The presented data from eight laboratories and different regions provide an insight into the distribution of TC results in the first step of the FH screening program in Croatia. Based on the result distribution, 9.2% of preschool children need to be involved in the second step of the screening program (family anamnesis, repeated TC determination or further clinical evaluation), while 90.8% have negative FH screening results.

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C-02

Does the type and duration of dialysis affect the concentration high sensitive troponin I?

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Introduction: Patients with end-stage renal disease (ESRD) on haemodialysis (HD) or hemodiafiltration (HDF) procedures have higher incidence of cardiovascular diseases with elevatedconcentrations of high sensitive troponin I (hsTnI). The aim of this study was to determine if HD or HDF as well as years of dialysis affect hsTnI concentrations before and after treatment.
Materials and methods: Our study included 73 patients divided on HD (N = 47) and HDF (N = 26) group as well as if they underwent the treatment for less than 3 years (N = 44) and more than 3 years (N = 29). We determined hsTnI concentrations before and after the treatment for entire cohort and in separate groups. We used Wilcoxon test for paired samples and Mann Whitney test for testing the differences between the defined groups (MedCalc statistical software, version 14.8.1)
Results: No statistically significant difference was found in hsTnI concentrations before and after thetreatment in the whole cohort (P = 0.701) and regardless of the type of the treatment (P = 0.182 for HD; P = 0.275 for HDF group) as well as the duration of the treatment (P = 0.415 less than 3 years; P = 0.156 more than 3 years). We found no statistically significant difference in hsTnI concentrations regardless of the type of the tretman before (P = 0.330) and after (P = 0.195) the treatment. Statistically significant difference of hsTnI concentrations before and after the treatment wasfound between the group that underwent the treatment for less than 3 years (P = 0.020) and more than 3 years (P = 0.001).
Conclusion: Our results showed that type and duration of the treatment do not affect hsTnI concentrationsbefore and after the treatment thus suggesting that HD and HDF have similar effects on cardiovascular status. Significant difference was found depending on the duration of the treatment suggesting that cardiovascular damage increases with the duration of the treatment.

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D Endocrinology

D-01

Verification of the TSH method on the Snibe Maglumi X3 analyser and comparison with Abbott Architect i1000SR

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Introduction: Croatian Thyroid Society guidelines recommend measuring TSH in serum as the first test to detect thyroid function disorders. Analytical verification of new assay is required prior to its introduction into routine practice. The aim was to verify the performance of the chemiluminescence immunoassay (CLIA) TSH test on the Maglumi X3 analyzer (Snibe, Shenzhen, China) as well as to compare it to the routinely used chemiluminescent microparticle immunoassay (CMIA) on Architect i1000SR analyzer (Abbott, Abbott Park, USA).
Materials and methods: Verification was performed according to the CLSI EP15-A3 guidelines. Coefficients of variation (CV) for repeatability and intra-laboratory imprecision were calculated and compared with EFLM criteria (optimal < 4.5%, desirable < 8.9%, minimum < 13.4%) same as bias (optimal < 5.0%, desirable < 10.1%, minimum < 15.1%). Sixty-seven patient samples were analysed using Maglumi X3 analyser and the results were compared with measurements obtained by Architect i1000SR analyser. Method comparison was performed with Bland-Altman and Passing-Bablok regression analysis. Statistical analysis was performed with MedCalc (MedCalc Software, Ostend, Belgium). P value < 0.05 was considered statistically significant.
Results: Calculated CV for repeatability for levels 1 and 2 were 1.48% and 1.03%, and for intra-laboratory imprecision were 1.23% and 1.15%. Bias were 2.74% and 0.34%. Comparison of TSH results measured by the Maglumi X3 and Architect method showed excellent agreement (rho = 0.98, 95% CI: 0.97 to 0.99). Passing-Bablok regression showed proportional difference (y = - 0.06 (- 0.12 to 0.01) + 1.3 (1.27 to 1.35) x. Cusum test for linearity showed no significant deviation from linearity (P > 0.10). Bland-Altman analysis revealed absolute bias of - 0.68 (95% CI: - 0.85 to - 0.51) and relative bias of - 20.9% (95% CI: - 25.64 to - 16.2).
Conclusion: Maglumi X3 TSH assay showed acceptable analytical performance. Because there is proportional difference and significant bias between two methods they can't be used interchangeably.

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D-02

The quality of OGTT in pregnancy

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Introduction: The oral glucose tolerance test (OGTT) is a test that measures glucose concentrations before and after consumption of 75 g glucose solution. OGTT is routinely performed on pregnant women with normoglycemia in first trimester of pregnancy. OGTT is performed between 24-28 weeks of pregnancy, and according to the recommendations of the gynecological and laboratory profession, glucose is determined at three points; before taking the glucose solution, one hour after taking the solution and two hours after. This way, gestational diabetes is diagnosed, which is the most common metabolic complication in pregnancy. It was noticed that laboratories do not comply with the stated rules for OGTT testing, and the aim of this research was to see what percentage of pregnant women were correctly tested.
Materials and methods: The anamnestic data of 155 pregnant women in the third trimester of pregnancy were collected in 3 hospitals in Slavonia: CMC Osijek, NMH Vukovar and GH Vinkovci. All pregnant women submitted the results of OGTT testing in primary health care laboratories.
Results: Out of 155 pregnant women, 34 of them (22%) were not referred by gynecologists for OGTT testing between 24-28 weeks of pregnancy, although it is recommended to do this for all pregnant women with normoglycemia in the first trimester. In 22 pregnant women (14%), OGTT testing was not done in 3 points; in 9 pregnant women (41%), glucose was not determined one hour after taking the solution, while in 13 pregnant women (59%) the third extraction was not performed because the first two points were normal.
Conclusion: Some laboratories choose to improvise OGTT testing in pregnant women with normal fasting glucose values, however improper performance of the OGTT test can lead to missed diagnoses of gestational diabetes, which can have a fatal outcome for mother and child.

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D-03 (Oral presentation)

Ratio of helpers/cytotoxic lymphocytes and NK/NKT cells as an early marker of gestational diabetes

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Introduction: Gestational diabetes mellitus (GDM) is a condition of abnormal glucose tolerance that appears during pregnancy and usually disappears after the delivery. GDM is usually diagnosed between 24-28 weeks of pregnancy using oral glucose tolerance test (OGTT) because no good early biomarker of GDM has yet been found. The goal of our research is to investigate if the ratio of helpers/cytotoxic lymphocytes and ratio of NK/NKT cells can be valuable early biomarkers of GDM.
Materials and methods: The study enrolled 61 pregnant women, divided into three groups: 13 pregnant women with GDM, 14 pregnant women with a glucose disorder in the first trimester and 34 healthy pregnant women in control group. GDM in pregnancy is defined with OGTT test. Lymphocyte, NK and NKT cells were analysed from the whole blood samples of pregnant women by flow cytometry (cytometer BD LSR II with acquisition software BD FACSDiva 8.0.1. and for FloJo analysis, Beckton Dickinson, Germany) in first and third trimester of pregnancy. The statistical program MedCalc Statistical was used for data analysis Software version 20.218 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023).
Results: Results of the analysis performed by flow cytometry showed that the control group of healthy pregnant women has more T- helper lymphocytes ($P = 0.020$) and a higher ratio of helper/cytotoxic lymphocytes ($P < 0.001$) compared to the other two investigated groups in first trimester of pregnancy. Pregnant women with fasting glucose disorders had a statistically significantly higher percentage of NK cells in both, first and third trimester of pregnancy ($P = 0.030$, both).
Conclusion: The ratio of helper/cytotoxic lymphocytes could be a potential early marker of GDM. The ratio of NK/NKT cells has not been shown to be a potential early biomarker for the prediction of GDM. Additional research on a larger sample population is needed to confirm our findings.

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D-04

The precision study for CMIA-Abbott TSH, T4, FT4, FT3 and Tg performed for Alinity i platform

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Introduction: The study aim was to test whether our within-laboratory precision data for CMIA-Abbott TSH, T4, FT4, FT3 and Tg for Alinity i platform may fulfill the requirements of EFLM biological variation database criteria for desirable precision.
Materials and methods: The precision study for was conducted for Alinity i platform (Abbott Laboratories, Abbott Park, USA) according to CLSI EP15-A3 (2014, Wayne, USA) protocol (five days, five measurements each day) in May 2024. Within-laboratory precision data were calculated and compared with EFLM BIOLOGICAL VARIATION DATABASE desirable goals for precision. The control material was Randox IAPremium Plus (lot 640929, EXP 2025-11-28, two levels). The calibrators and reagents lots were the same during entire study.
Results: For desirable coefficient of variation (CV < 8.9%) TSH measurements achieved agreeable CVs (mean 1.82 mIU/L, CV 3.0%; mean 16.42 mIU/L, CV 3.0%) as well as for thyroglobulin CV < 5.5% (mean 2.22 µg/L, CV 2.9%; mean 62.52 µg/L, CV 4.8%). The EFLM goal (CV < 3.2%) was fulfilled for one level of T4 measurement (mean 121.17 nmol/L, CV 3.1%), but not for the other (mean 199.19 nmol/L, CV 5.0%). For both levels of FT4 measurement EFLM goal CV < 2.4% was not achieved (mean 9.95 pmol/L, CV 4.2%; mean 22.70 pmol/L CV 2.8%) as well as for FT3 desirable CV < 2.5 (mean 3.26 pmol/L, CV 6.3% and mean 12.78 pmol/L, CV 4.1%).
Conclusion: The precision desirable EFLM BV database criteria were achieved for TSH and Tg but not for thyroid hormones (T4, FT4, FT3) measurements for two levels of third party Randox control material using Abbott-CMIAassays for Alinity i platform. Thyroid hormones are under highly stringent homeostatic control which resulted in low within-subject biological variation values. For thyroid hormone immunoassays conducted on platforms which are in every day maximum use, criteria for within-laboratory precision derived from within-subject biological variation might be difficult to achieve.

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D-05 (Oral presentation)

Effects of levothyroxine therapy on markers of endothelial dysfunction in patients with subclinical hypothyroidism

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Introduction: Subclinical hypothyroidism (SCH) is an independent risk factor for cardiovascular diseases due to endothelial dysfunction and atherosclerosis development. We hypothesized that patients with a mild form of SCH (TSH ≤ 10.0 mIU/L) had higher concentrations of endothelial dysfunction markers than healthy controls and that the levothyroxine introduction would have a beneficial effect on these parameters. The study aimed to determine the levothyroxine effect on endothelial dysfunction markers (endothelin-1 (ET-1), asymmetric dimethylarginine (ADMA) and endocan) in SCH patients since clinical importance and treatment of them is often discussed.
Materials and methods: The study included 61 patients with a mild form of SCH and 30 age and gender matched healthy controls. The concentrations of endothelial dysfunction markers (ET-1, ADMA and endocan) were measured by ELISA (R&D Systems, Elabscience and Abcam, respectively) in all subjects at baseline, and in 29 SCH patients after 6 months of levothyroxine treatment following the euthyroidism. Data (median (interquartile range)) were analyzed using the Wilcoxon rank sum test and Mann-Whitney test. $P < 0.05$ was considered statistically significant.
Results: Our study showed elevated ADMA (235.80 (168.33-516.55) vs. 166.30 (140.60-243.40) ng/mL, P = 0.003) and endocan (114.30 (63.45-194.65) vs. 67.26 (50.80-126.10) pg/mL, P = 0.004) concentrations, and no differences in ET-1 concentrations (1.24 (1.07-1.46) vs. 1.36 (1.09-1.56) pg/mL, P = 0.287) in SCH patients in comparison with healthy subjects. In SCH patients, after 6 months of levothyroxine treatment following the euthyroidism, we observed a significant decrease in endocan (91.47 (61.88-200.03) vs. 97.90 (55.18-154.88) pg/mL, P = 0.004), and no differences in ADMA (235.8 (160.8-588.6) vs. 326.25 (147.68-621.33) ng/mL, P = 0.456) and ET-1 concentrations (1.41 (1.23-1.52) vs. 1.42 (1.22-1.61) pg/mL, P = 0.681).
Conclusion: A mild form of SCH is associated with increased concentrations of endocan and ADMA. The potential benefit of levothyroxine was shown through the significant decrease of endocan levels in SCH patients, thus contributing to the prevention of endothelial dysfunction.

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D-06

Effects of levothyroxine therapy on dyslipidemia in patients with subclinical hypothyroidism

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Introduction: Dyslipidemia is commonly present in patients with hypothyroidism, even in its subclinical form (SCH), representing an additional risk factor for cardiovascular diseases. We hypothesized that patients with a mild form of SCH (TSH≤10.0 mIU/L) had higher dyslipidemia parameters than healthy subjects, and that levothyroxine introduction would have a beneficial effect on their concentrations. The study aimed to determine the effect of levothyroxine on lipid parameters in SCH patients.
Materials and methods: The study included 61 patients with a mild form of SCH and 30 age and gender matched healthy controls. Lipid parameters (triglycerides, total cholesterol, LDL-cholesterol and non-HDL cholesterol) were determined in all subjects at baseline, and in 29 SCH patients after 6 months of levothyroxine treatment following the euthyroidism. Data (median (interquartile range) or mean (standard deviation)) were analyzed using the Wilcoxon rank sum test and Mann-Whitney test or paired sample t-test and independent sample t-test. $P < 0.05$ was considered statistically significant.
Results: Our study showed higher LDL cholesterol (3.7 ± 0.9 *vs*. 3.3 ± 0.6 mmol/L, P = 0.043) and non-HDL cholesterol (4.2 ± 1.0 *vs*. 3.8 ± 0.7 mmol/L, P = 0.020) concentrations, and no differences in triglycerides (1.1 (0.8-1.5) *vs*. 0.9 (0.7-1.3) mmol/L, P = 0.064) and total cholesterol (5.9 ± 1.1 *vs*. 5.5 ± 0.9 mmol/L, P = 0.059) concentrations in SCH patients as compared to healthy subjects. In SCH patients, after 6 months of levothyroxine treatment following the euthyroidism, we observed a decrease in total cholesterol (6.2 ± 1.0 *vs*. 5.8 ± 1.0 mmol/L, P = 0.039) and no differences in triglycerides (1.1 (0.8-1.5) *vs*. 1.1 (0.9-1.4) mmol/L, P = 0.864), LDL cholesterol (3.9 ± 0.8 *vs*. 3.6 ± 0.8 mmol/L, P = 0.107) and non-HDL cholesterol (4.5 ± 1.0 *vs*. 4.2 ± 0.9 mmol/L, P = 0.144) concentrations.
Conclusions: A mild form of SCH is associated with elevated LDL cholesterol and non-HDL cholesterol. The potential benefits of levothyroxine were shown through the significant decrease of total cholesterol concentrations in SCH patients, thus contributing to the prevention of atherosclerotic process and cardiovascular disease development.

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D-07 (Oral presentation)

The stability study of thyroid function tests

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Introduction: Thyroid function tests (TSH, tT4, tT3, fT4, fT3, A-TPO and A-TG) are essential in diagnosing and monitoring of various thyroid dysfunctions. In laboratory, delayed analysis or subsequent test requests are sometimes needed, making it necessary to know test stability. Our aim was to determine the stability of thyroid function tests in serum during 72 hours.
Materials and methods: The study was conducted in accordance with the recommendations of the EFLM Working Group for Preanalytical Phase regarding stability testing. Residual samples from 54 patients (91% woman), median age 42 (11-73) were included. Venous blood was collected in clot activator gel tubes (Greiner Bio-One GmbH, Kremsmuenster, Austria). Ten samples of different concentrations spanning the entire analytical range were used for each test and were analysed in duplicate at each time point (0h, 4h, 8h, 24h, 48h, 72h) on Abbott Architect i2000. First 8h samples were at room temperature and remaining 64h in the refrigerator at 2-8°C. Test stability was determined by assessing the mean percentage difference (PD%), average of ten percentage difference from 0h) and comparing it to the maximum permissible difference (MPD%, calculated via $\sqrt{((2{,}77 \cdot CVa)^2+(0{,}5 \cdot CVb)^2)}$; analytical (CVa) and intraindividual (CVb) coefficient of variation.
Results: Respective PD% and MPD% at defined time points were: TSH (0.0; 3.0; 4.8; 4.5; 6.4; 4.2; ± 17.38), tT4 (0.0; - 0.9; 3.0; 1.1; 1.9; 3.7; ± 11.00), tT3 (0.0; 0.6; 1.6; 4.4; 2.2; 3.3; ± 11.02), fT4 (0.0; 1.2; 0.1; 2.6; 4.0; - 0.2; ± 16.25), fT3 (0.0; - 0.9; - 0.3; 0.9; 2.4; 4.1; ± 12.44), A-TPO (0.0; 3.8; 3.1; 2.8; 3.3; 2.6; ± 18.08), A-TG (0.0; 3.0 ; 3.9; 2.0; 1.5; 2.3; ± 12.91). PD% at all defined time points did not exceed the MPD% for any of the function tests.
Conclusion: Thyroid function tests remain stable in serum for 72 hours, meaning they can be postponed or performed subsequently following the tested protocol.

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D-08

The frequency of follicle-stimulating hormone receptor gene polymorphism and the risk of women and males infertility in Albanian ethnic population in Kosovo

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Introduction: Follicle-stimulating hormone (FSH) plays a major role in ovarian function and oocyte maturation in women as well as in enabling the normal function of cells involved in spermatogenesis in men. Since the secretion of FSH is in a negative feedback loop with the action of FSHR, the function of this receptor may vary depending on the background of the patient's FSHR genotype. Our aim was to investigate the association of the Asn680Ser FSHR polymorphism with the ovarian response in ethnic Albanian women, as well as to determine the prevalence of the Asn680Ser FSHR polymorphism in the Albanian male population with infertility.
Materials and methods: Asn680Ser polymorphism analysis was performed using the TaqMan SNP genotyping assay. Clinical and endocrinological parameters were analyzed based on genotype in 104 women and 114 infertile men. Hormonal assays were analyzed by the ELFA method with the Mini Vidas Analyzer (bioMérieux, France).
Results: Genotype frequencies in females: Asn/Asn 22.1%, Asn/Ser 47.1% and Ser/Ser 30.8%. Correlation analysis showed a statistically significant negative correlation ($P < 0.001$) of the number of oocyte retrieval in relation to age, and the dose of gonadotropins. Genotype frequencies in males: Asn/Ser 42%, Ser/Ser 33.9% and Asn/Asn 24.1% (χ2-test: $P = 0.08$). Adjusted logistic regression analysis revealed an increased odds ratio for male infertility among heterozygous carriers of the Asn/Ser genotype with lower values of sperm parameters.
Conclusion: FSH receptor polymorphism is associated with different ovarian response to controlled ovarian stimulation (COS), but is not an im- portant factor in increasing the degree of pregnancy. The presented results of FSHR Asn680Ser polymorphisms and male infertility show that the Asn/Ser genotype may increase the risk of male infertility in the Albanian population.

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F Chronic Diseases

F-01

Analytical verification of two automated immunoturbidimetric assays for determination of fecal calprotectin

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Introduction: Fecal calprotectin (FC) is a useful biomarker for diagnosis and monitoring of inflammatory bowel disease. The aim was the analytical verification of automated immunoturbidimetric assays Bühlmann fCAL turbo (Bühlmann Laboratories AG, Switzerland) and Calprest Turbo (Eurospital Diagnostic, Italy) on Alinity c analyzer (Abbott Laboratories, USA).
Materials and methods: Precision was determined following the CLSI EP15-A3 protocol by analyzing commercial controls at two levels (Bühlmann fCAL turbo L and H, Calprest Turbo C1 and C2) for five days in quintuplicate. Measurement uncertainty was estimated by analyzing assay-dedicated calibrators 10 consecutive times. Methods comparison included analysis of 46 extracted stool samples by both assays, as well as the chemiluminiscence immunoassay QUANTAFlash Calprotectin on Bioflash analyzer (Inova Diagnostics, USA).
Results: Within-run coefficients of variation (CVs) for Bühlmann fCAL turbo were 3.6% for the low and 4.1% for the high concentration level, while for Calprest Turbo 3.9% and 7.8%, respectively. Between-run CVs were 5.5% and 4.2% (Bühlmann fCAL turbo), and 7.2% and 2.3% (Calprest Turbo), while within-laboratory CVs were 6.4% and 5.6%, and 8.0% and 7.4%, respectively. Measurement uncertainties were 30.8%, 10.0% and 26.7% for Bühlmann fCAL turbo, and 39.0%, 29.6% and 41.6% for Calprest Turbo. Spearman's correlation coefficients ranged from 0.902 (Bühlman fCAL turbo *vs*. Inova) to 0.919 (Buhlman fCAL turbo *vs*. Calprest Turbo). Passing-Bablok regression indicated proportional difference for comparison of Buhlman fCAl turbo with both Calprest Turbo ($y = 0.45 x + 11.4$) and Inova ($y = 3.97 x - 99.9$) as well as between Calprest Turbo and Inova ($y = 1.85 x - 34.5$). Bland-Altman analysis yielded relative biases of 61.3% (95% CI: 40.9 to 81.7) (Bühlmann fCAl turbo vs. Calprest Turbo), - 66.1% (95% CI: - 83.9 to - 48.2) (Bühlmann fCal turbo *vs*. Inova), and -7.3% (95% CI: - 32.9 to 18.2) (Calprest Turbo *vs*. Inova).
Conclusion: Both assays demonstrated satisfactory analytical performance and are suitable for FC determination in routine practice. However, comparative analysis revealed that Buhlman fCal Turbo slightly outperformed Calprest Turbo.

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F-02

Clinical significance of FIB-4 index for MASLD-risk stratification in non-obese patients with diabetes mellitus

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Introduction: Diabetes is one of the major risk factors for developing Metabolic dysfunction–associated steatotic liver disease (MASLD). The American Diabetes Association (ADA) recommends using calculated FIB-4 index (FIB-4i) for universal screening of people with diabetes for MASLD. This study aimed to evaluate clinical significance of FIB-4i for MASLD-risk stratification in non-obese, well-controlled diabetic patients without diabetes-related complications.
Materials and methods: FIB-4i was calculated in 390 patients with type 1 (T1D) and type 2 (T2D) diabetes (202/188; 52%/48%, respectively) by a dedicated formula using patient's age, AST, ALT, and platelet count. FIB-4i ≤ 1.3 is considered a low risk, while range between 1.3-2.67, and FIB-4i > 2.67 are considered intermediate and very high-risk categories for clinically significant fibrosis, respectively. Statistical analysis was performed using the MedCalc statistical program (MedCalc Software, Ostend, Belgium).
Results: Based on FIB-4i, 272 of the patients (70%) had low risk, 113 (29%) had intermediate, and 5 (1.2%) high risk for developing MASLD. Aslightly lower FIB4-i was observed in patients with T1D in comparison to T2D (1.099 ± 0.598 vs. 1.208 ± 0.541, P = 0.050). There was no significant difference regarding the MASLD risk category distribution (low vs. cumulative intermediate+high-risk) in relation to the type of diabetes ($\chi 2$ = 0,170, df = 1, P = 0.678). Multiple linear regression analysis showed that FIB-4i was positively associated with patient's age, GGT and bilirubin (r- partial = 0.517, 0.125 and 0.127; P = < 0.001, 0.018 and 0.017, respectively), and negatively with leukocyte count and HbA1c (r-partial = - 0.170 and - 0.196, P = < 0.001).
Conclusion: Our results show that 70% of non-obese diabetic patients with well-controlled glycaemia and free from diabetes-related complications have low risk for developing MASLD, based on FIB-4i, with T1D patients having slightly lower FIB-4i than T2D patients. Among positive predictive variables, patient's age had the most prominent effect on FIB-4i, while leukocyte count and HbA1c were identified as weak, but significant negative predictors of FIB-4i in the studied cohort.

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F-03

Comparison of laboratory assays for diabetes screening in high risk individuals: which test is the most appropriate?

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Introduction: Diabetes screening is recommended in high risk individuals using either fasting plasma glucose (FPG), hemoglobin A1c (HbA1c) or 2-h oral glucose tolerance test (OGTT). Our aim was to investigate the comparability of these screening assays.
Materials and methods: Adult participants, without previously diagnosed diabetes, referred for evaluation to the endocrinology clinic, were prospectively enrolled. Inclusion criteria comprised clinical suspicion for diabetes or the presence of at least one risk factor (obesity, hypertension, dyslipidemia, positive family history for diabetes, cardiovascular or thyroid disease, previous gestational diabetes, polycystic ovary syndrome and other conditions associated with insulin resistance). Venous blood was drawn from each participant in sodium fluoride/potassium oxalate (for FPG and OGTT measurements) and K2EDTAtubes (for HbA1c testing) (Vacuette, Greiner Bio-One, Kremsmünster, Austria). Measurements were performed on the Abbott Alinity c analyzer (Abbott, Abbott Park, USA). The FPG was used to classify participants into 3 groups: diabetic (FPG ≥ 7.0 mmol/L), prediabetic (FPG 5.6-6.0 mmol/L) and healthy (FPG < 5.6 mmol/L). Diagnostic accuracy of HbA1c and OGTT were calculated using FPG as reference. The detection rates of diabetes and prediabetes based on FPG, OGTT or HbA1c using recommended criteria were compared. Statistical analyses were performed using Medcalc (v. 20.008, Ostend, Belgium).
Results: A 135 individuals participated in the study (aged 42 (19-78) years; females 84%). OGTT and HbA1c displayed comparable diagnostic performance for diabetes screening. Diagnostic accuracy measures (with corresponding 95% confidence intervals) were: 54 (37-69)%, 95 (88-98)% and 0.74 (0.66-0.81) for OGTT sensitivity, specificity and area under curve (AUC), respectively; and 59 (42-74)%, 94 (87-98)% and 0.76 (0.68-0.83) for HbA1c sensitivity, specificity and AUC, respectively. Furthermore, no differences in detection rates were found when the screened population was classified according to recommended criteria for FPG, OGTT and HbA1c (P = 0.259).
Conclusion: Our results demonstrate that FPG, OGTT and HbA1c display comparable diagnostic characteristics and detection rates, and might be used interchangeably for screening purposes.

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F-04

Comparison of two immunochromatographic tests for fecal occult blood detection

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Introduction: Immunochromatographic tests for fecal occult blood testing (iFOBT) are suitable for population screening of colorectal cancer. Various rapid immunochromatographic tests for the qualitative detection of traces of blood in stool samples are commercially available in Croatia. The aim of this study was to compare the results obtained using two iFOBTs - the CoproHemoGnost by Biognost (Zagreb, Croatia) and FOB Advanced Test by Ulti med (Ahrensburg, Germany). We hypothesized that the results will be comparable.
Materials and methods: A total of 20 stool samples (10 negative and 10 positive) were examined using firstly CoproHemoGnost and followed by FOB Advanced Test. Testing was performed as per manufacturers reccomendations. The declared analytical sensitivity of both tests was 40 ng/mL. The obtained results were analyzed using the inter-rater agreement test (kappa statistics) in the MedCalc program v20.008 (MedCalc Software Ltd, Ostend, Belgium).
Results: All 20 analyzed samples showed complete agreement: negative stool samples were found negative with both tests; positive stool samples were found positive with both tests. The obtained kappa coefficient was 1.00 (95% CI 1.00-1.00), indicating almost perfect agreement between the two tests.
Conclusion: Our results indicate almost perfect agreement between the CoproHemoGnost and FOB Advanced Test. Both test may be used interchangeably for the detection of blood in stool samples.

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G Autoimmune Diseases

G-01

Is it possible to harmonize anti-dsDNA antibodies? Comparison of six different reagents for anti-dsDNA antibodies determination

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Introduction: Anti-double stranded DNA (anti-dsDNA) antibodies play an important role in the diagnosis, classification and management of systemic lupus erythematosus (SLE). Our aim was to compare the results of six methods in different hospital centers.
Materials and methods: The study included 50 patients with history or suspicion of SLE. Anti-dsDNA was measured using 6 different reagents: Anti-dsDNA-NcX ELISAon Euroimmun Analyzer I-2P (EUROIMMUN AG, Lubeck, Germany), dsDNAchemiluminiscent immunoassay (CLIA) on IDS iSYS (IDS ISYS, Pouilly en Auxois, France), QUANTAFlash dsDNACLIAon BIO-FLASH (Inova Diagnostics Inc, San Diego, USA), Anti- dsDNAon Alegria2 ELISAmethod (Orgentec, Mainz, Germany), Anti-dsDNA ELISAon BEP 2000 analyzer (Orgentec, Mainz, Germany), and NOVA Lite dsDNACrithidia luciliae kit (Inova Diagnostics Inc, San Diego, USA). Results were categorized as positive or negative and we calculated kappa statistics as well as intraclass correlation coefficient (MedCalc version 14.8.1).
Results: Intraclass correlation coefficient showed good degree of consistency in average (0.80 (95% CI: 0.63-0.90)) but only moderate reliability on single ratings (0.44 (95% CI: 0.25-0.63). Separate Cohen`s kappa testings revealed fair to moderate agreement for most reagent combination and substantial agreement between Alegria2 ELISAanti-dsDNAand Orgentec anti- dsDNAELISA (kappa = 0.72) as well as between CLIAmethods, QUANTAFlash and IDS iSYS dsDNA (kappa = 0.63). NOVA Lite dsDNACrithidia luciliae kit showed substantial agreement only with Euroimmun Analyzer I-2P (kappa = 0.71) and fair to moderate agreement (0.34-0.59) with other reagent combinations.
Conclusions: Regardless of the efforts towards standardization of the anti- dsDNAmethod, obtained results still show substantial heterogeneity. Anti-dsDNA is widely used for diagnosing and following patient with SLE so future studies should focus on defining false positive results. Considering that we are still far from harmonization of the results it is important to follow up the patient with the same method.

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G-02

Analytical verification of autoimmune tests on the novel BioCLIA500 fully automated chemiluminescence analyzer

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Introduction: Autoimmune diagnostics as one of the fastest-growing parts of laboratory diagnostics needs a fast and reliable analytic platform. We aimed to perform analytical verification of BioCLIA500, a fully automated chemiluminescence analyzer (HOB Biotech Group, Suzhou, China).
Materials and methods: Analytical verification of autoimmune tests on BioCLIA500 analyzer included anti-dsDNA, anti- Ro60, anti-SSB, anti-Sm, anti-RNP/Sm, anti-Scl70, anti-Jo1, anti-ENA, and anti-CENP B. Precision was tested on two levels of manufacturer quality controls (QC) and pool of sera (PS) in triplicate over 5 days. Accuracy was assessed by comparison with the method in routine use, QUANTAFlash chemiluminescence method on BIO-FLASH analyzer (Inova Diagnostics, San Diego, USA) for all tests except anti-CENP B which was compared with Line immunoassay (LIA) and indirect immunofluorescence (IIF) on HEp-2 cells (both Euroimmun, Luebeck, Germany). Method comparison was performed on 23 to 43 serum samples with requests for routine autoimmune testing. Agreement between methods was assessed with an Inter-rater agreement kappa test with an acceptable kappa coefficient set at ≥ 0.60.
Results: Intra- and inter-assay coefficients of variation for low and high QC and PS were as follows: anti-dsDNA 8.45%, 10.10%, 4.33%, 9.26%, 2.78%, 3.67%; anti-Ro60 2.50%, 2.82%, 2.08%, 4.28%, 2.57%, 2.27%; anti-SSB 2.87%, 4.20%, 2.15%, 2.81%, 2.48%, 4.70%; anti-Sm 1.86%, 3.07%, 2.03%, 2.66%, 2.57%, 2.27%; anti-RNP/Sm 2.99%, 5.95%, 3.40%, 7.19%, 1.63%, 1.38%; anti-Scl70 3.16%, 3.94%, 3.96%, 4.86%, 1.24%, 1.82%; anti-Jo1 2.30%, 3.31%, 4.39%, 5.94%, 1.29%, 1.16%; anti-ENA 4.33%, 4.38%, 3.66%, 5.53%, 2.08%, 2.85%, anti-CENP B 1.25%, 1.60%, 1.59%, 2.84%, 2.10%, 2.07%. Kappa coefficients (95% CI) were as follows: anti-dsDNA 0.769 (0.583-0.954); anti-Ro60 0.806 (0.628-0.983); anti- SSB 0.876 (0.712-1.000); anti-Sm 0.920 (0.768-1.000); anti-RNP/Sm 0.899 (0.764-1.000); anti-Scl70 0.750 (0.488-1.000); anti-Jo1 0.933 (0.805-1.000); anti-ENA 0.945 (0.838-1.000); anti-CENP B 0.913 (0.746-1.000) for LIAand 0.806 (0.549-1.000) for IIF.
Conclusion: Results of verification showed very good analytical performances of the BioCLIA500 analytical platform.

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G-03 (Oral presentation)

Kappa free light chain index as a quantitative alternative to isoelectric focusing

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Introduction: Kappa free light chain index (KFLCI) is a marker of IgG intrathecal synthesis. Isoelectric focusing (IEF) is a gold standard to detect oligoclonal bands and support or dismiss the diagnosis of multiple sclerosis (MS). The aim of the study was to compare KFLCI and IEF diagnostic sensitivity and specificity in patients with multiple sclerosis.
Materials and methods: We measured KFLC in cerebrospinal fluid (CSF) and sera of 32 patients with confirmed MS diagnosis (N = 8) or other non- MS conditions (N = 24) with nephelometry on Atellica NEPH 630 (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). KFLCI was calculated as the ratio of kappa light chain/albumin quotient. The IEF results were collected from anamnestic data of patients. We performed receiver operating characteristic (ROC) analysis in order to select the optimal cut-off for KFLCI. Using 2x2 tables we calculated sensitivity and specificity for IEF and KFLCI and compared the obtained data (MedCalc version 14.8.1).
Results: ROC analysis revealed the area under the FKLCI curve as 0.808 +- 0.099 (95% CI: 0.642-0.919) and the best cut-off values to separate MS and non-MS patients was at 0.0641 (sensitivity 87.5% and specificity 75.0%). When categorised according to KFLCI > 0.064, and using 2 x 2 table we calculated sensitivity of 100% and specificity of 80%. Results of IEF were categorized as negative (without intrathecal synthesis) and positive (intrathecal synthesis) and yielded sensitivity of 100% and specificity of 61%.
Conclusion: Our results showed that KFLCI and IEF have the same clinical sensitivity but the specificity of KFLCI was higher compared to IEF suggesting that KFLCI has a potential as a good analytical tool in helping diagnosing MS due to reliability, automatization, as well as the rapid and objective determination. Future, more elaborate studies could confirm KFLCI role in diagnosing MS.

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G-04

Immunoglobulin G (IgG) N-glycome changes during immunosuppressive treatment of the anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis - a case report

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Introduction: IgG N-glycome represents an enzymatically attached oligosaccharides network, substantial for IgG functional "phenotype". Immunosuppression is the treatment cornerstone for ANCA-associated vasculitis (AAV), an autoimmune disease presenting with vascular loss and necrosis in various organs. Given the assumed IgG N-glycome alterations involvement in AAV pathogenesis, we evaluated IgG N-glycome changes during the immunosuppressive treatment in an AAV case.
Material and methods: Serum was collected from a 63-year-old female patient treated for myeloperoxidase AAV with lung and renal involvement. The first and second points were the immunosuppression induction (cy clophosphamide+methylprednisolone) and remission achievement (after three months). The third point was Rituximab administration (three months later) for maintaining immunosuppression, and the fourth sampling point was after one month. Following IgG isolation, N-glycans were enzymatically released, fluorophore-labeled, and separated using capillary gel electrophoresis. After measuring the relative abundances, the derived glycan traits were calculated.
Results: Until remission, the most prominent changes were the increase in digalactosylated glycans of 12.2% and the decrease in agalactosylated and afucosylated for 8.5% and 12.3%, respectively. The observed trends persisted between the second and the third sampling points but were less protruding: digalactosylated glycans rose by 3.8%, agalactosylated lowered by 4.4%, and afucosylated decreased by 2.0%. Amonth after the Rituximab administration, the most notable changes were the decreases in the disialylated (8.8%) and monosialylated (3.8%) glycans, while the changes in the (a) galactosylated and afucosylated glycans remained below 2%.
Conclusion: IgG N-glycome changed during immunosuppressive treatment administered in an AAV case. Some alterations, like the decrease in the agalactosylated glycans, most probably indicated anti-inflammatory IgG "phenotypic" changes during remission achievement. For others, such as afucosylation and sialylation, straightforward assumptions would be challenging. These pilot data merit validations in a cohort of patients to assess the IgG N-glycans biomarker potential in personalized AAV management.

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G-05

Diagnostic performance of a fully automated chemiluminescent immunoassay for glutamic acid decarboxylase autoantibodies (Maglumi anti-GAD)

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Introduction: The presence of autoantibodies to glutamic acid decarboxylase (anti-GAD) in the blood is considered one of the biomarkers for autoimmune diabetes detectable before the clinical onset of the disease. Markedly high levels of anti-GAD are also found in patients with stiff person syndrome, a rare autoimmune neurological disorder characterized by muscle rigidity and spasms. The traditional enzyme-linked immunosorbent assay (ELISA) used for the detection of anti-GAD was both time-consuming and complex to perform. A new fully automated chemiluminescence immunoassay (anti-GAD, CLIA) on the Maglumi X3 analyzer (Snibe Co. Ltd., Shenzhen, China) was recently introduced. The aim was to evaluate the diagnostic performance of the Maglumi anti-GAD for the detection of autoimmune diabetes, and to verify the transferability of the literature-provided diagnostic cut-off for GAD-positivity in the Croatian population.
Materials and methods: Serum samples were obtained from 207 Croatian patients with diabetes referred to our clinical laboratory for anti-GAD testing. Anti-GAD was analysed with both manual ELISAmethod (Euroimmun AG, Luebeck, Germany) and the fully automated Maglumi CLIAmethod. The results were classified into two categories (negative and positive) according to method-dependent cut-off values: 5 IU/mL for the ELISAmethod, as declared by the Euroimmun, and 5.1 IU/mL for the CLIAmethod, as published by Ziobrowska-Beck *et al.* (2019.) for the Northern European population.
Results: 104/207 samples were anti-GAD positive with ELISA, and 85/207 with Maglumi CLIAmethod (50% vs. 41%), respectively. There was a strong agreement between the two methods in anti-GAD positivity classification with a weighted kappa of 0.82 (CI 0.74 to 0.89).
Conclusion: The new Maglumi anti-GAD CLIAassay showed excellent diagnostic performance when compared to the Euroimmun ELISAmethod. Observed minor discordances in classification merely reflect the effect of the manufacturer's cut-off value since 15/19 discordant cases were in the very-low range of anti-GAD positivity, *i.e.* within the previously recommended cut-off of the Euroimmun ELISAmethod (< 10 IU/mL). An evidence-based cut-off value of the Maglumi anti-GAD (5.1 IU/mL) assessed for the Northern European population is applicable for the classification of anti-GAD positivity in the Croatian population as well.

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H Nutrition

H-01

Analytical verification of the 25-OH Vitamin D assay on the Snibe Maglumi X3 analyser and comparison with Abbott Architect i1000SR

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Introduction: Insufficiency or deficiency of vitamin D is more prevalent then ever. High-risk population should be screened in order to prevent fractures and bone loss, infections and many other diseases. The aim was to verify the performance of the chemiluminescence immunoassay (CLIA) 25-OH Vitamin D test on the Maglumi X3 analyzer (Snibe, Shenzhen, China) as well as to compare it to the routinely used chemiluminescent microparticle immunoassay (CMIA) on Architect i1000SR analyzer (Abbott Laboratories, Abbott Park, USA).
Materials and methods: Verification was performed according to the CLSI EP15-A3 guidelines using two control samples from the reagent kit (QC1 and QC2). Coefficients of variation (CV) for repeatability and intra-laboratory imprecision were calculated and compared with EFLM criteria (optimal < 1.7%, desirable < 3.4%, minimum < 5.1%) same as bias (optimal < 3.9%, desirable < 7.7%, minimum < 11.6%). Fourty patient samples were analysed using Maglumi analyser and the results were compared with measurements obtained by Architect analyser. Method comparison was performed with Bland-Altman and Passing-Bablok regression analysis.
Results: Calculated CV for repeatability for QC1 and QC2 were 0.64% and 0.88%, and for intra-laboratory imprecision were 2.11% and 1.27%. Bias were 0.14% and 1.64%. Comparison of 25-OH vitamin D results measured by the Maglumi and Architect method showed excellent agreement (r = 0.98, 95% CI: 0.97 to 0.99). Passing-Bablok regression showed no constant or proportional difference (y = - 0.5(-3.20 to 1.60) + 1.0(0.96 to 1.06) x. Bland-Altman analysis revealed absolute bias of -1.6 (95% CI: - 0.47 to 3.57) and relative bias of 5.6% (95% CI: - 0.48 to 11.61).
Conclusion: Results of this verification confirm that the evaluated CLIAmethod for 25-OH Vitamin D performed on Maglumi X3 analyzer meets the analytical acceptance criteria and is in good agreement with the routinely used method on Architect i1000SR.

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H-02

A comparison of two different immunoassays for assessing vitamin D status

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Introduction: Vitamin D is a hormone that plays a vital role in bone metabolism. However, its deficiency has also been linked to diabetes, cancer, cardiovascular, autoimmune and respiratory diseases. In clinical laboratories, the most common methods for determining vitamin D status are automated immunoassays, which show significant variability in results. This study aimed to compare two different immunoassays for assessing vitamin D status.

Materials and methods: Atotal of 76 serum samples, ranging from 10 to 169 nmol/L, were analysed using Enzyme Linked Fluorescent Assay (ELFA) on mini VIDAS (bioMérieux, France) and Electrochemiluminescence Assay (ECLIA) on Cobas e411 (Roche, Switzerland). The results obtained by both methods were compared by the Bland-Altman (BA) and the Passing-Bablok regression analysis (PB). Furthermore, based on the literature recommendations, three categories of vitamin D status (significant deficiency as < 50, deficiency from 50 to 75, and sufficiency as > 75 nmol/L) were observed. To assess agreement between methods among different categories kappa index (κ) was calculated. The statistical analysis was conducted using MedCalc v22.023 (MedCalc Software, Belgium).

Results: The arithmetic means (with a 95% confidence interval (95%CI)) in nmol/L were 88 (70-96) using ELFA- and 82 (74- 91) using ECLIA, with an average bias of -8.52%. The BArevealed constant and proportional differences between the methods ((5.76; 95% CI: 2.69-8.83) and (10.69%; 95% CI: 6.09-15.30), respectively). Additionally, the PB revealed a constant difference, with an intercept of - 10.88 (95% CI: -17.65 to - 3.96). The obtained κ of 0.82 (95% CI: 0.72-0.92) indicates a strong level of agreement between methods in the assessment of the vitamin D status.

Conclusion: The immunoassays evaluated are not comparable to each other and show significant variability in the results, mostly due to well-known differences in immunochemistry, such as dissociation from proteins and cross-reactivity. Despite this, they show a strong level of agreement considering assessment of vitamin D status.

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I Infectious Diseases and COVID-19

I-01

Quality control of in-house culture media for primary isolation of Bordetella pertussis duting outbreak of whooping cough in Croatia in 2023

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Introduction: Whooping cough or pertussis is a contagious, acute respiratory illness caused by the Gram-negative coccobacillus *Bordetella pertussis*. Laboratory confirmation is predominantly based on PCR tests but cultures considered the gold standard. In the early stage of outbreak of whooping cough in Croatia in 2023, we established new in-house selective media for testing *B. pertussis* in lack of ready to use media and PCR reagents. The aim of this study was twofold: (i) to detect variation in the detection rate with same lot due to date of preparation, and (ii) to compare physical and chemical characteristics commercially prepared media with those prepared in-house in order to validate media.
Materials and methods: The agar plates in 90 mm Petri dish used in this study were: charcoal agar prepared with dehydrated powder (Biolab, Budapest, Hungary) g/L: peptones 20.0, starch 10.0, sodium chloride 5.0, charcoal 4.0, niacin 0.001, agar 13.0 and in-house prepared charcoal agar plates using chemicals. Culture media were prepared suspending powder in reverse osmosis water, sterilized by autoclaving at 121°C for 15 minutes and supplemented with 10% sterile defibrinated horse blood and 0.04g of sterile cephalexin at pH 7.2 at 25 °C. Plates were incubated at 35°C in a humidified aerobic atmosphere, examined daily for suspect colonies for up to twelve days and number of colonies were recorded for each medium. The suitable performance of culture media is checked regard to physical and chemical characteristics (pH, volume, thickness).
Results: As part of the study on the influence of different agar plates on growth performance, chemical and physical characteristics, 96 tests were performed. The statistical elements were calculated using the StatEL, from Excel tables combining data sources. There is a different number of CFU for two agar plates, 84 CFU for commercial agar, 90 CFU for in house agar. The Friedman test on the performance of equipment shows that there was not a significant difference between the two media plates ($P > 0.05$).
Conclusions: From the results obtained, it seems obvious that the growth *B. pertussis* on in house prepared media is a good alternative to commercial plates.

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I-02

Metabolic changes in healthy subjects after first 8 months of coronavirus pandemic

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Introduction: COVID-19 emerged in December 2019 and in March 2020 WHO declared this infection as pandemic. The objective was to evaluate how pandemic related measures and lifestyle modifications effected lipid metabolism and blood total and LDL cholesterol as well as triglyceride levels in healthy subjects.
Materials and methods: We analysed lipid concentrations in 554 apparently healthy individuals, 258 males (47%) and 296 females (53%) who have been on preventive annual health examination. Mean age was 46 years (range 19-77). Aretrospective analysis was performed on subject's laboratory results taken at three time points: November 2018 to January 2019 (Visit 1); November 2019 to January 2020 (Visit 2) and November 2020 to January 2021 (Visit 3). Serum lipid concentrations were determined on AU 480 analyser (Beckman Coulter, Tokyo, Japan). Asignificant change was defined as a 5% difference between different time points.
Results: There was no significant change in lipid concentrations between prepandemic timepoints (V1 vs. V2). Conversely, during visit 3 (after first 8 months of coronavirus pandemic) a notable increase in serum total cholesterol, triglyceride and LDL cholesterol concentrations were observed in relation to prepandemic visit 2 ($P < 0.001$). Mean change (with SD) from visit 2 to visit 3 leves was for total cholesterol 12.7% (17.1), LDL-cholesterol 16.3% (26.3) and triglycerides 20.0% (54.3), respectively.
Conclusion: Our findings signify how isolation measures and lack of physical activity can negatively impact metabolic lipide profile and possible cardiovascular risk factors of otherwise healthy individuals.

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J Pediatric laboratory medicine

J-01

Vitamin D concentrations in paediatric population and comparison with the declared values of the manufacturer

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Introduction: Vitamin D is a well-known regulator of calcium and phosphate homeostasis. Current studies demonstrate it also affects numerous physiologic processes, therefore the number of requests for measuring vitamin D and its metabolites has increased. Arecommended value for 25-hydroxy vitamin D is > 75 nmol/L according to the reagent manufacturer (Elecsys Vitamin D total III Roche Cobas, Switzerland), regardless of the season and age. However, results may differ because of preanalytical factors like age, gender, season, geographical latitude, and ethnic groups. This research aimed to compare the established vitamin D concentrations in the paediatric population with levels from the manufacturer's study on adults in the United States.
Materials and methods: The results were taken retrograde (2021/09/02-2023/07/21) from laboratory information system BioNET, from one single center. After eliminating in-patients with acute or chronic disease, we had a total of 1313 vitamin D results (709 boys and 604 girls), sampled in summer (N = 591) and winter (N = 722). Vitamin D values were determined by Roche Cobas e411 analyser (Roche Diagnostics, Rotkreuz, Switzerland) using electrochemiluminescence immunoassay. The statistical analysis was performed in MedCalc software. P-value < 0.05 was considered statistically significant.
Results: The obtained values of vitamin D in summer and winter are statistically significantly different from each other (P < 0.001). The median for samples collected in summer is 75.7 nmol/L (95% CI: 74.0 to 78.6) with the first quartile (Q1) 59.6 and the third quartile (Q3) 92.0. For samples collected in winter, the median is 56.4 nmol/L (95% CI: 54.2 to 59.1) with Q1 = 41.6 and Q3 = 73.8. The manufacturer's median for summer (N = 245) was 69.2 nmol/L and for winter (N = 218) 57.1 nmol/L with no available 95% CI and quartile data.
Conclusions: The recommended value for 25-hydroxy vitamin D is suitable in the summer, but not in the winter. Alarger study is required to discover whether the new reference range or vitamin D supplementation for the winter is needed.

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J-02

Introduction of second-tier test for detection of homocystinuria in newborn screening

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Introduction: Homocystinuria is a rare metabolic condition, mostly caused by reduced activity of a cystathionine beta-synthase (CBS) that catalyzes methionine (Met) to homocysteine (Hcy) conversion. Infants with unrecognized homocystinuria develop symptoms of mental retardation, psychiatric disorders, tendency to thrombosis and atherosclerosis, as well as skeletal malformations and osteoporosis. Elevated Met concentration in newborn screening (NBS) can point to homocystinuria and enable early diagnosis and treatment of the disease, but it can also produce a large number of false positive results. Each positive NBS result requires additional, more specific follow-up tests, and if they are performed from the same dried blood spot (DBS) as the NBS, we refer to them as second-tier tests. Here we present a comparison of a possible second-tier test for homocystinuria from DBS with routinely used plasma Hcy method.
Materials and methods: Plasma and DBS samples of 30 patients were prepared using two commercial Recipe reagent kits, ClinMass for plasma, and ClinSpot for DBS Hcy. All samples were analyzed on tandem mass spectrometer coupled with high performance liquid chromatography (LC-MS/MS 8050-UPLC Nexera) by Shimadzu, and concentrations were calculated using calibration curves for each sample type. Methods were compared with Bland-Altman and Passing- Bablok regression analyses.
Results: The average of the differences on Bland-Altman plot revealed that plasma Hcy method measures 1.02 units more than the DBS method. In Passing-Bablok analysis, 95% confidence interval (CI) for intercept was 0.371 (from - 0.723 to 1.166), and 1.053 (from 0.983 to 1.139) for slope. 95% CI for intercept and slope includes 0 and 1, respectively, which means that there is no systematic nor proportional difference between the two methods. Cusum linearity test has showed that there is no significant deviation from linearity ($P > 0.10$).
Conclusion: Based on our results, plasma and DBS method for Hcy measurement are comparable. We conclude that the method for Hcy in DBS can be used as a second-tier test after a positive NBS result, as well as for treatment monitoring in patients with confirmed diagnoses.

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L Molecular diagnostics

L-01

FBN1 mutation profile in Korean patients with TAAD: data from AMC Hereditary Thoracic Aortic Aneurysm Registry

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Introduction: Marfan syndrome (MFS), caused by mutations in fibrillin-1 (FBN1), is a relatively common hereditary connective tissue disorder. The thoracic aortic aneurysms and acute aortic dissections (TAAD) are the leading causes of mortality in MFS patients. This study investigated the spectrum of FBN1 variants in Korean patients with TAAD, and the genotype - phenotype correlations.
Materials and methods: The study subject were enrolled from the Asan medical center (AMC) Hereditary Thoracic Aortic Aneurysm Registry between October 2016 and December 2021. MFS was diagnosed based on Ghent-criteria, clinical manifestations, and detection of FBN1 mutations using Sanger sequencing, and Next-Generation Sequencing (NGS).
Results: Total 302 patients (M:F = 203:99) were enrolled in AMC Hereditary Thoracic Aortic Aneurysm Registry and family members (N = 70) were also performed FBN1 genetic test. Pathogenic variants (PV) of FBN1 were detected in 50.3% (N = 152, M:F = 93:59). Most frequent type of PV was null variant (46.7%, N = 71), followed by other missesnse variant (28.9%, N = 44) and cysteine changed missense variant (27.0%, N = 41). 102 patients with PV underwent cardiovascular surgery. In familial study, 20 familial memebers (28.6%) with PV underwent cardiovascular surgery. There were 5 pediatric patients, who underwent cardiovascular surgery. In these cases, 3 variants were detected in exon 30-32 and 2 variants were in intron 46-47.
Conclusion: This study shows distributions of the genetic alterations of FBN1 in TAAD patients. Not only null variants, but also missense mutations with Cysteine alteration are the causes of the cardiovascular phenotype. TAAD in pediatric patients is caused by variants of specific-loci of the FBN1. This can be helpful for genetic counseling and preventive surgery in families with Marfan syndrome.

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L-02

Genetic profiling of patients with colorectal carcinoma and adenomas using next generation sequencing

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Introduction: Colorectal cancer is, among other things, caused by the accumulation of genetic modifications. This study aimed to determine whether there are differences in gene variants between colorectal carcinoma (CRC) and adenoma (pre-CRC).
Materials and methods: The study included 24 patients grouped according to pathologic diagnosis, 7 pre-CRC and 17 CRC. DNAwas extracted from sliced sections of formalin fixed paraffin embedded specimens using QIAamp DNAFFPE Advanced Kit (QIAGEN, Hilden, Germany). Sequencing was performed using Archer VARIANTPlex™ Solid Tumor Focus v2 panel (Invitae, USA) on NextSeq550 (Illumina, USA). The results are shown using Variant allele frequency (VAF). All detected gene variants were classified according to ClinVar.
Results: In CRC samples 10 different genes variants were identified. The most variants were detected in TP53 gene (11 variants), but only NM_000546.5:c.844C>T (VAF = 0.2756) is considered a somatic pathogenic variant, and it was detected in two samples. More somatic pathogenic variants were detected in the CRC group than in the pre-CRC group [BRAF NM_004333.4:c.1799T>A (VAF = 0.1504) in three samples, KRAS NM_004985.3:c.35G>T (VAF = 0.3109) in two samples, and KRAS NM_004985.3:c.436G>A (VAF = 0.1779) in one sample. Pre-CRC had variants in five different genes, but most variants were detected in the KRAS gene. The KRAS NM_004985.3.38G>A (VAF = 0.2723) variant was detected in three pre-CRC samples, the c.436G>Ain one sample and c.35G>Ain one sample. The KRAS NM_004985.3.38G>Ain considered a somatic pathogenic variant in CRC. Furthermore, the KRAS NM_004985.3:c.35G>Avariant detected in CRC and pre-CRC (VAF = 0.2749 vs. 0.0366) was also reported as a germline mutation.
Conclusion: Pathogenic variants in KRAS gene are found in both pre-CRC and CRC samples suggesting an early activation of EGFR signaling pathway via KRAS. Furthermore, more variants in CRC samples than in pre-CRC samples indicate greater cancer cell heterogeneity.

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L-03 (Oral presentation)

The pharmacogenetic influence on apixaban side effects occurrence

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Introduction: Apixaban is a direct oral anticoagulant with good safety and efficacy but there are cases of bleeding and thromboembolic events among users. Apixaban is a substrate of CYP3A4/5 enzymes and ABCB1/ABCG2 transporters. The polymorphisms in these genes could affect apixaban pharmacokinetics. This research aims to determine the association between relevant polymorphisms and the occurrence of apixaban-related side effects.
Materials and methods: These are preliminary results of the larger cohort (N = 470 expected). Adults with a new indication for apixaban treatment, without contraindications, were recruited. The genotyping for CYP3A4*22, CYP3A4*1B, CYP3A5*3, ABCG2c.421C>A, ABCB1c.1236C>T, c.2482-2236G>A, and c.3435C>T variants was performed with specific TaqMan Assays on ABI7500 Real-Time PCR system. Clinical and laboratory data were collected. Associations between carriers/noncarriers of variant alleles or haplotypes/phenotype and bleeding/thromboembolic events were analysed. Statistical analysis was performed by JASP0.17.1 software.
Results: 140 patients on apixaban treatment (Median age = 67 (20-89); f = 59, m = 81) were treated in dosages of 2 x 5 mg (N = 113) and 2 x 2.5 mg (N = 27). Eleven patients developed apixaban associated bleeding (serious = 3, anemia = 3, epistaxis = 2, hematoma = 2, hematuria = 2, hemoptysis = 1) and six thromboembolic events (insult = 4, thrombosis = 2). Genotype distribution is consistent with Hardy Weinberg's equilibrium. The analysis with the Chi-square test was performed and a significant association between ABCB1c.1236C>T variant carriers and smaller bleeding occurrence (OR = 0.27, 95% CI: 0.06-1.13, P = 0.046) was found.
Conclusion: There is no association between the CYP3A4*22, CYP3A4*1B, CYP3A5*3, ABCG2c.421C>A, ABCB1c.2482- 2236G>A, and c.3435C>T variants nor phenotype with the apixaban-related side effects, only for ABCB1c.1236C>T variant carriers with smaller bleeding occurrence. The results should be re-evaluated on the larger cohort.

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M POCT

M-01 (Oral presentation)

Comparison of intraoperatively measured haemoglobin concentration during liver transplantation between a POCT and a central laboratory method

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Introduction: Liver transplantation is characterized by significant hemodynamic instability of the patient. The bleeding risk requires frequent intraoperative haemoglobin concentration monitoring using a point-of-care testing (POCT) blood gas analyzer. Since samples for blood gas and complete blood count analysis are sampled simultaneously during each transplantation, this study aimed to compare these two methods for haemoglobin determination.
Materials and methods: This study included 260 arterial blood samples for blood gas and complete blood count analysis sampled during 185 liver transplantations performed at Merkur University Hospital from October 2021 to May 2024. Hemoglobin concentration was determined by the CO-oximetry method on the POCT blood gas analyzer (Radiometer ABL90 FLEX, Radiometer, Bronshoj, Denmark) and the sodium lauryl sulphate (SLS) method (Sysmex XN1000, Sysmex, Kobe, Japan) accredited according to ISO 15189. Passing-Bablok and Bland-Altman regression analysis were used for method comparison, with acceptance criteria of 1.7% (desirable specifications for bias according to the EFLM Biological Variation Database). Statistical analysis was performed using MedCalc Statistical Software (version 22.005, MedCalc Software Ltd, Ostend, Belgium).
Results: The hemoglobin concentration ranged from 48 to 143 g/L. The median hemoglobin concentration was 80 g/L for both methods (95% CI: 78 to 82, for both methods). Passing-Bablok analysis revealed a statistically significant systematic, but not proportional difference: y = 1.00 (95% CI: 1.00 to 1.00) + 1.00 x (95% CI: 1.00 to 1.00). Bland-Altman analysis revealed an absolute bias of -0.5 g/L (95% CI: - 0.7 to -0.3) and a relative bias of - 0.6% (95% CI: - 0.9 to - 0.3).
Conclusion: Statistical analysis showed consistent bias between the CO-oximetry method and the SLS method. Since the bias is not clinically significant according to the biological variation criteria, we conclude that the methods are comparable and can be simultaneously used for intraoperative haemoglobin concentration monitoring.

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M-02

Verification of a POCT Horiba Microsemi CRP hematology analyzer

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Introduction: Neonatology blood sampling often imposes a significant problem in terms of adequate volume and/or quality of samples for routine laboratory methods. Point-of-care hematology analyzers (HA) that can perform measurements of complete blood count (CBC), as well as of C-reactive protein (CRP) from the same sample are of indispensable value for neonatology units. We aimed to verify the analytical characteristics of such HA-Horiba Microsemi CRP.
Materials and methods: Verification protocol included: precision testing (both repeatability and total precision) performed on quality control materials, as well as on remnant patient EDTA-whole blood and serum samples in various concentrations, expressed as coefficient of variation (CV, %), linearity (by serial dilutions of samples close to the upper reportable range), comparability with the laboratory routine HA(Sysmex XN-1000) and chemistry analyzer (Siemens Atellica CH 930) on remnant patient samples spanning the reportable range of each assay, and sample carryover.
Results: Repeatability and day-to-day precision meet the acceptance criteria (minimal biological variability. The declared linearity was verified for leukocytes, erythrocytes, and hemoglobin. The average bias for MCV and platelet measurements was outside the manufacturer's permissible deviation, - 4.5% and 8.8%, respectively. Although the average bias for CRP in whole blood (23.5%) and serum (22.4%) measured on the Microsemi CRP analyzer is within the criteria of minimal biological variability (34.8%), a statistically significant proportional deviation is visible already at low concentrations of CRP (> 10 mg/L). The analyzer is not comparable to the reference analyzer Atellica CH 930 in CRP measurements above 10 mg/L. Carryover for erythrocytes, leukocytes, hemoglobin, and platelets were within the manufacturer's criteria.
Conclusion: POCT Horiba Microsemi CRP HAmeets most of the manufacturer's technical specifications and defined quality criteria. However, it is not recommended to use analyzers alternately to measure the concentration of CRP, but the monitoring dynamics in an individual child should be monitored exclusively on one analyzer.

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M-03

Analytical verification of the Dymind D7-CRP analyzer

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Introduction: The Dymind D7-CRP (Shenzhen Dymind Biotechnology Co., Shenzhen, China) analyzer combines analysis of complete blood count with C-reactive protein (CRP), making it suitable for near-patient testing. Here we present part of the results of its verification.
Materials and methods: Verification was performed according to the CLSI EP15-A3 protocol in the Clinical department of laboratory diagnostics, Clinical hospital center Rijeka during March 2024. Verification included all parameters of complete blood count and CRP out of which we present the data for leukocytes, erythrocytes, hemoglobin, platelets and CRP. Precision was evaluated using two-level CRP (C-reactive Protein Control, Shenzhen, China; LOT 2023092101) and three-level hematology commercial control materials (CBC-DH Hematology Control, R&D systems, Minnesota, USA; LOT: DH2403). Acceptance criteria were defined according to biological variation database. Accuracy was evaluated on 40 leftover patient samples using Beckman Coulter DxH800 and Beckman Coulter AU480 (Brea, USA). Data analysis was done using MedCalc statistical software (MedCalc, Ostend, Belgium).
Results: CVs for CRP were 10.7% for low and 6.3% for high control. CVs for leukocytes were 1.8%, 1.3%, 0.8%, erythrocytes 0.9%, 0.9%, 0.8%, hemoglobin 0.9%, 0.8%, 0.6% and platelets 6.1%, 2.3%, 2.3% for low, normal and high control respectively. They all met desirable criteria except for platelets in low control. Passing-Bablok regression analysis provided linear equation for tested analytes. Regression equations and 95% confidence intervals for intercept and slope were as follows: leukocytes y = - 0.138(- 0.367 to 0.005) + 1.043 (1.025 to 1.068) x; erythrocytes y = - 0.081 (- 0.151 to 0.020) + 1.029 (1.000 to 1.048) x; hemoglobin y = - 2.308 (- 4.032 to 0.340) + 1.039 (1.019 to 1.056) x; platelets y = 5.249 (1.041 to 12.558) + 1.044 (1.020 to 1.069) x; CRP y = - 1.738 (- 2.606 to 0.475) + 1.110 (1.059 to 1.191) x.
Conclusion: Verification results showed that analyzer Dymind D7-CRP meets the defined criteria for near-patient testing.

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O New Biomarkers

O-01

Assessing traumatic brain injury: the role of GFAP and UCH-L1 as diagnostic markers

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Introduction: Although imaging techniques, mainly computed tomography (CT), are considered a gold standard for diagnosing traumatic brain injuries, other diagnostic approaches, such as biomarkers, are constantly investigated. This study aimed to evaluate the diagnostic utility of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH- L1) in patients with traumatic brain injury.
Materials and methods: The study involved patients admitted to the Emergency Department of GH SKC from May to October 2022 with suspicion of brain injury secondary to head or neck trauma. All patients underwent Glasgow Coma Scale (GCS) assessment and received either a head CT or x-ray. Additionally, serum concentrations of GFAP and UCH-L1 were measured on Abbott's Alinity-i instrument and interpreted as follows: GFAP ≥ 35.0 ng/L and/or UCH-L1 ≥ 400.0 ng/L correspond to a positive result (TBI = 1), while values of GFAP and UCH-L1 below the aforementioned cut-off values indicate a negative result (TBI = 0). All statistical analyses were conducted using MedCalc (MedCalc, Ostend, Belgium).
Results: The research included 44 patients aged 58 (2-89) years. Positive CT or x-ray result was found in 34.1% of patients and following results for TBI were calculated (with respective 95%CI): sensitivity 100% (78-100), specificity 31% (15-51), positive predictive value (PPV) 43% (37-49), negative predictive value (NPV) 100% (66- 100) and accuracy (DA) 55% (39-70). ROC analysis for GFAP (cut-off: 60.5 ng/L) gave the following results: area under the ROC curve (AUC) 0.79 (0.64-0.90), sensitivity 80% (52-96), specificity 69% (49-85), PPV 57% (42-71) and NPV 87% (70-95). ROC analysis for UCH-L1 (cut-off 460.1 ng/L) yielded the following results: AUC 0.68 (0.52-0.81), sensitivity 87% (60-98), specificity 55% (36-74), PPV 50% (39-61) and NPV 89% (68-97). Additonal evaluation for TBI was performed using cut-off values from ROC analysis; sensitivity 100% (78-100), specificity 38% (21-58), PPV 45% (39-53), NPV 100% (72-100) and DA 59% (43-74).
Conclusion: Considering the exceptional NPV, the combination of GFAP and UCH-L1 is appropriate for ruling out TBI, but their low specificity makes them unsuitable for confirming the diagnosis.

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O-02

Determination of TGF-ß1 presence by liquid and tissue biopsy methods in patients with colorectal cancer

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Introduction: Early detection of colorectal cancer (CRC) is essential for improving prognosis and reducing mortality. Due to the invasiveness of colonoscopy and tissue biopsy as existing diagnostic methods, liquid biopsy was developed as a non- invasive and reproducible method allowing exosome isolation from the patient's peripheral blood. Exosomes carry molecules originating from tumor tissue, including transforming growth factor-ß1 (TGF-ß1). We hypothesized that levels of TGF-ß1 in exosomes could be comparable to those in tissue biopsy samples. This study aimed to determine the presence of TGF-ß1 in exosomes from CRC patients obtained by liquid biopsy and to compare the results with immunohistochemical analysis of tumor tissue samples obtained by biopsy.
Materials and methods: Liquid and tissue biopsy samples of 59 CRC patients were analyzed. Exosomes were isolated from plasma using miRCURY Exosome Serum/Plasma Kit (Qiagen, Germany). Protein concentration was determined by colorimetric method with bicinchoninic acid and CuSO4 (Sigma-Aldrich, USA). Western blotting was performed using mouse monoclonal antibody to detect TGF-ß1 (Invitrogen, USA) in exosomes. The same antibody was used for immunohistochemical analysis of formalin-fixed paraffin-embedded tissue biopsy samples.
Results: The presence of TGF-ß1 was proven in all exosomal samples and the intensities of obtained bands were relatively quantified (1.39 (1.21-1.75)). Immunohistochemical analysis of tumor tissue biopsy samples demonstrated a positive reaction (100%) for TGF-ß1 in all examined samples.
Conclusion: Results showed the presence of TGF-ß1 in both liquid and tissue biopsy samples. However, different intensities of TGF- ß1 obtained in exosomes represent a potential for introducing liquid biopsy into clinical practice as a non-invasive method that enables screening, diagnosis, and disease monitoring in patients with CRC, which should be further examined on a larger number of samples.

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O-03

Utility of blood ACE, chitotriosidase activity and CD4/CD8 ratio in bronchoalveolar lavage for pulmonary sarcoidosis diagnosis

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Introduction: Sarcoidosis is a systemic granulomatous inflammatory disease of yet unclear etiology. Three elements are important when diagnosing a patient with pulmonary sarcoidosis: histopathological evidence of noncaseating granuloma formation, clinicoradiological features of the pulmonary disease and exclusion of other diseases with similar presentation. No laboratory parameter is sufficiently specific and sensitive, so by combining certain parameters we can greatly facilitate the diagnosis of pulmonary sarcoidosis.
Materials and methods: This retrospective study included consecutive patients with diagnostic workup of benign mediastinal lymphadenopathy in whom all three biomarkers were requested within one month. The diagnosis of sarcoidosis was established by patohistological confirmation and exclusion of alternative causes of granulomatous disease with comparable clinical presentation. The angiotensin-converting enzyme was measured in the serum (ACE kinetic, Bühlmann Laboratories AG, Switzerland), chitotriosidase activity in EDTA plasma with an in house fluorimetric method and CD4/CD8 in BAL were analysed with flow cytometry (BD FASCLyric BD, USA). Differences in test values were tested with statistical tests for independent samples: Mann-Whitney test and t-test for non-normally and normally distributed data, respectively.
Results: In the evaluated period, the consecutive BAL samples of 189 patients with benign mediastinal lymphadenopathy suspected to be sarcoidosis were collected. Of those, 105 were excluded according to described exclusion criteria (missing blood biomarker data, multiorgan or skin sarcoidosis, sarcoidosis on therapy). Finally, 84 patients were included in the study. In 30 (36%) patients with median age 47 (30-69) years, the sarcoidosis diagnosis was established. A significant difference was demonstrated between patients with sarcoidosis and control patients regarding all evaluated biomarkers. The sarcoidosis patients had higher chitotriosidase ($P < 0.001$) and ACE ($P < 0.001$) activity, and a higher CD4/CD8 ratio ($P < 0.001$) in BAL compared to the non-sarcoidosis patients-
Conclusion: The statistically significant difference demonstrated for all parameters between patients with a similar clinical presentation but a different diagnosis and patients with sarcoidosis supports the usefulness of serum ACE, chitotriosidase and CD4/CD8 ratio in BAL in the diagnosis of lung and mediastinal lymph node sarcoidosis.

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O-04 (Oral presentation)

Is miR-193a-3p potential novel biomarker in colorectal cancer?

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Introduction: MicroRNAs (miRs) are non-coding RNAs involved in colorectal cancer (CRC) malignancy. We investigated miR-193a-3p from formalin-fixed paraffin-embedded (FFPE) tissue samples and from exosomes obtained by liquid biopsy as potential biomarker of CRC and compared it with the existing biomarkers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9).
Materials and methods: CA-19-9 and CEA were measured using the e401 analyzer (Roche Diagnostics) in serum samples from 52 CRC and 76 colorectal adenoma (preCRC) patients (control group). MiRNeasy FFPE Kit and miRNeasy Serum/Plasma Advanced Kit (Qiagen) were used for miR isolations. Reverse transcription was performed with the miRCURY RT LNAkit (Qiagen) and qPCR was performed on the 7500 Real-Time PCR System (Applied Biosystems) using the miRCURY LNASYBR GREEN PCR Kit (Qiagen) with miR-103a as reference and UniSp6 as internal control. Statistical analysis was performed in MedCalc (MedCalc Software Ltd, Ostend, Belgium) using the Mann-Whitney test, Wilcoxon matched-pairs test, ROC curve analysis and logistic regression.
Results: We found statistically significant differences for miR-193a-3p, CEA and CA 19-9 ($P < 0.001$, $P < 0.001$, $P = 0.002$) between CRC and preCRC patients, and for miR-193a-3p ($P < 0.001$) between tissues and exosomes. CEA and CA 19-9 had AUCs of 0.80 (95%CI = 0.71 to 0.87, $P < 0.001$) and 0.67 (95%CI = 0.57 to 0.75, $P = 0.001$). MiR-193a-3p had AUC of 0.73 (95%CI = 0.64 to 0.80, $P < 0.001$). Logistic regression analysis showed that when using CEA and miR-193a-3p, 71.3% of cases are correctly classified with the AUC 0.80 (95%CI = 0.71 to 0.87, $P < 0.001$).
Conclusions: MiR-193a-3p could be potential novel biomarker for discriminating CRC from preCRC patients, and CRC diagnosis marker using liquid biopsy, but its potential should be examined on larger number of samples.

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P New Technologies

Detection and characterization of circulating tumor cells in patients with colorectal cancer

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Introduction: Metastases are the leading cause of death associated with colorectal cancer (CRC). Circulating tumor cells (CTCs) can be detected in the blood of CRC patients before metastases.
Materials and methods: This study included 58 CRC patients from Sestre milosrdnice University Hospital Centre. CTCs were isolated from 3 mL whole blood using the ScreenCell Cyto kit system (Sarcelles, France), according to the manufacturer's instructions. The detection and characterization of CTCs were performed by immunofluorescence analysis using primary antibodies to cytokeratin (CK) and to CD 45, followed by secondary antibodies labeled with Alexa Fluor 488 and Alexa Fluor 568 was done. Finally, preparations were stained with 4',6-diamidino-2-phenylindole (DAPI) for nucleus visualization. Visualization was performed with ZEISS Axio Observer 7 fluorescent microscope. Tumor cells of epithelial origin, express the unique CK marker (CK+), are negative for the leukocyte lineage marker (CD45-) and have a recognizable nucleus (DAPI+).
Results: The results of testing the blood of patients with CRC using liquid biopsy showed the presence of CTCs in 41 patients (1–5 CTCs/3 mL of blood). None CTCs were found in 15 blood samples, while two blood samples, due to pronounced coagulation, were not suitable for CTC isolation. The presence and increased number of CTCs are associated with an increased risk of recurrence and a poor prognosis, and they are also biomarkers for monitoring the response to therapy. According to our results, 70.1% of the samples contained CTCs (1–5 CTCs/3 mL of blood); none CTCs were found in 25.9% of the samples, and only 3.4% of the samples were not acceptable for analysis.
Conclusion: The future research utilizing liquid biopsy and CTC in diagnostics, requires the improvement of the analysis and standardized protocols, the tasks of utmost importance.

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R Case Report

R-01 (Oral presentation)

Investigation into the interference of the monoclonal therapeutic drug ocrelizumab in electrophoretic and immunofixation techniques

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Introduction: Introduction of new biological therapies in the form of monoclonal antibodies brought previously unrecognized laboratory interference that, if not properly recognized, can have significant clinical repercussion. Ocrelizumab (Ocrevus) is a humanized anti-CD20 IgG kappa monoclonal antibody used for the treatment of multiple sclerosis (MS). We represent a case report of a MS patient threated with ocrelizumab. The aim of this study was to determine whether IgG kappa detected using serum immunofixation (IF) technique represents an ocrelizumab interference or newly detected monoclonal component.
Materials and methods: To determine migration position of ocrelizumab an aliquot of drug was obtained from the Department of Neurology and analyzed by serum protein electrophoresis (SPEP) and IF. Two monoclonal protein-free serum samples were spiked with 100 µL of the drug to a final ocrelizumab concentration of 27 mg/mL. All samples were analyzed by capillary electrophoresis (Minicap, Sebia, USA) and IF (Hydrasys, Sebia, USA).
Results: Ocrelizumab was identified on IF as an IgG kappa and migrated to the far cathodal end of the gamma zone. SPEP of spiked-serum samples showed a small gamma fraction abnormality, but no quantifiable monoclonal spike was observed. IF of spiked-serum samples showed a light IgG kappa band migrating to a same far cathodal end of the gamma zone as ocrelizumab. Patients SPEP/IF analysis showed presence of an IgG kappa band that migrated to the cathodal end of gamma zone close to the ocrelizumab, but with the IF migration position slightly closer to the anodal end.
Conclusion: For presented patient we ruled-out an ocrelizumab interference in SPEP/IF, and proved the presence of a monoclonal IgG kappa protein. Recognition of migration patterns on SPEP/IF is a model of identifying an interference of drugs with monoclonal antibody structure. To avoid the possibility of ocrelizumab interference in SPEP/IF, we recommend blood sampling prior to the initiation of drug application.

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R-02

IgG4-related disease in a patient with rheumatoid arthritis

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Introduction: Immunoglobulin G4-related disease (IgG4-RD) is a chronic immune-mediated fibroinflammatory disease that often manifests with tumor-like masses, lymphadenopathy, and painless enlargement of multiple organs. IgG4-RD has been described to involve almost every organ system. It is highly responsive to immunosuppressant therapy, but can have detrimental consequences if left untreated. High serum IgG4 concentration showed to have a high sensitivity and positive predictive value (> 90%). We report a case of a patient with IgG4-RD whose diagnose was guided by high serum IgG4 concentration.
Materials and methods: A 56-year-old patient was admitted to the Department of Rheumatology for a differential diagnostic of polyarthritis. Laboratory analysis were performed. C-reactive protein (CRP) was measured on a biochemistry analyzer DxC 700AU (Beckman Coulter, Brea, USA), antibodies to cyclic citrullinated peptide (CCP) on the immunochemistry analyzer Bioflash (Werfen, Spain), and immunoglobulin G and G4 on immunoturbidimetry analyzer Optilite (The Binding Site, UK).
Results: The patient complained of pain in both shoulders, knees, and hands, as well as breathing difficulties. His laboratory tests showed elevated concentration of CRP (112.6 mg/L), IgG (21.3 g/L), CCP (> 2777 CU), and IgG4 concentration of 3406 mg/L (RI 39.2-864). Patient was diagnosed with seropositive rheumatoid arthritis (RA). Because of the laboratory finding of elevated IgG4, the patient underwent a thoraco-abdominal CT scan, which demonstrated multiple nodes in the lungs and reactive mediastinal lymphadenopathy. The finding was confirmed by the PET/CT scan. Patient was treated with 1 mg/kg of methylprednisolone and his RAsymptoms improved, IgG4 levels reduced to 135 mg/L, and he experienced a complete remission.
Conclusion: The diagnosis of IgG4-RD is frequently challenging owing to its non-specific clinical manifestations and indolent nature. Early disease recognition is therefore mandatory. In presented case report laboratory finding of elevated IgG4 was crucial to obtaine early diagnosis of IgG4-RD.

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R-03

A case report of mantle cell lymphoma followed by chronic myelomonocytic leukemia: an immunophenotypic approach

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Introduction: Chronic myelomonocytic leukemia (CMML) and non-Hodgkin's lymphoma (NHL) are hematologic malignancies that originate from different progenitor stem cells. Mantle cell lymphoma (MCL) is a rare type of B-NHL, and concurrence with CMML is extremely rare. Most of the cases reported in the literature refer to patients who developed NHL after treatment for CMML, but not vice versa. Here, we present a case of a patient with MCL in remission who developed CMML, with special emphasis on immunophenotypic approach.
Materials and methods: A 58-year-old man with MCL in remission was admitted to the Department of Clinical Hematology at University Hospital Centre Osijek, due to general weakness. Laboratory analysis showed high white blood cells count (219 x10^9/L), anemia and thrombocytopenia. A review of the peripheral blood smear showed left-shifted myeloid maturation, monocytosis and occasional blasts. The morphological features of the bone marrow cells were consistent with CMML. Cytogenetic analysis results were negative for Philadelphia chromosome. Additional laboratory testing included flow cytometry immunophenotyping (FCI) analysis of the bone marrow aspirate. It was performed on FACSLyric flow cytometer with FACSuite software (BD Biosciences), using 6-color lyse/wash staining protocol.
Results: The FCI analysis of bone marrow cells showed a large population of 51% of cells with granulocytic differentiation at all stages of maturation, 25% of mature monocytes, only 4% cells with lymphocytic immunophenotype and 11% of cells with immunophenotype CD11c+CD13+CD33+CD34+CD117+HLADR+cMPO+, corresponding to myeloblasts.
Conclusion: Considering that this is a patient diagnosed with MCL in remission, the worsening of symptoms indicated a relapse of the underlying disease. Therefore, the appropriate marker panel for FCI analysis would be lymphoid rather than myeloid, leading to a misdiagnosis. This case showed the necessity of using a wider FCI markers panel than recommended, as well as the need for a multidisciplinary approach in diagnosing complex hematological malignancies like this one.

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R-04

Heavy chain disease - case report

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Introduction: Heavy chain diseases (HCDs) are B-cell proliferative disorders characterized by production of abnormal, structurally incomplete, immunoglobulin heavy chains without the corresponding light chains. These defective proteins can accumulate in various tissues and lead to a range of clinical symptoms depending on the subtype of HCD, which include gamma heavy chain disease, alpha heavy chain disease and mu heavy chain disease, making HCD a complex and heterogeneous disease.
Materials and methods: A 71-year-old woman was admitted to the emergency room due to persistent fever. She has a decreased appetite and has lost 10 kilograms in weight in the last 3 months. The patient has a history of rheumatoid arthritis and is being treated with TNF-alpha inhibitors (certolizumab).
Results: Measurement of serum proteins shows a decreased value of total proteins, high value of immunoglobulin G, and normal values of free kappa and lambda light chains with a normal kappa/lambda ratio. The serum protein values are as follows: total proteins 48.4 g/L (reference interval, RI 66.0-80.0), immunoglobulin G 28.66 g/L (RI 7.0-16.0), immunoglobulin A 1.21 g/L (RI 0.7-4.0), immunoglobulin M 0.18 g/L (RI 0.4-2.3), free kappa light chains 14.10 mg/L (RI 3.30-19.40), free lambda light chains 18.51 mg/L (RI 8.31-27.0), kappa/lambda ratio 0.76 (RI 0.31-1.56). Serum protein electrophoresis shows 2 peaks possibly indicating monoclonal protein. Immunofixation electrophoresis of serum proteins confirms 2 fractions of gamma heavy chains. One fraction migrates in the alpha-2 region and the other in the gamma- globulin region. Both fractions contain monoclonal protein but without the corresponding light chain.
Conclusion: In this patient, immunofixation electrophoresis confirmed the presence of abnormal gamma heavy chains without the corresponding light chains, which is characteristic of gamma heavy chain disease (γ-HCD).

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R-05

Neonatal thyrotoxicosis caused by maternal autoimmune thyroiditis

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Introduction: Neonatal thyrotoxicosis (NT) is rare, life-threatening disorder caused by transplacental passage of thyroid- stimulating hormone receptor antibodies (TRAbs). Transport of maternal TRAbs to the fetus occurs early in gestation with peak rate in last trimester. The higher the maternal TRAb concentration in the third trimester, the higher the risk of NT. We report on a neonate with NT due to TRAbs, born to a mother with autoimmune thyroid disease (AIT).
Materials and methods: Our patient was born at 35-weeks` gestation (BW 2830 g, BL 49 cm, Apgar score 8.9). Except mild polycythaemia and thrombocytopenia he had no signs of disease until 6th day of life when he developed severe tachycardia, excessive sweating, irritability, hepatomegaly, diarrhea and proptosis. Thyroid function tests revealed overt hyperthyreosis (TSH < 0.01 mIU/L, FT4 > 64.35 pmol/L, FT3 > 30.72 pmol/L, TRAb > 50 IU/L, Alinity i ABBOTT-CMIA immunoassay). Treatment with metimazol, Lugol solution and propranolol was introduced immediately and led to normalization of symptoms and lab results within 6 days. Treatment was gradually tapered and discontinued at the age of 6 weeks. Detailed history revealed that the mother was diagnosed with Hashimoto thyroiditis (HT) (positive anti-TGAband anti-TPO) 5 years prior to pregnancy. She was on levothyroxine (82.5 mcg) before and during first two trimesters. In third trimester, she developed subclinical hyperthyreosis (TSH < 0.01 mIU/L, fT4 22.6 pmol/L) with unmeasurably high TRAbs, hence levothyroxine was discontinued. Several days after labor she developed severe hyperthyreosis (TSH < 0.01 mIU/L, fT4 41 pmol/L, fT3 26 pmol/L, TRAb > 50 IU/L).
Conclusion: AIT usually presents either with HT or Graves disease phenotype. Although rare, clinical and antibody overlap between these conditions can occur, with some patients converting from hypo- to hyperthyroidism, as observed in the mother of our patient. Therefore, meticulously documenting the mother`s clinical history and laboratory evaluation, regardless of AIT type, is essential. This thorough approach can aid in diagnosing the newborn and prevent severe complications.

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R-06

Painful crisis in a boy with sickle cell anemia - case report

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Introduction: A case of a boy with sickle cell anemia in acute attack of pain is presented. Sickle cell anemia is a genetic disorder in which hemoglobin S (HbS) is formed due to a mutation at the 6th position of the β-globin chain, glutamine is replaced by valine. Hb S polymerizes and causes erythrocytes to deform into a sickle shape, which are promoted by hypoxia, hypercapnia and hyperthermia, leads to painful, vaso-occlusive crises. The aim is to present the results of laboratory tests and microscopic images of sickled erythrocytes in a peripheral blood smear.
Materials and methods: A 7-year-old boy, a British citizen of African ethnic origin, was admitted to hospital presenting with severe abdominal and back pain, which started two hours before admission. The boy was physically active for three days before the onset of symptoms. After he was born, he was diagnosed with sickle cell anemia.
Results: Normocytic, normochromic anemia was observed: erythrocytes count were 2.72 $x10^{12}$/L, hemoglobin 83 g/L, hematocrit 0.238 L/L, MCV 87.3 fL, MCH 30.5 pg, MCHC 349 g/L, distribution of erythrocytes by volume 19.2 %, reticulocytosis of 224.94 $x10^{9}$/L (82.7‰), 3 erythroblasts /100 leukocytes. Erythrocytes description: anisocytosis +++, poikilocytosis ++, target cells ++, drepanocytes +. CRP was 6.2 mg/L, increased lactate dehydrogenase activity 639 U/L, as well as the concentration of total 41 µmol/L and conjugated bilirubin 11 µmol/L with a negative direct and indirect antiglobulin test, which excludes acquired hemolytic anemia. D-dimers were 4.77 mg/L. Using the hemoglobin capillary electrophoresis method, as a high-resolution method for screening for hemoglobinopathies, increased values of HbS of 83.6% and hemoglobin F of 12.5% were obtained.
Conclusion: Diagnostic treatment of the described subject confirmed the suspicion of a painful crisis due to his sickle cell anemia, caused by increased physical activity.

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S Pre - and Postanalytical Phase of Laboratory Work

S-01

The influence of different storage conditions on the stability of C-peptide and insulin

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Introduction: The stability of C-peptide and insulin is affected by various preanalytical factors. The aim of this study was to examine the influence of different storage conditions on the stability of these polypeptides in order to transport samples from collaborative laboratories.
Materials and methods: The study included 10 serum samples with c-peptide (nmol/L) and insulin concentrations (µU/mL) spanning the Roche Cobas e801 ECLIA measuring range. The zero value (t0) was measured within an hour of sampling, after which the sample was divided into two aliquots. One aliquot was stored at room temperature in light and reanalyzed after 6 (t6) hours, and the other was stored in the refrigerator and analyzed after 24 (t24hl) and 48 (t48hl) hours. The difference from t0 was calculated (t6/24hl/48hl-t0/t0 (%)), and the results were analyzed by nonparametric Wilcoxon's steam test and Friedman ANOVA using MedCalc statistical software ver. 20.104. P-value < 0.05 was considered statistically significant, and the 24% and 33% difference for c-peptide and insulin, respectively, was considered clinically significant (Croatian Centre for Quality Assessment in Laboratory Medicine performance specifications).
Results: Median (interquartile range) concentrations of c-peptide were: t0 0.48 (0.41-0.95), t6 0.48 (0.42-0.95), t24hl 0.48 (0.41-0.93), t48hl 0.48 (0.41-0.94) and insulin: t0 6.9 (5.0-23.9), t6 6.7 (4.8-23.9), t24hl 6.8 (4.8-23.1), t48hl 6.9 (4.9-23.5). P-value and mean differences from t0 were: t6 P = 0.910; 0.40%, t24hl P = 0.922; 0.30%, t48hl P = 0.846; 0.13% for c-peptide and t6 P = 0.008; -3.23%, t24hl P = 0.322; -0.57%, t48hl P = 0.164; -1.17% for insulin. Astatistically significant difference in samples analyzed after 6 hours at room temperature for insulin was found (P = 0.025), while c-peptide (P = 0.916) showed no statistically significant difference.
Conclusion: Although a statistically significant difference for insulin was found in samples stored at room temperature after 6 hours, the observed difference was not clinically significant. Both c-peptide and insulin showed acceptable stability under the tested conditions.

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S-02

Comparison serum ferritin immunoassays on Vitros ECI and Alinity ci analyzers

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Introduction: The main objective of this study was to compare two immunochemical methods for ferritin. The specific objectives of the paper were to investigate the comparability of analytical methods, and to determine the constant and proportional deviations between methods.
Materials and methods: The research was conducted in the Department of Laboratory Diagnostics of the Clinical Hospital Centre Mostar in Bosnia and Herzegovina and included 130 respondents, older than 18 years. All participants signed an informed consent. The methods used to analyze serum ferritin concentration were commercially available assays; VITROS Ferritin test, chemiluminescent sandwich method, antigen-antibody complex, and Alinity i Ferritin chemiluminescent microparticle immunoassay. The reference range for ferritin; on the Vitros ECI analyzer is 14-400 ng/mL for man and 7-133 ng/mL for women of reproductive age, 14-295 ng/mL for women of non-reproductive age. The reference range for ferritin on the Alinity ci is 21-00-274.66 for man and 4.63-204.00 ng/mL for women.
Results: For Bland-Altman analysis mean difference between two measurements was 56.87 (41.22-72.52) with limits of agreement D ± 1.96. Passing Bablok regression equation - 1.049 (- 1.735 to - 0.330) + 1.711 (1.675 to 1.740) x and showed statistically significant constant and proportional deviation.
Conclusion: It is concluded that the methods are not comparable. Bland-Altman analysis showed that the limits of the confidence interval are different from zero. Regression equations, obtained by Passing Bablok analysis, also confirmed that there is a statistically significant difference between these methods. When making clinical decisions, using ferritin results from the same assay in patients is critical. If the assay is changed, it is essential to assess agreement between results from the new and old assays. Apart from the statistical difference in measurements, these differences are also clinically significant and further studies to harmonize ferritin assays are required.

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S-03

Interference of icteria on drug and immunochemistry tests

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Introduction: Aim of this study was to examine interference of icterus on drug and immunochemistry tests.
Materials and methods: In total, 40 mL of fresh serum pool from leftover samples was prepared. To obtain elevated drug concentration, TechnopathMultichem S L3 (Technopath, Tipperary, Ireland) control sample was added to serum pool. Bilirubin conjugate, Ditaurate salt (Sigma-Aldrich, Chemie GmbH, Germany) was dissolved in distilled water to obtain target bilirubin concentration of 2000 µmol/L (stock solution). Serum pool was diluted with stock solution to obtain bilirubin target concentrations: 25, 50, 75, 100, 300, 450, 575, 675, 800 and 1000 µmol/L. Icteria index (I index) and each analyte were measured in duplicate. Included analytes were: lithium, carbamazepine, gentamicin, vancomycin, phenobarbital andvalproic acid. Immunochemistry tests were: myoglobin, anti-cyclic citrullinated peptide (anti-CCP), human chorionic gonadotropin (hCG), ferritin, folic acid, high-sensitivity troponin I (hsTnI), N-terminal pro-brain natriuretic peptide (NT- proBNP), procalcitonin (PCT) and 25-hydroxi vitamin D (25-OHD). For each icteric sample (I), bias against native (0) sample was calculated as: ([valueI - value0] / value0) x 100%. Data were analysed in Excel 2016 (Microsoft, Redmond, Washington). All measurements were performed on Abbott Alinity i and c analysers (Abbott, Abbott Park, USA). External quality assessment acceptance criteria were selected for decision limits. Cut-off value was defined as the highest value of I index with corresponding bias lower than acceptance criteria. Results were compared with manufacturer´s declarations.
Results: No significant interference was observed for almost all immunochemistry tests. Acceptance criteria were not met for: lithium (12.4%) at 100, PCT (16.7%) at 300 and carbamazepine (17.3%) at 800 µmol/L of bilirubin, respectively.
Conclusion: There is no significant interference in immunochemistry tests and drugs performed on Abbott Alinity i and c up to 1000 µmol/L, except for PCT. Lower I index from manufacturer´s declaration was observed for PCT and higher for carbamazepine. Significant interference at lower I index from declared value was observed for lithium.

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S-04

Comparison of chromogranin A concentrations in serum and plasma

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Introduction: Chromogranin A (CgA) is regarded as a major, pan-neuroendocrine marker, because of its location in the dense core secretory granules of many neuroendocrine cells. It has been used widely for diagnostic purposes and monitoring of the therapy in patients with neuroendocrine tumors. However, there are many various factors, drugs, and coexisting diseases that should be considered when interpreting CgA values. One such factor is the type of biological sample, whether it is plasma or serum. The aim of our study was to compare CgA concentrations measured in serum and plasma, and to determine CgAd ifferences between genders.
Materials and methods: Blood was collected from 94 subjects (47 males) into two tubes: one with K2EDTA (plasma) and one with a clot activator (serum) (Beckton Dickinson, USA). The subject's exclusion criteria were: treatment with proton pump inhibitors, corticosteroids, chronic renal or hepatic diseases, and prostate cancer. CgA was measured using a time- resolved amplified criptate emission (TRACE) assay on Kryptor Compact Plus (Brahms, Thermo Fisher Scientific, USA). Differences between groups were assessed using the Mann-Whitney test. Significance was assumed at $P \leq 0.05$.
Results: Median values of CgA in serum were 62.1 µg/L (42.8-97.7), and in plasma 44.3 µg/L (29.8-75.4), $P < 0.001$. Average difference in measured CgA was 19.2 µg/L (min-max: 0.9-48.3). There was no significant difference in CgA values between males and females, nor in serum ($P = 0.164$), nor in plasma samples ($P = 0.234$), but the difference in CgA values between two different sample types was greater in males than in females (21.3; 13.3-29.1 *vs*. 14.0; 10.1-20.1, $P = 0.005$).
Conclusion: CgA values were markedly higher in serum than in plasma samples. There was no significant difference between two gender. The cut-off value for CgA in serum of < 100 µg/L is not suitable for interpretation of CgA in EDTA- plasma samples. 95th percentile for CgA values in plasma showed to be 75 µg/L.

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S-05

The stability of parathyroid hormone (PTH), osteocalcin and C-terminal telopeptides of type I collagen (CTX-I) in serum and plasma

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Introduction: Besides verification of analytic performances, it is important for the laboratory to assess the stability of analites in their preanalytical settings. This study aimed to compare the results of selected analites obtained from serum and plasma immediately after sampling and after storing whole blood for 4 hours at room temperature.
Materials and methods: Two sets of serum and K3EDTAplasma tubes (Greiner Bio-One) were collected from each of the 27 volunteers. The first sets of serum (S0) and plasma (P0) tubes were centrifuged and analyzed immediately after sampling, while the second sets of serum (S4) and plasma (P4) tubes were first stored at room temperature for 4 hours. PTH was measured with Roche Cobas e801, osteocalcin, and CTX-I with IDS iSYS. The results (S0 vs. P0, S0 vs. S4, and P0 vs. P4) were compared using the Passing-Bablok regression analysis (PB). For constant and proportional differences, the intercept (95% CI) and slope (95% CI) are presented. Clinically significant difference was assessed by comparing mean bias with desirable criteria for bias from the EFLM Biological Variation Datebase (PTH: 7.5%; osteocalcin: 8.4%; CTX-I: 12.1%).
Results: PB between S0 and P0 showed a constant difference for PTH (0.26 (0.09-0.51)) and a proportional difference for CTX-I (1.06 (1.01-1.15)), which were not clinically significant. When comparing S0 vs. S4 and P0 vs. P4, for PTH and CTX-I, statistically significant proportional differences were obtained in serum (0.92 (0.88-0.97) and 0.89 (0.84-0.94), respectively), while for osteocalcin, statistically significant constant and proportional differences were obtained in serum (0.80 (0.11-1.70) and 0.86 (0.80-0.90), respectively) and plasma (0.80 (0.09-1.44) and 0.90 (0.85-0.95), respectively). However, the obtained differences were clinically significant for PTH and osteocalcin between S0 and S4, with average biases of - 10% and - 11%, respectively.
Conclusion: The obtained results suggest a higher stability of the selected analites in plasma in the investigated preanalytical settings.

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S-06

Heterophilic antibodies - a hidden danger in CA 19-9 immunoassay and use of heterophilic blocking tube

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Introduction: Heterophilic antibodies (HA) are immunoglobulins which bind to animal antibodies used in immunochemistry assays. They can produce false positive or false negative interferences, although positive interference is more likely. In our hospital CA 19-9 assay is often requested without sufficient justification as a screening test for malignancy, and due to the possible interference of HAit can result in false management of the patient. Heterophilic Blocking Tubes (Scantibodies, USA) (HBT) contains a blocking reagent composed of specific binders which inactivate heterophilic antibodies. In patients with isolated increased results of CA 19-9 with no previous diagnosis, we process the serum using HBT. If the HBT CA 19-9 results differed by more than the reference change value (RCV) from those obtained on the untreated sample, we can suspect on interference from heterophilic antibodies. The aim was to analyse the number of observed HA interferences on CA 19-9 assay in General hospital Varaždin.
Materials and methods: Patient results from January 2023 to March 2024 which had requested CA 19-9 assay were analysed. CA 19-9 was measured on Abbott Alinityi (Abbott, Abbott Park, USA) using chemiluminescent microparticle immunoassay (CMIA). The patients with isolated increased CA 19-9 were processed in HBT, 500 µL of serum was placed in HBT, incubated in room temperature one hour, and measured again. RCV was calculated using formula $RCV = \sqrt{2} \times Z \times \sqrt{(CVa^2 + CVi^2)}$.
Results: Out of 11,145 patient samples according to specified criteria, 172 (1,5%) serums were further processed with HBT. Using RCV of 21.6%, out of these 172 patients HA were detected in 41 (21%) with 100 patients that had absolute difference greater than 100 U/mL.
Conclusion: The analysis revealed presence of interference of HAin CA 19-9 assay. Awareness of the possibility of interference is important to prevent inappropriate management of patients.

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S-07

Vitamin B1 stability in whole blood sample

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Introduction: Vitamin B1 in whole blood sample is stable for 7 days at the temperature 2-8°C according to the manufacturer of the reagent. The goal of the research was to examine the stability of vitamin B1 following European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) WG-PRE CRESS checklist.
Materials and method: The study included 20 remaining whole blood samples from patients admitted to the Department of Clinical Chemistry, Sestre milosrdnice University Hospital Center, Zagreb. Vitamin B1 concentrations were determined on an LCMS-8050 analyzer (Shimadzu, Kyoto, Japan) using the commercial kit ClinMass Vitamins B1, B2 and B6 in whole blood (Recipe, Munich, Germany). After analysis, the samples were stored in a refrigerator at 2-8°C and vitamin B1 concentration was determined again after 7, 14 and 35 days. For each sample, the percentage deviation (PD%) between the initial measurement and each subsequent determination point was determined. For each point, the mean PD% with 95% CI was determined. The maximum permissible deviation (MPD) was defined according to the equation: $MPD = \pm \sqrt{(1{,}65 \times CVa)^2+(0{,}5 \times CVi)^2}$, CVa is analytical variability and CVi is intraindividual variability obtained from the EFLM biological variation database. Stability limit (SL) was obtained following equation SL = MPD/a, where a is slope of the instability equation.
Results: Mean PD% with 95%CI for vitamin B1 at time points 7, 14 and 35 days was - 22.9% (- 13.8 to - 32.1), - 45.5% (- 37.2 to - 53.7) and - 69.1% (- 63.5 to - 74.7), respectively. Calculated MPD (%) was 12.1% and instability equation was y = - 2.181x. Instability equation does not have intercept, as there is no difference in starting point. Extrapolation of instability equation shows a stability limit of 6 days.
Conclusion: Whole blood sample for vitamin B1 determination is less stable at 2-8°C (6 days) than declared by the manufacturer (7 days).

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S-08

Stability of iron on the gel after two days

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Introduction: For iron (Fe) analysis manufacturer's recommendation for storage of separated specimens are at 2-8 °C up to 7 days. The aim of this study was to evaluate stability of Fe in the serum on gel after storage at 2-8 °C up to two days (48h).
Materials and methods: Samples of 17 patients were analyzed for Fe on Dimension Xpand Plus (Siemens, Dublin, Ireland) biochemistry analyzer. Blood was collected for routine laboratory analysis in BD Vacutainer tube SST II Advance (REF 367975), PET tube with additive (silica clot activator) and gel separator. Analysis was performed upon arrival into laboratory after which samples were stored at 2-8 °C for further analysis. Coefficient of variation (CV) of normal analytical quality control for Fe was 4.35% and CV pathological control was 1.13%. Statistical analysis was performed using MedCalc statistical software (Mariakerke, Belgium).
Results: Results of measurement were compared using paired t-test where mean difference (MD) and standard deviation (SD) of MD were used for the difference assessment. MD were compared with CV of daily analytical quality controls. Fe concentration expressed as mean ± SD at 0h and 48h was 16.59 ± 7.05 µmol/L and 16.76 ± 6.92 µmol/L, MD ± SD = 0.17 ± 0.13 (1.02%).
Conclusion: Mean difference between two measurements for Fe (1.02%) is lower than both CV of quality controls. The result implies that serum on gel samples can be used for Fe analysis after prolonged storage if necessary.

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S-09

Hemolysis in blood gas samples: do we need to pay more attention to preanalytics?

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Introduction: Hemolysis can distort blood gas analysis results, leading to incorrect patient evaluations or treatment decisions. Current procedures do not screen for hemolysis blood gas samples, so our research aims to determine how often it occurs in arterial blood gas samples.
Materials and methods: Atotal of 502 arterial whole blood samples were analyzed. The samples were taken directly from the arterial puncture using a blood gas syringe with balanced heparin (samples from the Department of Pulmonary and Cardiac Functional Diagnostics). Initially, the samples were analyzed with the ABL90 Flex blood gas analyzer (Radiometer, Bronshoj, Denmark) as a part of routine clinical diagnostics. The residual volume of each sample was then aliquoted into an Eppendorf tube and centrifuged. The hemolysis index (HI) was measured using the Alinity c analyzer (Abbott Laboratories, Abbott Park, USA). All samples with HI > 70 were classified as hemolysis positive, and results are reported as numbers and percentages.
Results: Of the total 502 samples, 12 had a hemolysis index greater than 70, representing 2.39% of the total samples.
Conclusion: In the University Hospital Centre Zagreb, the majority of arterial blood gas samples are analyzed using point-of-care devices. Our hemolysis rate is 2.39%, which is above the quality indicator of 1% set by the Department of Laboratory Diagnostics. Blood gas analysis is an emergency diagnostic and we can not perform centrifugation or measure HI on a biochemistry analyzer as this is too time-consuming. It's therefore essential to train clinical staff to be more careful when interpreting blood gas analysis results. One potential solution could involve developing a method within the blood gas analyzer to rapidly detect hemolysis in whole blood samples and provide reliable information on result accuracy.

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U Laboratory Management

U-01

Changes in clinical laboratory practices following the implementation of LSD neonatal screening in Korea: a single laboratory experience

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Introduction: Lysosomal Storage Disorders (LSDs) are rare genetic metabolic diseases that can lead to severe health problems if not diagnosed and treated early. Recently in Korea, LSD neonatal screening was included in the national health insurance program. Previously, screening was conducted only for suspected cases or high-risk groups, but now all newborns are screened. This study investigates the impact on clinical laboratory practices, focusing on test volumes, positivity rates, and cut-off values.
Materials and methods: We analyzed enzyme activity data from dried blood spots (DBS) collected over three years before (2021-2023) and four months after the screening became covered by insurance. The screenings were performed using the Neo-LSD kit (Perkin-Elmer) by MS/MS. The study included samples from various obstetric hospitals. We compared the increase in test volumes, positivity rates (positive for at least one of six enzymes), and cut-off values before and after the insurance coverage. Our laboratory uses the 0.25 percentile as the cut-off.
Results: With the expansion of LSD screening to all newborns, the monthly test volumes increased significantly by 25.5 times after the insurance coverage. The positivity rate decreased from 3.55% to 0.32%, a 9.1-fold decrease. Although the use of the 0.25 percentile cut-off resulted in a decreased positivity rate, there is a need to review the cut-off criteria to improve specificity and accommodate the expanded screening population.
Conclusion: The expansion of the screening population necessitates the establishment of an optimal cut-off value to maintain the accuracy and efficiency of the screening process. Future research should focus on developing a screening system that satisfies both sensitivity and specificity requirements to ensure effective early diagnosis and treatment.

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U-02 (Oral presentation)

How much time can we save with pneumatic tube transport in emergency department?

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Introduction: In last several years there has been a steady increase in laboratory tests ordered in our laboratory, and in 2023. Emergency laboratory tests took up to 45% of total test ordered that year. Increased demand put a pressure on the total laboratory process, especially in the processing of emergency samples that have turnaround time (TAT) of 60-90 minutes. One of the challenges that we faced was sample delivery from emergency department (ED). Our laboratory is in pavilion type hospital which requires hospital staff to walk between the buildings in order to deliver the samples to laboratory which led to prolonged delivery and overall longer TAT. To address this problem and shorten TAT we decided to automate the sample delivery and introduce pneumatic sample transport (PTS). In 2020 TEMPUS600 (Sarstedt, Denmark) PTS was installed. The system transports individual samples in tube from the building where ED was located to core laboratory through 200 m of underground pipes in 20-30 seconds which assures quick and safe sample transport. In the beginning of 2023 ED relocated to a new building which meant that staff had to manually deliver samples again until the installation of a PTS from the new building.
Materials and methods: We analysed time from test order in the hospital system to the sample registration in the laboratory form ED in 3 different time periods. Six months in 2019. Before installation of PTS, in 2021. when PTS was in use and in 2023 when ED relocated to the new building. Median of sample delivery in 3 time periods was calculated using Microsoft Excel (Microsoft Office, USA).
Results: Median of sample delivery time significantly reduced in 2021 (7 min) in comparison with 2019 and 2023 (21 and 19 min respectively).
Conclusion: Implementation of PTS significantly reduced sample delivery time of samples from ED.

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V Management in the Health Care System

Comparison of four immunochromatographic tests for the fecal occult blood detection

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Introduction: Population screening programs are widely adopted in most developed countries and are proven to be cost-effective and reduce mortality. Fecal occult blood (FOB) test is a first step in the national screening programs for the early detection of colorectal cancer (CRC) and as such represents a sort of a guideline for further diagnostic steps. The aim of this work was to compare the results of four FOB immunochromatographic tests.
Materials and methods: The following immunochromatographic FOB tests were used to detect human hemoglobin in 30 stool samples: a) HEXAGON OBTI (Human, Germany), b) ACCU-TELL FOB Cassette (AccuBio Tech Co. Ltd, China), c) iColo-Rectal Test (Ameritech Diagnostics Reagent Co. Ltd, China), d) CerTest FOB Blister test (Biotec S.L, Spain). The analysis procedure was carried out in accordance with the manufacturer's recommendations. The reading and interpretation of each result was performed by two laboratory employees. The stool samples for comparison were selected based on the results of the Hexagon test, as a test routinely used in our laboratory. Besides positive samples, either with weak or strong line intensity, 6 negative samples from patients who had a positive result in one of the three stool samples were also used.
Results: In 30 selected stool samples in which the HEXAGON test had 24 positive results (16 with strong line, 8 with weak line), the ACCU-TELL test showed 22 positive results (16 with strong line, 6 with weak line), the iColo-Rectal test detected 13 positive results (9 with strong line, 4 with weak line), while the CerTest FOB Blister test had only 3 positive results (1 with strong line, 2 with weak line).
Conclusion: Immunochromatographic tests should be verified before introduction into routine laboratory work. The sensitivity of the FOB immunochromatographic tests can have a significant impact on the results of CRC screening programs.

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Z Other

Z-01

Comparison of the direct and indirect potentiometric method for electrolytes measurement in serum samples of hospitalized trauma patients

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Introduction: An electrolyte panel is one of the most common requests in the laboratory, either as a part of a routine blood screening or a comprehensive metabolic panel. This study aimed to compare the concentration of serum sodium (Na), potassium (K), and chloride (Cl) measured on blood gas analyzer ABL90 flex (Radiometer, Bronshoj, Denmark) (BGA) and automated chemistry analyzer Alinity c (Abbott, Abbott Park, USA) (AA).
Materials and methods: The study was performed on 200 randomly selected trauma patients. Serum samples were processed within one hour by both analyzers and electrolyte concentrations were determined utilized two different methods, indirect on AAand direct potentiometric method on BGAanalyzer. The results between methods were compared by Bland-Altman plots (BA) and Passing-Bablok regression analysis (PB). Statistical analysis was performed with MedCalc version 11.5.1.0. (MedCalc Software, Ostend, Belgium). P-value < 0.05 was considered statistically significant.
Results: The median values (interquartile range) in mmol/L obtained for Na were 140 (137-142) using AA and 141 (139-143) using BGA ($P < 0.001$), for K were 4.1 (3.8-4.4) using AA and 4.1 (3.9-4.4) using BGA ($P = 0.379$), and for Cl 104 (102- 106) using AA and 103 (101-105) using BGA method ($P < 0.001$). BA analysis showed constant and proportional difference for Na (-1.1 (95% CI: - 1.4 to - 0.8) and - 0.8% (95% CI: - 1.0 to - 0.5), respectively) and Cl (1.2 (95% CI: 0.9 to 1.4) and 1.1% (95% CI: 0.9 to 1.4), respectively). By PB analysis, complete agreement between methods was obtained for Na and K, whereas a constant significant difference (intercept 2; 95% CI: 2 to 2) without clinical significance was obtained for Cl. The average bias between Cl methods (- 1.1%) was within the eligibility criteria of 4.0%
Conclusion: Investigated methods for the determination of electrolytes in serum samples could be interchangeably used in trauma patients, which is important to maintain the turnaround time in the case of unexpected technical difficulties with AA.

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Z-02

Evaluation of the accuracy of FUS-II strips for urine analysis in the screening of proteinuria and leukocyturia

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Introduction: The FUS-1000 Urinalysis Hybrid (DIRUI Industrial, China) offers comprehensive urinalysis, including routine analysis and formed element counting, in one device. We evaluated the accuracy of FUS-II Urinalysis Strips for proteinuria and leukocyturia screening, alongside other tests.
Materials and methods: Twenty-seven urine samples from Croatia Polyclinic, Split Laboratory, were randomly selected. The weighted kappa coefficient, computed using MedCalc, compared FUS-II Urinalysis Strips with quantitative techniques on AU480 (Beckman Coulter, Brea, USA) and Sysmex XN 550 (Sysmex, Kobe, Japan). Proteins were categorized: 0 for neg and < 0.15; trace for 0.15-0.5; 3 for +/++ or 0.5-3; and 5 for +++/++++ or > 3 g/L. Leukocytes were categorized: 0 for neg or 15; 1 for 15-70; 2 for 70-200; and 3 for 200-500 leukocyt/µL.
Results: Protein results showed moderate concordance (weighted kappa = 0.69) with a 95% CI of 0.44 to 0.94. Samples with poor concordance (22%) were classified by AU480 as 0.3 g/L but as neg by DIRUI FUS 1000. Leukocyte results exhibited poor concordance (weighted kappa = 0.31) with a 95% CI of 0.12 to 0.50.
Conclusion: The proteins result of the comparison are satisfactory and we consider that FUS-II Urinalysis Strips can be used in the routine screening for proteinuria. Testing the accuracy of leukocyte esterase showed a weak concordance between the results of the DIRUI FUS 1000 and SYSMEX XN 550 analyzers. Due to the difference in methodology and measurement of intact leukocytes by SYSMEX XN 550 and leukocyte esterase determined by the DIRUI FUS 1000 analyzer, we nevertheless consider the method acceptable in the screening semiquantitative test of urine leukocyte esterase.

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Z-03

Verification study of four ELISA reagent kits for chromogranin A measurement

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Introduction: The acid protein chromogranin A (CgA) is present in the intracellular granules of neurons and neuroendocrine cells. This study aims to compare analytical characteristics and results of serum analysis for four different CgAELISA reagent kits.
Materials and methods: In addition to the routinely used LDN reagent (Nordhorn, Germany), the study also included reagents of Demeditec Diagnostics (Kiel, Germany), DRG Instruments (Marburg, Germany), and DIASource Immunoassay SA (Louvain-LA-Neuve, Belgium). Results of the manually performed analyses were obtained using the SPECTROstar Nano (BMG LABTECH, Ortenberg, Germany) reader. Repeatability and accuracy were verified by analyzing two levels of commercial QC materials in pentaplicates: 60 µg/L and 240 µg/L (LDN, Demeditec, DRG), 144 µg/L and 810 µg/L (DIAsource). Acceptable criteria were established based on manufacturer›s data: CV-L1 4.2%, CV-L2 5.1% (LDN, Demeditec, DRG), CV-L1 7.3%, CV-L2 6.6% (DIAsource). Comparability study was conducted using 80 serum samples with CgArequest (32 to > 900 µg/L) and processed by MedCalc Statistical software (version 20.023, Ostend, Belgium) wherein P-value <0.05 was considered significant.
Results: Measured CVs were in the range 1.3-5.6%. The highest estimated bias for L1 (- 11.9%) was obtained with DIAsource reagent, whereas for L2 (- 5.3%) with Demeditec reagent. The level of data comparability ($P < 0.05$) in relation to the LDN reagent (IQR 49.68-142.75 µg/L) was evaluated using Bland Altman statistics: Demeditec – IQR 47.87-135.58 µg/L, 8.74 (7.80-10.42)%; DRG – IQR 51.45-149.90 µg/L, 2.85(2.32-3.61)%; DIAsource – IQR 56.74-241.03 µg/L, 17.70(11.44-21.82)%. Correlation and Passing-Bablok results were: Demeditec – $R = 0.995$ ($P < 0.05$), $y = 3.91$ (1.15 to 6.54) + 0.85 (0.81 to 0.89)x; DRG – $R = 0.994$ ($P < 0.05$), $y = - 1.17$(- 2.76 to - 0.08) + 1.01 (1.00 to 1.04) x; DIAsource – $R = 0.867$ ($P < 0.05$), $y = 1.61$(- 4.42 to 7.06) + 1.03 (0.98 to 1.13) x.
Conclusion: The manufacturer's statements for repeatability were confirmed. In comparison to LDN, Demeditec and DRG reagents demonstrated minimal result difference while due to clinically significant differences in individual samples, as well as median deviations, comparative measurements are mandatory before introducing DIAsource reagent into routine practice.

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Z-04 (Oral presentation)

Lactate as a predictor of early graft failure after liver transplantation

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Introduction: Arterial blood lactate concentration rises during liver transplantation. This study aimed to determine the prognostic value of lactate in liver transplantation regarding early graft failure (EGF).
Materials and methods: A retrospective observational study included data from 165 patients who underwent liver transplantation at Merkur University Hospital from October 2021 to February 2024. Patients were divided into two groups regarding 90-day survival after liver transplantation. Arterial blood lactate concentration was determined by the amperometric method accredited according to ISO 15189 on the Radiometer ABL90 FLEX analyzer (Radiometer, Bronshoj, Denmark). The normality of the data was tested with the Kolmogorov-Smirnov test. The difference between the two groups was tested with the Mann- Whitney test with $P < 0.05$ considered statistically significant. Receiver operating characteristic (ROC) curve analysis was performed to calculate the optimal intraoperative and postoperative lactate cut-off values predicting EGF. Statistical analysis was performed using MedCalc Statistical Software, version 22.005 (MedCalc Software Ltd, Ostend, Belgium).
Results: 134 patients had a successful outcome and 31 patients had EGF. Patients with EGF exhibited significantly higher intraoperative (5.8 (IQR: 5.4-7.9) mmol/L vs. 3.4 (IQR: 2.8-4.2) mmol/L, $P < 0.001$) and postoperative (5.7 (IQR: 4.9-7.8) mmol/L vs. 2.3 (IQR: 1.5-3.0) mmol/L, $P < 0.001$) lactate values compared to patients with successful outcomes. The optimal cut-off value for intraoperative lactate was 4.3 mmol/L (sensitivity = 82%, specificity = 94%, AUC = 0.94 (95% CI: 0.89 to 0.97, $P < 0.001$)) and for postoperative lactate 4.3 mmol/L (sensitivity = 93%, specificity = 94%, AUC = 0.97 (95% CI: 0.93 to 0.99, $P < 0.001$)).
Conclusion: Intraoperative and postoperative lactate concentrations are indicators of hepatic metabolic function restoration and liver tissue damage. The optimal cut-off values for intraoperative and postoperative lactate concentrations can be used as predictive factors for early postoperative outcomes including EGF and patient survival.

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Z-05

Evaluation of the accuracy of LabStrip U mALB/CREA test strip for routine screeining of albumin/creatinine ratio in urine

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Introduction: DocUReader 2 Pro is a semiautomatic analyzer for analysis of the chemical examination of urine with LabStrip U11 Plus and LabStrip U mALB/CREAtest strips. We assumed the accuracy of the LabStrip Urinalysis mALB/CREAstrip, which semiquantitatively determines the presence of albumin, creatinine and their ratio in urine is satisfactory and that DocUReader 2 Pro with Lab U mALB/CREAstrip can be used in the routine determination.

Materials and methods: Thirty-two urine samples from the Sestre milosrdnice University Hospital Center and Croatia Polyclinic, Zagreb were randomly selected and tested. The Weighted Kappa Coefficient, which was computed using the MedCalc program, was used to compare the data with the results of the quantitative technique applied to Alinity C analyzer. Three groups of results are identified: first, < 3 mg/mmol; second, 3-30 mg/mmol; and third, > 30 mg/mmol.

Results: The results show a moderate concordance, as indicated by the weighted Kappa of 0.66. Arange of poor to good fit is indicated by a value between 0.45 and 0.87. The samples with the biggest variances were those classified by the Alinity C analyzer in the second group, but DocUReader 2 Pro placed them in the third category (3/32 samples; 9%).

Conclusion: The results of the comparison DocUReader 2 Pro and Alinity C analyzer are satisfactory and we consider the semiquantitative method of the DocUReader 2 Pro is adequate for routine use.

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Z-06

Performance characteristics of four tumor markers on Maglumi X6 analyzer

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Introduction: The aim of this study was to perform a verification of S100, CYFRA21-1, HE4, and NSE on the high throughput analyzer Maglumi X6 (Snibe, Shenzhen, China) and a comparison study with CobasPro e801 (Roche Diagnostics, Rotkreuz, Switzerland).
Materials and methods: Analytical verification included assessment of precision, as repeatability and reproducibility, and estimation of bias in two levels of control samples following CLSI guidelines EP15-A3. Acceptance criteria were defined as upper verification limit (UVL) and calculated using the manufacturer's precision claims and factor F determined by degrees of freedom. The highest calculated UVL for repeatability was 5.1% and for reproducibility 2.7%. Passing-Bablok analysis with MedCalc software (version 20.023, Ostend, Belgium) was used for method comparison on 34 to 40 serum samples per analyte, P-value < 0.05 was considered significant.
Results: According to Grubbs' test, one CYFRA21-1 precision result was an outlier. Obtained repeatability data were from 1.6-8.4% and reproducibility 1.2-3.8%. At least one CV per analyte did not meet the criteria. Passing-Bablok analysis showed no significant differences for HE4 (y = - 4.97(- 8.98 to 0.00) + 1.08 (1.00 to 1.14) x, R = 0.983, P < 0.05), a minor proportional, but not constant difference for S100 (y = - 0.01 (- 0.02 to - 0.01)+1.18 (1.00 to 1.33) x, R = 0.789, P < 0.05), and minor both constant and proportional differences for CYFRA21-1 (y = - 0.30 (- 0.61 to - 0.05) + 0.81 (0.71 to 0.98) x, R = 0.926, P < 0.05) and NSE (y = - 2.37 (- 3.82 to - 1.00) + 1.12 (1.01 to 1.24) x, R = 0.976, P < 0.05) between Maglumi X6 and CobasPro e801. In CYFRA21-1 results > 100 µg/L and S-100 > 0.5µg/L, greater differences were observed.
Conclusion: Due to the low CV values declared by the manufacturer, even when UVL is accepted as precision criteria, the manufacturer's precision data were not confirmed. Furthermore, according to BV data, all CVs are within a desirable imprecision criterion. The comparison study revealed excellent correlation results for HE4 and NSE, as well as the need for comparative patient sample measurements before introducing CYFRA21-1 and S100 on Maglumi X6 into routine practice.

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Z-07

Comparison of creatinine results measured by the Jaffe and enzymatic method in patients with spinal cord injury

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Introduction: Creatinine is derived from the metabolism of creatine and phosphocreatine in muscles. Patients with spinal cord injury (SCI) often have a reduced muscle mass because of muscle atrophy and for this reason the concentration of serum creatinine is often decreased in such patients. Enzymatic method is more specific for creatinine and less susceptible to chemical and chromogenic interferences then Jaffe method. The aim was to compare creatinine results measured by the Jaffe and enzymatic method in patients with SCI.

Materials and methods: The study included serum samples from patients with SCI (N = 57). Creatinine was measured on Siemens Dimension Xpand using Jaffe method and Snibe Biossays 240 Plus using enzymatic method. Method comparison was performed with Bland-Altman and Passing-Bablok regression analysis. Statistical analysis was performed with MedCalc (MedCalc Software, Ostend, Belgium). P value < 0.05 was considered statistically significant.

Results: Comparison of creatinine results measured by the Jaffe and enzymatic method showed excellent agreement (r = 0.98, 95% CI: 0.97 to 0.99). Passing-Bablok regression showed constant difference (y = 3.89 (1.00 to 7.39) + 0.95 (0.89 to 1.00) x. Cusum test for linearity showed no significant deviation from linearity (P > 0.10). Bland-Altman analysis revealed absolute bias of - 0.45 (95% CI: - 1.51 to 0.71) and relative bias of -1.6% (95% CI: - 3.43 to 0.29).

Conclusion: Because there is constant difference between two methods they can't be used interchangeably for measuring creatinine in SCI patients.

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Z-08

Sensitization profile to birch tree pollen - an importance of recombinant allergens

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Introduction: One of the most common tree type pollens in Europe is the birch tree of Betulaceae family. Birch pollens are leading causes of asthma, rhinoconjunctivitis, and allergic rhinitis. Birch molecular characterization has reported 7 allergen components: Bet v1 (major allergen) and minor Bet v2, Bet v3, Bet v4, Bet v6, Bet v7, Bet v8. Sensitization profiles to birch allergens differ among European countries and patient's sensitization is useful in allergen-specific immunotherapy. The aim of our study was to assess sensitization profiles in a cohort of birch pollen allergic individuals.

Materials and methods: Total of 45 patient data was extracted from laboratory information system in the period from November 2023 until May 2024. Total IgE (tIgE), specific IgE (sIgE) to birch (Betula verrucosa, t3), specific IgE to rBet v1 (sIgE-t215) and rBet v2 (sIgE-t216) were identified using Siemens Immulite 2000XPi (Siemens Healthcare Diagnostics, Erlangen, Germany) in a two-step chemiluminescence (CLIA) immunoassay. Used reagents (Total IgE and 3gAllergy Specific IgE Universal Kit) and consumables were from the same manufacturer. According to the standard classification system, quantitative values of sIgE resulted in class numbers 0-VI. Correlation between tIgE and sIgE was performed using MedCalc v12.5.0.0 (Ostend, Belgium).

Results: Out of 45 tested sera, 36 contained sIgE-t3 (class I or higher). Out of positive sera, 32 were sensitized to t215. Spearman correlation coefficient between tIgE and sIgE-t3 showed poor correlation ($r = 0.42$, $P = 0.005$), between sIgE-t3 and sIgE-t215 showed excellent correlation ($r = 0.95$, $P < 0.001$) and between sIgE-t3 and sIgE-t216 showed no significant correlation.

Conclusion: Our results suggest high prevalence of Bet v1 among patients sensitized to birch which is in accordance with epidemiological studies in central Europe. Mapping of sensitization profiles helps understand exposure to various allergens and allergy development in a given geographic region, but also gives an insight to allergen-specific prevention and therapy strategies.

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Z-09

Methodological differences in the monitoring of inflammatory cytokines

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Introduction: Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are frequently tested cytokines in scientific research but have also entered the clinical practice. The aim of the study is to compare enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) method for determining TNF-α and IL-6 patients experiencing changes in inflammatory status.
Materials and methods: We analyzed 52 sera samples of patients that underwent isolated aortal stenosis surgery on cardiopulmonary bypass (CPB). Blood was collected at admission, before the start of CPB, at the end of CPB, 5 h after the end of CPB and 12 h after the end of CPB in order to obtain samples before the activation as well at the onset and decrease of the inflammatory response. The concentration of IL-6 and TNF-α were determined using ELISA method (Bender MedSystems GmbH, Vienna, Austria) and by CLIA on the Maglumi 800 analyzer (Snibe, Shenzhen, China). Results were categorized and Cohen's kappa coefficient was calculated (MedCalc version 14.8.1).
Results: The results are divided into two categories according to the limit values (< 8 pg/mL (Snibe) and < 7.8 pg/mL (ELISA) for TNF-α and < 7 pg/mL (Snibe) and < 5.8pg/mL (ELISA) for IL-6. Cohen`s kappa coefficient revealed barely fair agreement for TNF-α (0.20 (95%CI: 0.13-0.52) and substantial agreement for IL-6 (0.74 (95%CI: 0.61-0.88).
Conclusion: Our results show that two tested methods are not in agreement for TNF-α but have substantial agreement for IL-6. Due to the lack of harmonization in different immunochemical methods as well challenging preanalytical factors, awareness of the methodological differences and limitations is required when choosing a methodology for IL-6 and especially TNF-α.

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Z-10 (Oral presentation)

Lysosomal acid lipase activity in dry blood spots: the influence of platelet and leukocyte count

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Introduction: Lysosomal acid lipase deficiency (LAL-D) is a lysosomal storage disease with two clinical phenotypes. Wolman's disease begins at an early age and progresses rapidly, while cholesterol ester storage disease has variable course and progression. The disorders are result of deficiency of lysosomal acid lipase (LAL), an enzyme necessary for the hydrolysis of triglycerides and cholesterol esters. Enzyme replacement therapy is available for the treatment of LAL-D and the disorder needs to be diagnosed in time. The measurement of LAL activity in a dry blood spot (DBS) is the first screening method which determines future diagnostic procedures. The aim of this study was to determine the influence of leukopenia, leukocytosis, thrombocytopenia and thrombocytosis on the LAL activity in DBS.
Materials and methods: EDTA-blood samples were used (Sysmex XN-3000). DBS were prepared from blood samples with leukopenia, leukocytosis, thrombocytopenia and thrombocytosis. The LAL activity in DBS was measured by an in-house fluorimetric method with the synthetic substrate 4-methylumbelliferyl-palmitate and enzyme activator cardiolipin. The activity was calculated from the difference between the measured total acid lipase activity and the acid lipase activity after incubation with lalistat-2, a specific inhibitor of LAL.
Results: The results were statistically processed and expressed as median activity (nmol/punch/h) and 95% confidence interval (CI). In the case of patients with thrombocytopenia (N = 24), the median and 95% CI are 0.19 (0.17 to 0.31) nmol/punch/h; with thrombocytosis (N = 27) 0.65 (0.57 to 0.71) nmol/punch/h; with leukopenia (N = 25) 0.37 (0.32 to 0.94) nmol/punch/h; with leukocytosis (N = 24) 0.52 (0.41 to 0.63) nmol/punch/h. The correlation of LAL activity with the number of leukocytes in leukocytosis was $\rho = 0.24$ ($P = 0.253$), and in leukopenia $\rho = 0.13$ ($P = 0.535$). The correlation with number of platelets in thrombocytopenia was $\rho = 0.07$ ($P = 749$), and in thrombocytosis $\rho = 0.26$ ($P = 0.199$).
Conclusion: The total number of platelets and leukocytes in whole blood does not statistically significantly affect the LAL activity in DBS. The method can be used for the screening after clinical suspicion of LAL-D.

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Z-11 (Oral presentation)

Laboratory diagnostics of cerebrospinal fluid (CSF) in Croatia: a dual perspective

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Introduction: To improve cerebrospinal fluid (CSF) analysis we aimed to assess the current practice among Croatian medical-biochemistry laboratories (CMBLs), as well as to explore physician's expectations by collecting data through two separate surveys conducted during February and March 2024.
Materials and methods: An invitation to participate in the survey was sent to CMBLs who performed CSF diagnostics (N = 38). The physician's survey was sent to various specialists in secondary and tertiary health care (i.e. neurologists, pediatricians, hematologists, intensivists, infectologists). Both questionnaires were designed as questions and statements, with possible multiple answers.
Results: The response rate of the CMBLs survey was 100%. 121 answers were collected through the physician's survey, mostly specialists (82%, mainly neurologists) working in tertiary health care (49%). The majority CMBLs (22/45) use a combination of manual and automated counting. Morphological cell analysis is not carried out by a significant proportion of CMBLs (10/35). The majority of clinicians (55/98) stated that it is sufficient to express the number of mononuclear and polymorphonuclear leukocytes, at least in the case of an elevated leukocyte count. Also, most (79/98) want a leukocyte correction in the case of traumatic puncture, which only some laboratories provide (6/32). The majority of MBLs (24/31) report cytological analysis results in standard units, while the majority of physicians (69%) receive results in non-standard units. If the remaining sample is insufficient for biochemical analysis, 58% of physicians (mainly neurologists) consider total proteins to be the highest priority test, while 30% (mainly pediatric neurologists and neonatologists) prefer glucose analysis. CMBLs mostly use harmonized reference intervals (27/34), but mainly pediatricians are not satisfied with the indicated reference intervals.
Conclusion: There is a substantial heterogeneity regarding CSF analysis and results reporting. Some physicians' responses shed new light on their expectations regarding CSF diagnostics, thus confirming the need for harmonization.

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Z-12

Verification of serum Kloneus-S-FLC-kappa and Kloneus-S-FLC-lambda turbidimetry method on Abbott Alinity c analyzer

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Introduction: The NICE, IMWG and NCCN Guidelines for myeloma diagnosis and management recommend the use of FLCs concentration for initial workup of multiple myeloma, as well as in monitoring of disease progression and patient management. The aim of this study was verification of κloneus-S-FLC-kappa and κloneus-S-FLC-lambda turbidimetry method (TRIMERO Diagnostics, Barcelona, Spain) on Alinity c analyzer (Abbott Laboratories, Abbott Park, USA) before it's implementation in laboratory routine practice.
Materials and methods: Verification of κloneus-S-FLC-kappa and κloneus-S-FLC-lambda turbidimetry method was performed according to CLSI EP15-A2 protocol guidelines by analyzing commercial samples of κloneus-S-FLC-K- control and κloneus-S-FLC-L-control in three concentrations levels. Repeatability (precision in series), validation of intermediate precision from repeated measurements, intra-laboratory precision, measurement of uncertainty and reference intervals (N = 20) were tested. For precision verification manufacturer's criteria were accepted, while for other analytical perofrmances EFLM criteria were used. Statistical analysis was performed using Microsoft Excel (Microsoft Office, USA).
Results: Obtained results for S-FLC-kappa were according to QC level: for precision L1 = 3.14%, L2 = 3.08%, L3 = 2.11% (manufacturer declares 7.6%); for reproducibility L1 = 2.74%, L2 = 1.45%, L3 = 1.46%; for intermediate precision from repeated measurements L1 = 2.21%, L2 = 2.85% L3 = 1.74% and for initial measurement uncertainty L1 = 6.29%, L2 = 6.17%, L3 = 4.22%. Observed results for S-FLC-lambda were according to QC level: for precision L1 = 3.31%, L2 = 1.83%, L3 = 1.86% (manufacturer declares 6.5%); for reproducibility L1 = 3.31%, L2 = 1.46%, L3 = 1.34%; for intermediate precision from repeated measurements L1 = 1.91%, L2 = 1.39%, L3 = 1.51% and for initial measurement uncertainty L1 = 6.62%, L2 = 3.66%, L3 = 3.73%. The reference interval check did not fulfill the criteria of 18/20.
Conclusion: All three levels of control samples for both analytes fulfilled all the accepted criteria of analytical performance. We can conclude that tested TRIMERO κloneus-S-FLC-lambda and κloneus-S-FLC-kappa trubidimetry method can be implemented in our laboratory practice. However, de novo reference intervals should be established for our population.

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Z-13

Establishing of method-related reference intervals for serum free light-chains

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Introduction: Free light-chain (FLC) measurement has brought an important advance in diagnosis and monitoring of plasma cell dyscrasias, particularly monoclonal light-chain diseases. In association with electrophoretic techniques S-FLC measurement plays a central role in screening of this diseases. It represents a step of diagnostic algorithm, provides following of clonal substructure changes over time and assessing the patients response to treatment protocols. While introducing the sFLC assays we found out that reference intervals declared by the manufacturer were not appropriate for our population. The aim of this study was to establish our own method-related reference intervals.

Materials and methods: S-FLC values were determined in sera of 92 patients with negative clinical history as well as with normal results of immunoglobulin quantification, serum protein electrophoresis and immunofixation. The samples were collected in clot activator tube (CAT) (Beckton, Dickinson and Company, Franklin Lakes, USA), centrifuged at 3040xg for 10 minutes and analysed on Alinity c analyzer (Abbott Laboratories, Abbott Park, USA) with κloneus-S-FLC-kappa and κloneus-S-FLC-lambda reagents for turbidimetry (TRIMERO Diagnostics, Barcelona, Spain). Age was presented as median (minimum-maximum). MedCalc version 19.0.3 (MedCalc Software, Belgium) was used to calculate reference intervals (C.Roubust method CLSI C28-A3).

Results: Age of the patients was 57 (14-82), including 36 males and 56 females. Calculated reference interval for S-SFLC-kappa was: 1.96 mg/L (95% CI: 1.51 to 2.40) - 7.72 mg/L (95% CI: 7.22 to 8.21), for S-FLC-lambda was: 0.67 mg/L (95% CI: 0.41 to 0.94) - 4.52 mg/L (95%CI: 4.20 to 4.82) and ratio S-FLC-kappa/S-FLC-lambda was: 0.68 (95% CI: 0.47 to 0.91) - 3.15 (95% CI: 2.91 to 3.37).

Conclusion: The reference intervals for serum FLC were established on our own population. They are applicable to Alinity c analyser using κloneus-S-FLC-kappa and κloneus-S-FLC-lambda TRIMERO turbidimetry reagents.

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Z-14

Verification of the analytical system Labureader Plus 2 and Urised Mini for the qualitative analysis of urine

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Introduction: Qualitative analysis of urine is one of the first steps in the diagnosis of a patient and can be crucial for establishing a diagnosis in many asymptomatic cases, including infection of the urinary tract and various kidney diseases. We examined the precision and analytical accuracy of the urine chemistry analyzer LabUReader Plus 2 and the UriSed mini for microscopic urinalysis, and their comparability with the IRIS Iricell 1500 system currently in use.
Materials and methods: Precision was tested in 20 replicates using commercial Quantimetrix Dip&Spin control samples. Comparability with previously used iCHEM Velocity and IQ 200 Select systems was tested using patient samples (N = 30) for each parameter. The results were statistically processed with MedCalc statistical software version 22.021 using the inter-rater agreement kappa test. Comparability of specific gravity was evaluated by Passing-Bablok regression analysis. Analytical accuracy for total protein was tested by comparison with quantitative measurements on Beckman Coulter AU480 chemical analyzer.
Results: The obtained precision is acceptable for all chemical parameters and erythrocyte and leukocyte in urine sediment (acceptable 90%, 18/20). Comparability with the iCHEM Velocity system showed the best concordance for leukocytes (κ = 0.93) and nitrites (κ = 0.93), the worst for bilirubin (κ = 0.73), while comparability with IQ 200 showed the worst concordance for bacteria (κ = 0.59). Other sediment parameters were satisfactory (κ-coefficient > 0.60). Passing- Bablok regression analysis found that there is no constant or proportional deviation between the methods for specific gravity (y = - 1.01 (- 4.07 to 0.01) + 2.00 (1.00 to 5.00) x). The obtained sensitivity and specificity for total protein in urine is 75% and 100%.
Conclusion: Based on the results of the verification, the tested systems for chemical analysis of urine LabUReader Plus 2 and microscopic analysis of urine UriSed mini meet the set criteria for precision, accuracy and comparability with IRIS Iricell 1500 and can be used in routine work.

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Z-15

Establishing method-related reference intervals for anion gap

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Introduction: The anion gap (AG) represents the difference between the cations and anions in blood. It is commonly calculated as: AG = $[Na^+]$ - $[Cl^-+HCO3^-]$. Other ions are low in concertation and difficult to measure and quantify in an equation. The "unmeasured" proportion of serum ions represents the AG. It is used for diagnosis and evaluation metabolic acidosis and intoxication. Historically, there has been several different reference intervals used for setting the "normal" values of AG. As the values of ions used for calculation vary upon method and sample (serum, plasma, whole blood) used for determination, method-specific reference intervals should be determined for each laboratory. We aimed to determine reference intervals for AG from whole blood samples in General hospital Varaždin.
Materials and methods: Residual whole blood samples from 80 apparently healthy outpatients were analysed on Rapidlab 1265 (Siemens, Germany) no more than 15 minutes after collection. The blood was collected by venepuncture in lithium heparinized BD Preset syringes (BD, USA). $[Na^+]$ and $[Cl^-]$ were measured by ion-selective electrode (ISE) and $[HCO3^-]$ was determined from pH and pCO2 values. AG was calculated with common formula mentioned above and presented in mmol/L. Age was presented as median (minimum-maximum). MedCalc version 19.0.3 (MedCalc Software, Belgium) was used to calculate reference intervals.
Results: Age of the participants was 53 (2-93), including 20 males and 60 females. Calculated reference interval for AG was 4.1 mmol/L (95% CI: 3.6 to 4.7) to 11.5 mmol/L (95% CI: 10.9 to 12.0).
Conclusion: The reference interval for AG in General hospital Varaždin measuring whole blood samples on Rapidlab 1265 is 4.1-11.5mmol/L which is lower compared to 6-14mmol/L which is a reference value found in literature.

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