SPEECH TEXTS

WORLD PATHOLOGY FOUNDATION
Emerging Trends in Laboratory Medicine

Mining the Microbiota for Microbial-Based Therapeutic Strategies

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The past two decades witnessed a revolution in our understanding of host–microbiota interactions that led to the concept of the super-organism consisting of a eukaryotic part and a prokaryotic part. Owing to the critical role of gut microbiota in modulating the host immune system, more and more evidence indicated that the shift of gut microbiota influences disease outcome and response to the therapy in humans. Thus, targeting gut microbiota is becoming a promising strategy to improve therapy of human diseases. The challenge now is how to translate the body of knowledge into effective strategies for prevention and treatment of human pathologies. Identifying effector microorganisms that causally affect host phenotype and deciphering the underlying mechanisms have become foci of microbiome research and have begun to enable the development of microbiota-based therapeutics. Two complementary, reductionist approaches have commonly been used: the first starts with an immune phenotype and narrows down the microbiota to identify responsible effector bacteria, while the second starts with bacteria-derived molecules and metabolites and seeks to understand their effects on the host immune system. Together, these strategies provide the basis for the rational design of microbial and metabolite-based therapeutics that target and ameliorate immune deficits in patients.

Our studies on microbial tryptophan metabolites acting as important regulators of host-microbial symbiosis by activating xenobiotic receptors at the host:microbe interface, are a proof-of-concept demonstration that targeting gut microbiota is a realistic strategy to which molecular pharmaceutics essentially contributes.

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WORLD PATHOLOGY FOUNDATION
Emerging Trends in Laboratory Medicine

The Landscape of Digital Pathology and Artificial Intelligence in Malaysia

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The landscape of digital pathology in Malaysia has been evolving over the last 3 decades and has escalated in the last 5 years due to several compelling paradigm shifts. The recent Covid-19 Pandemic was a major driver in the adoption of digital technologies in daily life (communication, businesses, work and leisure): currently Malaysia has a mobile internet penetration of 98%, and broadband speed that is the third highest in SouthEast Asia. Being a signatory of the Sustainable Development Goals (SDGs), Malaysia has committed to both environmental and human health and the achievement of Universal Health Coverage. These commitments are intertwined with several strategic initiatives, among them, the 76th World Health Assembly (May 2023) resolution on strengthening diagnostics capacity, against the background of the *Lancet* Commission on Diagnostics (2021) which pointed out that diagnosis is the weakest gap in the cascade of care, and the adoption of digitization and artificial intelligence can transform access and affordability of diagnostics. Malaysia's Health White paper (2023) commits to the harnessing of digital technologies within its pillar of Healthcare reforms. Meanwhile, the call of Malaysia's National Planetary Health Action Plan (2024) includes commitment to environmentally friendly activities in the move towards reducing carbon footprint.

Today almost all pathology and laboratory medicine laboratories in Malaysia have adopted the electronic (digital) mode for handling of medical testing results and reports (communication, storage and retrieval). Laboratory information systems (LIS) is a norm and usually linked to the Hospital Information System (HIS). The remaining challenge is in the area of digital pathology imaging. In anatomical pathology, the use of digital images through virtual microscopy has been well-adopted for education (slide seminars, teaching sets), conferencing (multidisciplinary team discussions, tumour boards, diagnostic consultations) and quality assurance, signifying a crucial mindset change. However, the establishment of digital archives to replace glass slides repositories is rudimentary and fragmented due to the high cost of whole slide scanners. In the developmental space, a dichotomy of approaches is observed in pathology laboratories: in Ministry of Health laboratories, digital workflow and primary diagnosis using digital images is being piloted, whereas the university laboratories have a focus towards development of artificial intelligence (AI). The AI for Digital Pathology (AI4DP) Research Excellence Consortium and Vidanex (a University spin-off company) are examples of Malaysian initiatives in digital pathology research and AI respectively. Among them, studies into screening of blood smears for cancer, screening of fine-needle aspiration cytology smears for pancreatic cancer, Allred-scoring of estrogen receptor immunohistochemical expression by breast cancer and assessment for HER2 gene amplification in in-situ hybridization images of breast cancer are some examples. Various approaches of machine learning, ground truth annotations and development of convolutional neural networks, deep neural networks and DenseNet have been explored. In the process, interdisciplinary collaborations between pathologists, computing and informatic scientists have heightened, as have between local, regional and global players. The findings are encouraging towards the adoption of AI to value-add to anatomical pathology practice in Malaysia in the near future.

PLENARY SESSION

Unlocking the Power of Laboratory Medicine: Creating Virtual Patient Biorepositories in the Electronic Medical Record

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Clinical laboratories generate a copious amount of actionable data in the in- and out-patient settings. Studies report that the value of clinical laboratories to support precision medicine lies in the field actively identifying biomarkers to aid in disease detection and therapeutic targeting. Healthcare systems are using laboratory-generated data to develop clinical algorithms, disease risk models (detection, diagnosis, and management), and real-time care alerts to empower patients, physicians, and healthcare providers. Precision medicine commonly relies on assessing data from large-scale human biorepositories linked to annotated clinical data. These traditional biorepositories have limitations including manual enrollment with limited enrollment time periods, high staffing costs, compensation for consented study participants, lack specimen diversity (e.g., specimen type, population representation, and high maintenance and repair costs of freezers. The presentation will highlight the value of laboratory medicine in designing and supporting clinical trials and the feasibility of clinical laboratories collaborating across medical and research disciplines to embed scalable, virtually enabled biorepositories within the electronic medical record.

EUROPEAN UNION of MEDICAL SPECIALISTS
EU Standarts for Medical Specialist in Laboratory Medicine

EU Training Standards for Specialists in Laboratory Medicine

Vesna Kusec

UEMS Section for Laboratory Medicine/ Medical Biopathology
EU Board of Laboratory Medicine

The European Union of Medical Specialists (UEMS) is European non-profit organisation representing **more than 1.6 million specialist doctors,** from all EU countries and beyond. The UEMS objective is to *improve patient care throughout Europe* by developing and supporting excellence in specialist medical practice. Training of medical specialists and life-long continuous education have a direct implication on setting of healthcare standards and creating patient safety. The UEMS is comitted to work through its specialist sections and other groups of medical interest on preparing and updating the content and other modalities of specialist training according to the progress of medical sciences and the highest standards of methodology of education for medical doctors. This is achieved by the work of specialist sections or groupings on European Training Requirements (ETR) for a specific specialty training. This UEMS document is updated to conform to the progress of the specialty and also with regard to the current approach to training standards. The ETR includes CanMeds approach to competency-based training (including Entrustable Professional Activities), with syllabus, curriculum, criteria for trainees, for trainers, for training institutions, tools for monitoring trainee progress, and also information on the UEMS EU Board medical specialist examination. Embracing and implementing the ETR by national authorities will support the harmonization of core medical specialist training in the EU countries, create prerequesites for corresponding healthcare standards and also support the free movement of medical specialists.

EUROPEAN UNION of MEDICAL SPECIALISTS
EU Standarts for Medical Specialist in Laboratory Medicine

The Medical Aspect of Laboratory Medicine

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Healthcare is complex and patient safety is essential. Medical laboratories in Europe can be organised quite differently; this can be based on local requirements, historical and other reasons. Irrespective of this, every request to medical laboratories represents a patient. The latest version of ISO15189 Medical Laboratory standards rightly place a significant emphasis on patients and risk. The UEMS objective is to improve patient care throughout Europe by developing and supporting excellence in specialist medical practice. This presentation will address the key aspects that medical specialists play in Medical Laboratories to ensure high healthcare standards.

THE INTERNATIONAL FEDERATION of CLINICAL CHEMISTRY AND LABORATORY MEDICINE
The Place of Sustainable Laboratory Medicine in Patient Centered Health Care

Pathology and Laboratory Medicine – Integrating the Sustainability Pathway for Greater Effectiveness

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Abstract

Aim: Pathology and laboratory medicine are at the heart of the understanding of the pathogenesis and management of disease. The focus of the discipline had hitherto been on high quality laboratory medicine practice to provide superior optimal care for the patient. Recent developments are instructive on the responsibility sustainable or green laboratory medicine imposes on laboratorians and the need to embrace this desirable pursuit. Sustainability was originally defined as meeting "the needs of the present without compromising the ability of future generations to meet their own needs". Sustainability has been broadened and its relationship to the true mandate of pathology and laboratory medicine in adding value to patient care is incompletely elucidated. This presentation seeks to delineate the pivotal role of sustainability in pathology and laboratory medicine underscoring it as the overarching driver of the field in the future.

Methods. High quality publications in sustainable science and medicine with focus on pathology and laboratory medicine were selected from sources such as PubMed, Science Direct, Google Scholar and Scopus, employing electronic data bases from 2012 to present Numerous reviews were conducted including gray literature for good evidence on sustainability.

Comments/ Results

Research and practice of laboratory medicine contribute significantly to anthropogenic activities culminating in climate change but has only received limited attention until recently. This is a great challenge to the health sector, particularly to pathology and laboratory medicine which use larger amounts of resources; water, and energy than the average office and generates huge quantities of hazardous and non-hazardous wastes. The field of pathology and laboratory medicine has a responsibility to contribute to sustainable healthcare system by ensuring that resources are used efficiently, guaranteeing that ecological and economic benefits are balanced, but also providing high quality laboratory services to the patient. This approach reduces the environmental impact of laboratory medicine, enthroning sustainability. The ensuing paradigm shift constitutes a pathway to reduce environmental damage and associated consequences. Sustainability also embraces the concept of 'Green Chemistry', generally referring to the "design of chemical products and processes that reduce and/or eliminate the use of or generation of hazardous substances". The sustainability concept should be a key feature in the dynamics of the healthcare sector across the globe and must be integrated, into the curricular of the next generation of pathologists, scientists and laboratory medicine specialists. It should also be borne in mind, that although embracing sustainability is initially capital intensive, on the long run, it is cost saving, due to the concomitant efficient use of energy and other resources, leading to greater effectiveness. Implementation of ISO 14001 in Australia for instance led to a saving of over AU$ 0.80M. Other studies show that embracing sustainability could lead to 30% to 50% reduction in resource consumption, leading to minimal environmental impact, including reducing carbon footprint.

Conclusion: Sustainability in pathology and laboratory has great environmental, economic and societal benefits that should be integrated into the teaching and practice of the discipline. Indeed it should be the overarching driver of future pathology and laboratory medicine for greater effectiveness in patient centered laboratory medicine services and healthcare generally.

THE INTERNATIONAL FEDERATION of CLINICAL CHEMISTRY AND LABORATORY MEDICINE
The Place of Sustainable Laboratory Medicine in Patient Centered Health Care

Eco-Friendly Clinical Biochemistry: A Green Future and Sustainable Laboratory Practice

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Abstract:

The increasing use of fossil fuels, which began during the industrial revolution, has led to a proportional rise in harmful gas emissions associated with these fuels, continuing up to the present day. Due to industrial activities, particularly the consumption of fossil fuels, carbon dioxide emissions from human activities are rising much faster than oceans and forested areas can absorb. If current societal habits persist, severe consequences related to climate change are expected, leading to significant environmental degradation, potential mass casualties, and humanitarian disasters.

The foundation for global cooperation against climate change was first established with the United Nations Framework Convention on Climate Change (UNFCCC) in 1992. The Paris Agreement, adopted in 2015 and coming into force in November 2016, represents a turning point in climate change efforts, catalyzing intensive international cooperation.

The European Green Deal, a set of policy initiatives approved by the European Commission, aims to make the European Union (EU) the first climate-neutral continent by 2050. Similarly, Türkiye has announced its goal of achieving net-zero carbon emissions by 2053.

An Environmental Management System (EMS) is a set of processes and practices that enable organizations to reduce their environmental impacts and improve operational efficiency.

The UI GreenMetric World University Ranking, initiated by Universitas Indonesia in 2010, evaluates universities on green campus initiatives and environmental sustainability. Using 39 indicators across 6 criteria, the UI GreenMetric ranks institutions based on their commitment to and initiatives for environmental sustainability.

The health sector, whose mission is to protect and promote health, significantly contributes to the climate crisis — the greatest health threat of the 21st century — and therefore plays a vital role in addressing it. Health care's climate footprint accounts for 4.4% of global net emissions, equivalent to 2 gigatons of carbon dioxide.

The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) established a Task Force on "Green and Sustainable Laboratories" to support medical laboratories in adopting sustainable practices and enhancing their sustainability standards, not only in Europe but globally.

Although no universal standard exists for green and sustainable laboratory certification, various institutions and organizations offer such certifications. Medical laboratories that apply to these institutions and meet the necessary criteria can obtain certification.

Antalya Training and Research Hospital’s Clinical Chemistry laboratory applied for and received the EFLM Green and Sustainable Lab Certificate after meeting the necessary requirements.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

Role of scientific training in Laboratory Medicine Specialists

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The World Association of Societies of Pathology and Laboratory Medicine (WASPaLM) aims to promote education, research, and international quality standards through WASPaLM committees and secretariats as well as the World Foundation of Pathology. It also seeks to foster training and cooperation among pathology and laboratory medicine societies worldwide.

The WHO, on 76th Assembly, states that pathology laboratory services are fundamental to the application of scientific knowledge, playing a crucial role in ensuring equitable healthcare across all countries and thus in global population health. Besides, it highlights in its resolution on strengthening diagnostic capacity the need to increase investment in research to improve the training of laboratory medicine specialists in a disparate world.

The definition of the specialty in clinical pathology/laboratory medicine varies around the world, but all maintain the spirit of the definition given in 1954 by the SUPAC. “The specialty in clinical pathology/laboratory medicine is understood as a branch of medicine that applies the scientific method and clinical laboratory techniques for medical decision-making…” Scientific training enriches medical practice and drives the evolution of knowledge, being essential for laboratory medicine specialist to perform their tasks with precision, effectiveness, and innovation, contributing to advancements and improving patient care.

The integration of new technologies and the contribution of scientific knowledge with Artificial Intelligence (AI) will enable the creation of new algorithms interdisciplinary, that will allow for more accurate and integrated interpretation of data, leading to better to medical decision-making and health outcomes.

In the field of clinical pathology training, nearly all programs include scientific research methodology, laboratory research stages, statistical methodology, and the development and preparation of scientific articles. Additionally, in most programs, the completion of a final scientific project is required This final scientific project allows for the acquisition of the aforementioned knowledge and serves as a foundation for pursuing professional postgraduate activities, such as specialization diplomas and academic graduate programs, including doctorates.

The curricula should facilitate exchanges between postgraduates and residents, deepen training, and strengthen scientific research, scientific work, and publications, as well as create professional networks that enhance the training of specialists in pathology/laboratory medicine.

In summary, the clinical laboratory specialty applies scientific knowledge, new technologies, and advancements in artificial intelligence to medical decision-making. Laboratory medicine services form the foundation of scientific knowledge applied to medical practice and play a crucial role in achieving a balanced healthcare approach for the global population.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

Improving Clinical Biochemistry Residency Training in Turkey:

Challenges and Possible Solutions

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Abstract: Residency training of medical biochemistry in Turkey is provided at training and research hospitals affiliated with the Ministry of Health and at university hospitals. After taking the higher education entrance exam, students who are accepted into medical school graduate after a tough 6-year education, which includes 3 years of theoretical training and 3 years of practical training. After this 6-year education, doctors take the medical specialization exam, known by its abbreviation TUS. The TUS exam consists of two sections: basic medical sciences and clinical medical sciences. Based on the scores they receive, doctors can choose their desired specialties and hospitals.

Doctors who choose the medical biochemistry department as their field of specialization receive the title of medical biochemistry specialist after completing a 4-year specialization program. The fundamental principle of medical biochemistry residency training is to train high-quality specialists who can discuss the mechanisms of health and disease, manage pre-analytical, analytical, and post-analytical processes, be proficient in laboratory management, relate laboratory findings to relevant clinical information to provide consultation to clinicians, plan and conduct research, possess the skills to communicate their knowledge, adhere to ethical standards and patient rights, and adopt lifelong learning.

In the first year of the 4-year specialization program, the aim is to develop basic laboratory knowledge, techniques and applications, including laboratory safety, sample collection, preparation for analysis, complete blood count, urine analysis, photometric analyses, and emergency biochemistry analyses, in terms of clinical and interventional competencies. In the second year, routine and advanced laboratory tests and techniques, such as general clinical chemistry, hormones, coagulation, electrophoresis, blood gases, and HPLC, are applied. According to regulations, a thesis advisor and thesis topic are determined.

In the third year of the specialization program, applied skills are developed in the laboratory while collaboration with clinics is increased. During this period, mandatory rotations are conducted, and thesis studies are continued. In the fourth year, activities such as report approval, result interpretation, preparation of specifications, and clinical consultation activities are carried out. After completing the 4-year program, the specialization thesis is defended, and the specialization exam is taken. At the end of all these processes, the doctor receives the title of Medical Biochemistry Specialist (MD).

Medical biochemistry residency training around the world consists of similar processes. However, in some countries, it is considered a subspecialty of clinical pathology, or clinical biochemistry specialization training is pursued under a clinical specialty such as internal medicine or pediatrics. In some countries, the distinction and boundaries between PhD and MD titles are clearer. However, in some places, this distinction does not exist, which can lead to complications.

There are, of course, areas that need improvement in medical biochemistry residency training in Turkey. For example, practical training on every device cannot be conducted at every faculty. National and international rotations could be planned for specific studies such as HPLC, LC-MS, and flow cytometry.

Being open to innovations will always be more effective for improving medical biochemistry specialization training, which requires a holistic approach.

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3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

Get out of your Lab… to learn about Lab Medicine!

Angela Maresch

Coimbra Hospital and University Centre

In respect to the diversity of areas of knowledge and also of techniques involved, Clinical Pathology is one of the richest specialties in Medicine. Subjects as broad as haematology, transfusion medicine, clinical chemistry, immunology and genetics may be comprised in the typical training. Due to Lab Medicine's comprehensive nature, it's essential for the trainee to meet experts on the different matters in order to absorb the full content of a rotation. Even in a time where technology is able to shorten geographical distances, there is no match for experiencing an immersive observership to further improve the trainee's skills and future prospects.

That being said, qualified personnel are often scant for the educational requirements. Small hospitals often need to rely on bigger centres to complete the teaching requirements. Larger institutions are therefore also frequently overcrowded, with potential impact of the quality of tutoring. Trainees from bigger institutions can also be tempted to go outside their comfort zone, to grasp different work environments and organisations. In this context, international internships/observerships is a great opportunity to learn specific matters all while apprehending different working cultures and problem-solving strategies.

The destination of an observership should be carefully chosen. Older colleagues, the tutor responsible for the trainee's formation and the head of the laboratory, can all contribute with their opinions and experiences, aiming for the best choice that favours both that particular trainee and the institution. There might be local bursary programs worth watching out for, namely associations within and beyond the current teaching institution. of importance, multiple online platforms pertaining to recognised scientific societies offer suggestions for observerships, facilitating the connection between the trainee and his dream professional exchange programme. EFLMLabX, for example, was specifically created to provide this connection (see also ISTH, ESCMID, IFCC, etc…). Most of the observerships are free, and some even contemplate accommodation.

Controversially, I would say that selecting a centre of excellence is everything the intern needs to assure the best learning experience, independently of the subject! The best internship is one that teaches both hardskills and softskills, but the ideal internship in laboratory medicine is the one that also dwells on good laboratory management strategies, embraces scientific research and demonstrates a smooth, systematic articulation with the remaining heathcare services. This kind of short experience can change one's work life for the better, for years to come.

SOUTH AFRICAN ASSOCIATION FOR CLINICAL BIOCHEMISTRY AND LABORATORY MEDICINE
New Developments in the Assessment of Cardiovascular Risk

Laboratory tests for lipid disorders using new guidelines

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Cardiovascular disease (CVD) continues to be a leading cause of death and illness worldwide, creating a strong demand for more precise methods to assess and measure cardiovascular risk. Lipids are central to the development of atherosclerotic cardiovascular disease (ASCVD), a major contributor to global morbidity and mortality. Measuring plasma lipids and lipoproteins is a critical tool for identifying individuals at high risk of CVD who may benefit from lipid-lowering treatments. Recent advancements in laboratory diagnostics have significantly improved the accuracy of cardiovascular risk assessments.

The lipid profile, which includes total cholesterol, HDL cholesterol, triglycerides (TGs), and LDL cholesterol (LDL-C), remains vital in assessing cardiovascular disease risk. LDL-C is traditionally calculated using the Friedewald equation, although this method becomes unreliable with triglycerides levels above 4.5 mmol/L and assumes a fixed ratio of VLDL Cholesterol-to-TG ratio of 1:5 (measured in mg/dL). However, this assumption breaks down with elevated triglyceride levels, necessitating the use of fasting samples. In the presence of chylomicronaemia, VLDL-C may be underestimated, leading to an overestimation of LDL-C. The formula is unreliable for triglyceride levels above 4.5 mmol/L and in cases of type 3 hyperlipidaemia. Its accuracy diminishes when triglycerides exceed 1.69 mmol/L (150 mg/dL) or when LDL-C levels fall below the target of 1.8 mmol/L (70 mg/dL). With the advent of LDL-C lowering agents such as Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) inhibitors, which can significantly lower LDL-C when combined with statins, the Friedewald equation may produce inaccurate results due to the misestimation of VLDL cholesterol. Underestimating LDL-C can lead to inappropriate therapy and misclassification of cardiovascular disease (CVD) risk.

The gold standard for LDL-C measurement is beta quantification, which involves ultracentrifugation and polyanion precipitation to separate lipoproteins. Recently, LDL-C calculations have been improved with newer methods such as the Sampson-NIH and Martin-Hopkins equations, reducing the need for direct LDL-C measurement in most cases. Additional markers, including non-HDL-C, ApoB, and small dense LDL-C, have further refined risk assessment. This presentation will review the optimal use of lipid testing and also examine further refinements in this area based on our recent research.

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SOUTH AFRICAN ASSOCIATION FOR CLINICAL BIOCHEMISTRY AND LABORATORY MEDICINE
New Developments in the Assessment of Cardiovascular Risk

Practical Management of Common Dyslipidaemia

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CVD is a major cause of mortality and morbidity in the developed world and the incidence is increasing in the developing world. Dyslipidaemia is a common laboratory measured CVD risk factor that has several different components. This presentation will provide an insight into both classification and treatment of common dyslipidaemias.

LABORATORY TESTS IN THE DIAGNOSIS AND FOLLOW-UP of AUTOIMMUNE DISEASES

Clinical Biochemist Perspective on Autoimmune Disorders of the Central Nervous System

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Neuroimmune interactions are a crucial area of scientific research involving the intricate communication between the central nervous system (CNS) and the immune system. Traditionally, the CNS was considered an immune-privileged site. However, contemporary studies have shown that peripheral immune cells are capable of infiltrating the brain during pathological conditions, contributing to the pathophysiology of neurological diseases. These findings provide valuable insights into how the immune system influences CNS diseases.

Neuroimmune interactions are known to play a role in the onset and progression of various diseases, including autoimmune diseases such as autoimmune encephalitis and multiple sclerosis (MS), as well as conditions like depression, chronic pain, and metabolic syndrome. In autoimmune encephalitis, the role of specific autoantibodies against receptors like NMDAR and AMPAR has been characterized, leading to the exploration of new treatment avenues aimed at neutralizing these antibodies. In MS, autoreactive T cells attacking myelin sheaths induce inflammation and demyelination in the CNS. Ongoing research is investigating the effects of hematopoiesis in the bone marrow and potential targets for myelin repair, highlighting the dynamic relationship between immune responses and CNS pathology.

Furthermore, neuroimmune interactions extend beyond the CNS, with peripheral organs such as the gastrointestinal system and microbiota playing significant roles in these processes. The gut-brain axis has been shown to exert important regulatory effects on both the immune and nervous systems through microbiota-derived metabolites. For instance, short-chain fatty acids and tryptophan derivatives produced by gut microbiota are crucial in modulating immune responses and preventing metabolic disorders.

Future perspectives in this field will focus on the enhanced understanding of neuroimmune interactions, leading to the development of new biomarkers and therapeutic strategies. Approaches aimed at preventing the infiltration of immune cells into the CNS, immunotherapies for neurological disorders, and the modulation of gut microbiota's influence on neuroimmune responses are promising areas of exploration in neuroimmunology.

In this context, clinical biochemistry emerges as an essential discipline in understanding neuroimmune interactions and investigating the biochemical underpinnings of diseases. Future studies in neuroimmunology are expected to facilitate the development of more targeted and personalized approaches for treating both CNS and systemic disorders.

LABORATORY TESTS IN THE DIAGNOSIS AND FOLLOW-UP of AUTOIMMUNE DISEASES

Methods Used in Autoantibody Analyses

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Autoimmune diseases occur when the body's immune system begins to attack its own antigens. When self-tolerance is disturbed as a result of inflammatory pathogens, genetic background, environmental effects (radiation, drugs etc.), it leads to a immune system dysfunction, resulting an increase in autoantibodies. The autoantibodies may represent a status of disease activity or predict a future pathogenic condition. Laboratories analyze and measure autoantibodies employing broad spectrum of techniques and methods.

Old methods such as immunodiffusion assays, passive haemagglutination and western blotting have been replaced by currently used traditional methods such as indirect immunofluorescence (IIF), ELISA and dot blot/line blot immunoassay, as well as advanced methods such as antigen microarray or multipled bead based immunoassay.

IIF, for the detection of ANAs and other autoantibodies has been in use for more than 50 years and allows the detection of antibodies that react against nuclear, nucleolar or perinuclear antigens. ELISAs have been widely used since 1980s as; 1) a quantitative screening test to detect a wide variety of ANAs without indicating the specificity of a positive test result; and 2) an approach to identifying specific autoantibody targets. Dot blot/Line immunoassays are considered as a variation of immunoblots. Detection strips are coated with parallel lines of highly purified antigens, allowing simultaneous detection of multiple antibodies. Antigen microarray is a nanotechnology, in which antigens are printed on polystrene chip and chemiluminescent signals are captured by a chip reader. In multiplexed bead based array, beads with different sizes and/or fluorochromes with different colors or intensities are coated with different specific antigens and mixed to allow detection of each specific autoantibody by gating on beads with certain characteristic.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE

Validity and Requirements for Speciality Training in Pathology and Laboratory Medicine in Türkiye

Pathology Residency in Turkiye: Pros and Cons

Ömer Faruk Dilbaz

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Introduction: The speaker, Ömer Faruk Dilbaz, represents the Young Pathologists of the Federation of Turkish Pathology Societies. The focus is on pathology and being a resident doctor in Turkey.

Pathology Overview: Pathology investigates cellular changes at tissue and cytological levels and is considered a surgical specialty in Turkey. Residency lasts four years, with 80 institutions providing training, and cytopathology being the only subspecialty offered.

Pathologists' Role: Pathologists diagnose diseases and guide treatment through macroscopic, microscopic, and digital pathology techniques.

Federation of **Turkish Pathology Societies**: The organization aims to advance pathology through collaboration, education, and national and international standards.

Pathology Training: Residency includes structured and independent study methods, with evaluations based on theoretical and practical exams. Residents must examine a minimum of 8,000 specimens.

Skills Development: Residents gain skills in case management, pathology reporting, and integrating molecular tests. They are trained in laboratory management, quality control, and archiving.

Challenges: Residents face issues such as excessive time spent on grossing, limited interaction with clinicians, and lack of public awareness of pathologists' roles. However, most residents are satisfied with their career choice.

Pros and Cons of Pathology in Turkey:

Pros: High-quality theoretical education, international congresses, workshops, and balanced work-life.

Cons: Excessive time on grossing, salary discrepancies, uneven case distribution, and limited subspecialty options.

Conclusion: The presentation ends by discussing the importance of pathology in healthcare, the role of social media for professional networking, and the speaker's positive outlook on the future of pathology.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE

Validity and Requirements for Speciality Training in Pathology and Laboratory Medicine in Türkiye

Assessing the Validity and Essential Requirements of Medical Biochemistry Residency in Türkiye: With a Comprehensive Overview of Current Residency Conditions

Dr. Beyazıt Semih Yeşil, Dr. Zeynep Şule Evren

In our country, medical biochemistry specialization constitutes an important area of laboratory medicine, and this field requires well-trained specialists to improve the quality of healthcare services. The medical biochemistry residency training consists of a four-year education process that includes the implementation of a core training program, rotations in specific branches, and the completion of a specialty thesis. Entering this process first requires the completion of a six-year medical school. Afterward, doctors who wish to become medical specialists choose the specialty fields they are interested in, based on various criteria. Some of these criteria include the type of institution, the city where the institution is located, financial income, whether or not night shifts are required, the current number of residents and trainers, and whether the service provided overshadows the education received. One important point is that medical biochemistry is neither the first nor the last choice for many physicians. While there are many reasons behind this, the foremost reasons are expectations and criteria. We believe that the main reason why many of our colleagues who choose our department do not select medical biochemistry as their final branch is that during the six-year medical education, laboratory medicine branches, particularly medical biochemistry, are not sufficiently recognized. of course, we are involved in various observations in research laboratories during medical school, but we do not have enough insight into the routine services provided by a medical biochemistry resident. This leads some colleagues, who did not choose our department with broad information, to change their specialty.

There are many international and national curriculum programs for medical biochemistry residency training. One of the main goals of these curricula is to ensure standardization and harmonization in the education and job descriptions of residents working in different institutions. The first core curriculum studies for medical biochemistry specialization training in our country began with the Education Curriculum Commission established according to the Medical Specialization Regulation published in 2002. At that time, the curricula of the European Union of Medical Specialists (UEMS) Division of Chemical Biopathology (Clinical Biochemistry) and the American Board of Pathology Clinical Chemistry section were utilized. The curriculum, whose foundations were laid by our esteemed educators at that time, was most recently published in draft form on May 6, 2024, as part of the TUKMOS (Specialty Education Board Curriculum Development and Standardization System) and is currently being revised based on feedback from educators and residents. Its scope includes: Definition of the curriculum, core competencies, learning and teaching methods, practical training activities, independent and exploratory learning activities, assessment and evaluation methods, rotation branches and durations, training objectives, and educational standards.

Before we move on to the verbal evaluation, we want to touch on two topics according to these standards. The first is rotations. We can state that there are two types of rotations during our residency. Internal and external rotations. Our internal rotations take place within the medical biochemistry laboratory, including urine analysis, complete blood count analysis, flow cytometry etc. Our external rotations are those we carry out in branches other than medical biochemistry. According to our curriculum, these are three branches: Internal Medicine, Pediatrics, and Medical Microbiology. Although the duration and learning objectives of each branch are outlined in our curriculum, there are different requests and expectations in this regard. For example, the rotation period for internal

medicine, which was four months until six months ago, was reduced to three months in the most recent draft curriculum, and we have requested it to be reduced to two months. There are various reasons behind this: First and foremost, the fact that these clinical branches, independently of our curriculum, may employ us in unsuitable locations under inappropriate working conditions to fill their shortage of residents. We would also like to add that, according to our curriculum, we can only start our external rotations in the second half of our residency, often near our third year. During this period, we have been away from clinical settings for patient management, and we do not find it appropriate for medical biochemistry residents to be left alone in emergency and inpatient services.

Our second topic is the type of institution. Although our curriculum aims to ensure standardization, there are different types of institutions in our country. Universities can be classified as private/foundation, state, or affiliate; educational and research hospitals can be divided into those that are city hospitals and those that are not. There are also variations in terms of whether education or service provision takes precedence, whether they provide academic support, monthly salaries, whether shifts are required, and if so, whether these shifts last 16 or 24 hours. These differences also respond to the selection criteria we mentioned earlier.

Lastly, we would like to share the results of our verbal survey, conducted with one representative from each of approximately 30 different institutions. These 30 institutions reflect a sample of about 200 residents. The questions we asked and the responses we received are as follows:

***The distribution of residents and educators in the institutions we asked is as follows:**

City Hospitals: 55 residents, 29 educators

Private/Foundation Universities: 3 residents, 14 educators

Education and Research Hospitals (Non-city hospitals): 46 residents, 21 educators

Universities: 103 residents, 99 educators

Total: 207 residents, 163 educators

We would like to highlight a statement from our previous curricula. The standards planned for one educator for every three residents and this rate is maintained in most institutions.These questions were asked only for situation assessment purposes.

***What is more prioritized in your department, education or routine services?**

Routine: 127, Education: 46, Balanced: 34

*Does your routine/service interfere with your education?

Yes: 86, No: 121

We also received feedback for a question we did not ask. Some colleagues from institutions where education is prioritized stated that they were deprived of the routine experience they needed.

***What does routine/service mean to you?**

Approval of test results: 133

Education: 17

Evaluation of internal and external quality results: 25

***Does your department have a research laboratory?**

Yes: 125, No: 82

According to the standards, approximately forty percent of our colleagues do not have a research laboratory in their institution.

***Do you participate in the education of medical students?**

Yes: 112, No: 95

***Do you work night shifts in your department?**

Yes: 157, No: 50

This is an interesting finding since many residents choose Medical Biochemistry thinking it is a specialty without night shifts, yet many of our colleagues work 16 or 24-hour shifts at their institutions.

***Is there a separate room for residents in your department?**

Yes: 186, No: 21

This is also an important item in our curriculum standards. The definition of a room for the resident physician, especially one working night shifts, is clearly made.

***How would you rate the academic or scientific support in your department, on a scale of one to five?**

5 points: 66, 4 points: 82, 3 points: 36, 2 points: 23

***How often are educational seminars held at your institution during your training period?**

Once a week: 114, Twice a week: 62, Three times a week: 10, Once a month: 19, Never: 2

***What would you like to say about your gains from clinical rotations?**

Many of our colleagues responded to this open-ended question by saying that they were focusing on the work of the relevant clinical rotation. Some noted that they had the opportunity to learn tests such as flow cytometry and electrophoresis during their hematology rotations in internal medicine or pediatrics, which are not present in medical biochemistry. But these gains do not align with the learning objectives of our curriculum.

Based on what we have heard from our resident colleagues, despite the significant efforts of our esteemed trainers to date, it is evident that both trainers and residents still need to make efforts to achieve standardization and harmonization.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE

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Clinical Biochemistry National Board in Türkiye: 2004 to 2024

Tülay Köken

Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Biochemistry

The Clinical Biochemistry Proficiency Board was established in 2004 to raise, standardize and ensure continuity of medical biochemistry specialization training. The duties of the Proficiency Board are grouped under 3 headings:

• To establish and develop national standards of specialization training,

• To test and document the professional competencies of specialist physicians,

• To carry out the accreditation of institutions providing Specialization Training.

The Competency Executive Board is elected by the general assembly of competencies formed with the participation of specialist physicians who have received a certificate of competency. The Education Program Development Commission, the Education Program Evaluation (Accreditation) Commission, and the Measurement and Evaluation Commission are sub-commissions. In 2009, trainings were started nationwide to ensure standardization in specialization training. Dozens of “continuous professional development” trainings were provided until 2024. After seven years of training in various provinces of the country, preparations for the Board Exam began in 2016. A guide was prepared for this exam. A Measurement-Evaluation and Accreditation course was organized for academics who will prepare questions for the exam. The Core Education Program (CEP) was updated. A question pool was created. The distribution of exam questions according to CEP was ensured. After all preparations were completed, the first board exam was held in Tekirdağ in May 2017 after the continuous professional development activity. To date, 4 board exams have been held and 15 specialists have received a certificate. The program evaluation sub-commission completed its preparations for the accreditation of educational institutions in 2019. This year, an institution's accreditation application was received. The institution was evaluated and the accreditation certificate was presented. In 2023, another institution's application was received and the process is ongoing.

When we come to 2024, the Qualification Board has become able to both issue a specialist proficiency certificate and conduct institution accreditation. These activities, which are based on the principle of volunteering, continue with dedication.

THE ASSOCIATION of CLINICAL BIOCHEMISTS of INDIA
Metabolic Syndrome: South East Asia and Beyond

Metabolic Syndrome in South East Asians: Risk Factors and Causes

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Metabolic Syndrome (MetS) has gained significant attention due to its substantial impact on cardiovascular diseases (CVD) and its high prevalence among patients with type 2 diabetes (T2D). MetS is defined as a cluster of cardio-metabolic dysfunctions characterized by elevated fasting blood glucose (FBG), increased waist circumference (WC), high blood pressure (BP), elevated triglycerides (TG), and reduced levels of high-density lipoprotein cholesterol (HDL-C). It is estimated that 20–25% of the adult population worldwide suffers from MetS, which triples the risk of coronary heart disease and stroke, and doubles the mortality risk from cardio- and cerebrovascular diseases compared to those without the syndrome.

The global rise in MetS is closely associated with the obesity epidemic and the increasing prevalence of T2D. As a substantial contributor to non-communicable diseases globally, MetS represents a significant health and economic burden for both developing and developed nations. If not adequately addressed, the rising prevalence of MetS is expected to lead to increased morbidity and mortality due to cardiovascular disease, which could result in an estimated 22.2 million deaths by 2030.

The COVID-19 pandemic has further highlighted the pressing concerns regarding MetS and its related conditions, which are linked to increased infection risk, greater severity of COVID-19, and poorer prognoses. The syndrome is predominantly driven by sedentary lifestyles and excessive consumption of high-calorie foods, particularly in populations adopting Westernized lifestyles characterized by greater travel convenience, sedentary leisure activities, and a reliance on fast food.

Several factors contribute to an individual's predisposition to MetS, including upstream determinants like socioeconomic status and geographic residence, alongside individual factors such as genetics and lifestyle choices. Southeast Asia, represented by the Association of Southeast Asian Nations (ASEAN)—including Singapore, Thailand, Malaysia, Indonesia, the Philippines, Laos, Vietnam, Cambodia, Myanmar, and Brunei—faces a particularly acute challenge. As of 2020, the United Nations estimated that Southeast Asia is the third most populous subregion in the world, with a population nearing 670 million.

This ethnically diverse region has experienced rapid growth and development in recent decades, transitioning many traditionally agricultural areas into vibrant urban centers and shifting job markets from farming and fishing to office-based work. While these changes have brought prosperity, they have also acted as catalysts for the rise of MetS and its components. According to the World Health Organization (WHO), a higher percentage of the population in most Southeast Asian countries exhibits elevated blood glucose and blood pressure levels compared to those in the United States or the United Kingdom. Moreover, with the exception of Singapore, citizens in all other nations within the region face a considerably higher risk of premature death due to non-communicable diseases, primarily cardiovascular diseases such as stroke and coronary artery disease.

Addressing the growing prevalence of MetS in Southeast Asia will require targeted public health initiatives focused on lifestyle modification, increased awareness, and improved access to healthcare resources to mitigate its health impacts and reduce the associated economic burden.

THE ASSOCIATION of CLINICAL BIOCHEMISTS of INDIA
Metabolic Syndrome: South East Asia and Beyond

Molecular Mechanisms of Metabolic Syndrome

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Metabolic syndrome (MetS) has multifactorial etiology. These include genetic predisposition and multiple environmental or lifestyle factors. Environmental factors are considered the leading cause of MetS and its pathogenesis; however, genetic factors contribute significantly. Among the genetic factors, defects in the signaling pathway, insulin receptor defects, and defective insulin secretion are the major players. Children of obese parents have a higher chance of obesity than healthy individuals. Epidemiologic studies have shown that environmental factors in fetal life and the early postnatal period influence disease risk and pathogenesis in adults. Mother's lifestyle and intrauterine and postnatal nutrition play an important role in the etiology and pathogenesis of the MetS.

Endothelial dysfunction characterized by fluctuations in Plasminogen activator inhibitor-1 (PAI1), lectin-like oxidized lowdensity lipoprotein receptor-1 (LOX-1), vasoconstrictor agent endothelin-1 (ET1), and tissue plasminogen activator (tPA) are postulated to be associated with MetS. Proinflammatory cytokines, such as tumor necrosis factor, leptin, adiponectin and resistin are other molecules associated with MetS. Another important neurohormonal pathway in the development of MetS is the involvement of the renin–angiotensinogen system (RAS). Other contributory factors are Fetuin-A, also referred to as α2-Heremans–Schmid glycoprotein (AHSG), a protein with pleiotropic metabolic effects secreted by the liver; Mitochondrial Dysfunction and PGC-1α; and circulatory MicroRNAs. The IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) is a large multicenter population-based European cohort study that aims to explore the causes and prevent the development of diet and lifestyle-related diseases in children and adolescents with a special focus on overweight and obesity. A significant association affecting the repeated MetS score is seen in five SNPs of the fat mass and obesity associated (FTO) gene on chromosomal region 16q12. Lastly, epigenetic effects and the role of various metabolites are also being explored in the recent years.

THE ASSOCIATION of CLINICAL BIOCHEMISTS of INDIA
Metabolic Syndrome: South East Asia and Beyond

Metabolic syndrome and cancer

Dr Mala Mahto

AIIMS Patna

The Metabolic Syndrome (Met S) is defined as a cluster of cardio-metabolic dysfunctions which is characterized by hyperglycaemia, increased waist circumference (WC), blood pressure (BP), triglycerides (TG) and reduction in high-density lipoprotein cholesterol (HDL-C). Apart from the association of Met S with cardiovascular diseases (CVD) and Type 2 Diabetes mellitus, its coherence to increased incidence of cancer has been also noted. A number of factors such as, underlying inflammation, visceral adiposity, hyperinsulinemia, hyperglycemia, interaction between Insulin like growth factor (IGF) and IGF-Receptor and estrogen signalling play a possible role in development of cancer. Literature suggests that chronic inflammation may be strictly connected with aging processes, aptly named inflammaging. Many age- related diseases like diabetes mellitus, hypertension, dyslipidemia are associated with chronic inflammation. These inflammatory processes induce oxidative stress with reduced anti-oxidant capacity leading to an overproduction of free radicals which set in a vicious cycle. The altered regulation of cytokine release from adipocytes,increased advanced glycation end products, insulin resistance , altered composition of gut microbiota and decreased microbial diversity, activation of aberrant signalling pathway with consequent cell proliferation and growth all contribute towards development of a tumorigenic environment. Future prospective studies with a large sample size are called for to dissect these interrelated factors and to elucidate the interplay of these risk factors in carcinogenesis. Therapeutic interventions targeting the IGF receptor, peroxisome proliferating activated receptors (PPAR) in addition to thiazoilidines and metformin in the treatment of cancers maybe introspected further.

MEDICAL SOCIETY of CLINICAL LABORATORY
One Health Approach, Diagnostic Stewardship in Microbiology and Use of POCT for Value-based Laboratory Medicine in Chile

One Health Approach in Antimicrobial Surveillance

Juan Carlos Hormazabal

Chilean Public Health Institute, Chile

The One Health concept is based on the collaborative, multisectorial, and trans-disciplinary approach, working at the local, regional, national, and global level, with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, and the environment. The antimicrobial resistance (AMR) is a main clinical and Public Health concern. The global recognition of epidemic AMR mechanisms, and the harmonization of laboratory surveillance standards for detection in humans, animals and the environment is relevant for the early detection and the understanding of AMR spread in ecologic terms. One Health Approach in Antimicrobial Resistance Surveillance, is a challenge for Chile and Latin American countries.

MEDICAL SOCIETY of CLINICAL LABORATORY
One Health Approach, Diagnostic Stewardship in Microbiology and Use of POCT for Value-based Laboratory Medicine in Chile

Diagnostic Stewardship, the challenge for clinical microbiology laboratory

Dona Benadof
Roberto del Rio Children Hospital

Diagnostic stewardship aims to deliver the right test to the right patient at the right time. This strategy integrated with antimicrobial stewardship, allows making correct decisions in the use, restriction or suspension of antimicrobials,also helps the efficiency in the use of health resources.

Diagnostic stewardship allows the entire process related to laboratory diagnosis to be involved from preanalitical to postanalaitical, in addition to involving all professionals involved in interpretation and timely decisions in infectious disease to have the best outcomes in patients care.

MEDICAL SOCIETY of CLINICAL LABORATORY
One Health Approach, Diagnostic Stewardship in Microbiology and Use of POCT for Value-based Laboratory Medicine in Chile

Remote testing with POCT in Chiloé islands: from 4 weeks to 20 minutes. A value-based laboratory medicine solution for Chilean population

Carolina Prieto

DIPRECA Hospital, Chile

The lecture will cover the impact of the use of POCT to give access and equity in rural distant communities, with cost/effectiveness data and VBHC results of applied laboratory medicine in the improvement of care in diabetes and cardiovascular risk. This study aimes to deliver robust data for policy making in Chile, for better serve this type of communities living in remote places.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

New Challenges in Molecular Biology: What Clinical Pathology Residents Need to Know

Dr. Vania Medina

Hospital de Clinicas - "Dr. Manuel Quintela", Uruguay

This presentation explores the rapidly advancing field of molecular biology and its critical relevance for clinical pathology residents. As science and technology evolve, it becomes essential for pathologists to have a solid understanding of molecular and cellular biology to keep pace with new diagnostic tools and methodologies. This knowledge enables them to adapt to emerging technologies, such as next-generation sequencing (NGS) and advanced PCR techniques, which are increasingly important in modern clinical practice.

The presentation emphasizes the importance of continuous learning to meet the demands of these advancements, with a particular focus on how PCR serves as the foundation for many current molecular diagnostics. By understanding these core concepts, residents will be better equipped to apply these techniques in their day-to-day clinical work, ultimately improving diagnostic precision and patient outcomes.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

The Clinical Pathology Residency: Narrowing the Gap Between the Patient and Laboratory Diagnosis

Gonçalo Torres

Hospital Guimarães - Portugal

Clinical Pathologists have a significant impact on the success of healthcare, as they are involved in the entire clinical process, from pre-diagnosis to the outcomes of therapeutic decisions. In Portugal, this medical specialty is responsible for leading all activities within the clinical laboratory and acting as a consultant for other physicians. Advantages and drawbacks of such a broad scope of skills and activities will be analyzed.

The Portuguese clinical pathology residency program is designed to prepare future specialists for the multifaceted demands of the field. Residents undergo medical laboratory training (in clinical chemistry and microbiology, hematology, immunology and genetics) together with clinical internships alongside physicians from various medical specialties in order to better understand clinical contexts. The artificial intelligence era will see future clinical pathologists take on pivotal roles emphasizing leadership, management and communication skills.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

Prevalence of Hepatitis C Virus Infection at the Blood Bank of Hospital Civil Fray Antonio Alcalde: A concordance of reactive screening tests with complementary and confirmatory tests for HCV from January 2015 to December 2023.

Ebed Orozco

Mexico

Introduction
Hepatitis C Virus (HCV) represents significant public health issue. It is estimated that approximately 58 million people are infected globally, with 1.5 million new infections annually.
HCV is the principal cause of cirrhosis and hepatocelular carcinoma, which significantly impacts morbidity and mortality.
Blood transfusions are a route of transmisión for HCV, highlighting the importance of screening programs in blood Banks.

Backround
HCV was identified in 1989, but its existence had been suspected since the 1970s.
HCV infection was a major cause of post-transfusión hepatitis.
Screening tests for HCV, such as chemiluminescence immunoassays (CMIA) and NAT, have reduced the transmisión of the virus through transfusions.

Problem Definition
The comparison between screening test results for HCV, confirmatory tests and be able to perform complementary tests is critical to ensuring blood bank safety.
Screening tests are very sensitive but can it can generate false positives, confirmatory tests, such as RIBA and complementary tests, such as NAT, are essential to reducing these errors and confirming true cases of infection.
The estimated prevalence of HCV in a blood banks in México is 0.43%

Theoretical framework
Screening blood donors in blood banks is essential to maintain the safety of blood transfusions. The Mexican Official Standard establishes this in point 9.4. Blood samples taken from each blood donation and blood components must be tested for transfusion-transmissible agents, invariably before therapeutic use.

The Mexican Official Standard establishes in point 9.4.11 that screening must be carried out using tests for the detection of antibodies against the virus or simultaneous detection of viral antigens and antibodies against the virus, with a sensitivity ≥99.5% and specificity ≥99%. Among the established methodologies are the Enzyme-Linked Immunosorbent Assay (ELISA), chemiluminescent immunoassays, or others with equal or greater sensitivity and specificity.

At the blood bank of the Antiguo Hospital Civil, the chemiluminescent immunoassay method is used for screening tests. The **Alinity i Anti-HCV Reagent** Kit is employed, which is a third-generation chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibodies against the virus.

Reactive samples in the detection of antibodies (>1 s/co) must be confirmed with serological tests established in the Mexican Official Standard in point 9.4.11.2. The confirmatory test must be performed using the recombinant immunoblot assay or other tests with equal or greater sensitivity and specificity.

At the Blood Bank, **DeciScan HCV PLUS** is used for the algorithm-based confirmation of Hepatitis C virus infection through immunoblot and **RIBA** (Recombinant Immunoblot Assay) techniques. It is a single-strip or membrane test that utilizes an enzyme immunoassay technique, where the antigens (core, NS3, NS4, and NS5) are pre-fixed.
The Mexican Official Standard establishes in point 9.4.13 that blood banks performing nucleic acid amplification tests, such as transcription-mediated amplification techniques or polymerase chain reaction (PCR), must comply with specific regulations.
Complementary tests, such as nucleic acid amplification (**NAT**), detect the genetic material of a virus rather than waiting for the body to produce antibodies. This offers the opportunity to reduce the window period during which an infectious agent is undetectable by conventional tests, thereby further enhancing blood safety.
NAT also provides indirect information, allowing infected donors to be notified and referred to a specialist for evaluation.
At the blood bank, the qualitative methodology of Reverse Transcriptase Polymerase Chain Reaction (**RT-PCR**) is used, along with Real-Time Amplification Detection (**q-PCR**) through a test called TaqScreen Multiplex, which detects HIV-1 RNA HCV RNA, and HBV DNA in plasma.

General Objective
Analyse the concordance between screening tests (CMIA), confirmartory tests (RIBA), and molecular tests (NAT) in the bood bank.

Specific Objectives
1. Identify the prevalence of false positives and false negatives in the screening tests.
2. Determine the true positives and true negatives by correlating the HCV screening tests with the confirmatory tests.
3. Determine the association of the results of reactive screening tests (s/co) compared to the results of the molecular tests (NAT).
4. Identify donors who may be in a window period using molecular tests.

Material and Methods
Design: Retrospective observational and analytical study.
Population: 123, 302 donors from the Blood Bank (2015-2023).
Tests: Screening: CMIA (Alinity i), Confirmatory: RIBA (DeciScan PLUS), NAT (RT-PCR) (COBAS Ampliprep).

Inclusion Criteria
- All donors who met the minimum requirements established for blood donation according to the acceptance criteria in point 6 of the Mexican Official Standard NOM-253-SSA1-2012, for the disposal of human blood and its components for therapeutic purposes.
- Donors with reactive results in the HCV screening test.
- Samples from the complementary NAT tests that were reactive in the screening test.
- Samples with positive screening results that were subjected to confirmatory tests.

Exclusion Criteria
- Donors whose samples do not meet the established quality criteria for testing.
- Samples with indeterminate results or that cannot be adequately processed.
- All screening tests with results <1 (s/co) that are interpreted as non-reactive.

Data Analysis
Frequency Measures, Percentajes, Measures of Association (Kappa index), Rates, Demographic Analysis, Measures of Central Tendency.

Expected Outcomes
- Accurate estimation of HCV infection prevalence.
- Identification of the concordance between screening, confirmatory, and molecular tests.
- Data to optimize screeening and confirmation protocols in blood Banks.

Results

From January 2015 to December 2023, a total of 123,302 donors were received at the blood bank of the Antiguo Hospital Civil Fray Antonio Alcalde. As part of the blood donation process, screening is performed through serology to detect HCV, HCB, HIV, syphilis, and T. cruzi, using the chemiluminescence methodology. This research focuses solely on the detection of HCV.

Out of the total donors during the specified period, 627 blood samples tested reactive for HCV; of these, 89 were positive for NAAT. Among the 627 reactive samples, the confirmatory RIBA test was positive for 96, indeterminate for 159, and negative for 372 samples.

Of the 89 samples reactive for NAAT, RIBA was positive in 86, indeterminate in 3, and none were negative.

URL S/CO	RIBA Positive	RIBA Indeterminate	RIBA Negative	NAAT Positive
1 – 5 (492)	1	134	357	1
5.1 – 10 (53)	16	21	16	11
>10.1 (82)	77	5	0	76

The concordance between chemiluminescence serology with low antibody titers and RIBA is insignificant (0.14).

1-5 URL s/co (Null Concordance)

5-10 URL s/co (Adequate Concordance)

>10 URL s/co (Excellent Concordance)

The concordance between RIBA and NAAT is optimal (0.942), which means that the results of RIBA and NAT agree in most cases, suggesting that both tests are consistent in identifying the presence or absence of HCV.

- The **prevalence of HCV** infection based on true positives by RIBA is 0.078%. This reflects the proportion of donors confirmed as positive in the total population of 123,302.

- The **false positive rate** in the evaluated donors is 84.69%. This indicates that a high percentage of donors who initially tested reactive by screening were not confirmed as positive by the RIBA test.

- The **false negative rate** in the evaluated donors is 0%, indicating that there were no donors incorrectly classified as negative when they actually had Hepatitis C.
- The percentage of reactive samples for NAT, based on the 627 reactive samples from antibody screening, is 14.35%. This indicates that only a portion of the samples initially reactive by screening are confirmed as reactive by NAT.
- The prevalence of HCV infection, using antibody screening followed by confirmation with NAT, in relation to the total number of donors (123,302) is 0.073%.

The prevalence of HCV infection by sex is as follows:

- **Male:** 0.095% (62 true positives out of 65,000 donors).
- **Female**: 0.058% (34 true positives out of 58,000 donors). The prevalence is slightly higher in men than in women.

The prevalence of HCV by age groups is as follows:

18-24 years: 2.08%

25-44 years: 56.25%

45-65 years: 41.67%

The average age of true positives is 42 years. This indicates that most positive cases are found in the 25-44 age group, followed by the 45-65 age group.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

Investigating Causes of Sudden Death in Infants Using Next-Generation Sequencing

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Sudden unexpected death in infants (SUID) and the young remains a significant global health issue with complex and poorly understood aetiologies. Despite routine medicolegal autopsies in most countries, 70% to 80% of cases remain unexplained even after comprehensive postmortem examinations. These unexplained deaths in otherwise healthy infants or young people have a profound impact on families and communities. Recent advancements in next-generation sequencing and whole-exome sequencing have implicated inherited cardiac arrhythmic disorders, known as cardiac channelopathies, as potentially preventable causes of SUID. Evidence suggests that certain genetic variants may predispose infants to sudden death, particularly when combined with external factors such as sleep position or respiratory infections. Environmental stressors, including prone sleeping and tobacco smoke exposure, may exacerbate underlying genetic vulnerabilities, leading to fatal outcomes.

Postmortem molecular analysis plays a pivotal role in identifying the cause of death and recognizing family members who may carry the same inherited disorder. Since inherited cardiac arrhythmias are treatable, the integration of molecular techniques into SUID investigations is crucial for improving diagnostic accuracy and providing families with closure as well as for pre-emptive diagnosis of relatives.

We undertook a postmortem analysis of a cohort of 66 cases of sudden death and analysed 49 genes related to inherited cardiac arrhythmogenic disorders using next generation sequencing. We found a total of 178 different missense variants of which 164 were known, documented variants whereas the remaining 14 were novel. A total of 127 variants were of benign significance, 36 were variants of unknown significance, whereas the remaining two variants were of likely pathogenic and / or pathogenic significance. In this cohort post mortem genetic testing provided evidence of a genetic arrhythmic/cardiac conduction disorder as the probable pathogenic basis for 3% of sudden unexpected death / sudden unexplained infant death cases.

Combining molecular findings with traditional forensic methods allows for more precise risk factor identification and lays the groundwork for preventive strategies. The need for international collaboration is emphasized to further unravel the complex aetiology of these deaths and enhance public health strategies aimed at reducing infant mortality.

3rd INTERNATIONAL MEETING of RESIDENTS IN PATHOLOGY AND LABORATORY MEDICINE
Improving Residency Training in Clinical Pathology

A Clinico-Haematological and Molecular profiling in Chronic Lymphocytic Leukaemia patients – A single centred experience

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Background: Chronic Lymphocytic Leukemia (CLL) is one of the commonest adult leukemia occurring in elderly individuals but has a variable presentation in Indian population.

Aim and Objectives: A combined analysis of patient's genetic profile will provide comprehensive prognostication and risk stratification for the treatment approach.

Material and Methods: 45 newly diagnosed, relapsed and follow-up cases of CLL patients enrolled in AIIMS Rishikesh. As per European society of medical oncology (ESMO) CLL practical guidelines, clinical staging (Rai and Binet), hematological parameters and comorbidities are considered for treatment initiation. Prognostic factors, including cytogenetic aberrations (del 13q, del 11q, del 17p and trisomy 12) by Fluorescent in-situ Hybridisation (FISH) analysis and mutational variants of TP53, ATM, NOTCH1 by Next Generation Sequencing (NGS) and Immunoglobulin Heavy chain gene Variable region (IgHV) Somatic Hyper mutation Analysis by PCR Sequencing, are analyzed.

Results: In this study result 80% males and 20% females, having a mean age incidence 62years, and a median 60years in which 32% cases are ≤ 55 years. Based on Modified Rai and Binet staging, 16%, 40%, 44% of the study group fall under low-risk, intermediate-risk and high-risk respectively. FISH analysis showed positive for 56% del13q, 20% del11q, 31% trisomy12, 7% del 17p and 40% Unmutated IgHV, 2% mutated TP53 and 11% mutated ATM were tested and compared against the clinical stage to comprehend disease progression and its prognostic relevance to overall survival (OS).

Conclusion: NGS is being increasingly used for better management, treatment decisions and time to first treatment (TTFT) of the disease. FISH, NGS analysis of genes and IgHV are essential for better prognostication and risk stratification.

Keywords: Chronic Lymphocytic Leukemia- CLL, European Society of Medical Oncology- ESMO, Fluorescent in-situ Hybridization- FISH, Immunoglobulin Heavy chain gene Variable region- IgHV, Next Generation Sequencing- NGS, Overall Survival- OS, Time To First Treatment- TTFT.

PLENARY SESSION
Symbiosis Between Lab and Clinic: Hand-Holding Strategies Taken a Notch Higher

Atypical Laboratory Results: Where the Clinician Needs You

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Discordance between results of laboratory investigations and the clinical symptomatology exhibited by patients to the clinician, often lead to the laboratory and the clinic ending at inevitable yet potentially avoidable cross-purposes with one another. The laboratory's role in helping the clinician out of the dilemma, becomes crucial in such cases. This is a particularly common scenario with hormonal assays namely those involving prolactin, growth hormone and ACTH which are commonly affected by a plethora of extraneous factors such as body stress or stimulation and drugs, to name a few, with a resultant significant error in prolactin and cortisol estimations while timing of draw often proves to be the game-changer in estimations of TSH, insulin, cortisol and growth hormone. In addition, transportation and storage conditions have been found to be of paramount importance in the estimations of parameters like ACTH and rennin, both of which are extremely sensitive to temperature changes and also vulnerable to protease degradation. Patient education on consuming regular medication such as oral hypoglycemic agents (OHAs) on the day of testing as well as accurately noting the time gap from the start of meal until actual testing of blood glucose levels, keeping in mind their impact on the dual nature of post-prandial insulin release (rapid but short followed by slow and sustained), is vital to correct reporting. While testing for PP-sugar following a loading dose of 75 gm oral glucose, the possibility of occurrence of "Dumping Syndrome", must be borne in mind, wherein on sudden load of glucose there occurs an abnormal sharp rise of insulin and sudden hypoglycemia. In such cases insulin levels are usually sky-high. There are also instances where an insulin assay is not of much utility even in the setting of severe hypoglycemia. In such cases it is a C-peptide assay which can prove to be the missing link as its levels can clearly establish whether or not the high insulin in circulation is of endogenous or exogenous origin.

Apart from hormonal assays there are numerous instances of lab reports leaving the patient and physician thoroughly high and dry. Be it cases of known diabetics presenting with sudden loss of consciousness expectedly hypoglycaemic, yet on laboratory investigations exhibiting sky-high blood sugar levels and concomitant absurd hyponatremia, or high phosphate levels incompatible with human life which on changing the assay platform from wet to dry chemistry modified itself to normal value, there are no dearth of examples where such dilemmas may be solved by rational thinking and strategic corrective actions. There are also instances which highlight how crucial pre-test counselling of patients is; which may be as simple as advising the patient to refrain from attending gym prior to giving a sample for creatine kinase testing or advising against over-enthusiastic exercising that can lead to potential rhabdomyolysis. Finally, the significance of correlating biochemical test results with haematological parameters like high potassium values with high platelet counts to rule out pseudo-hyperkalaemia cannot be over-emphasized enough.

The message on the wall is thus amply evident that laboratory results and clinical profile may often be in contrast to one another. Yet a keen insight and strategizing with a problem-solving attitude at both ends can often yield the simplest and correct solutions to otherwise perplexing scenarios.

SOCIETY ITALIANO PATOLOGICA MEDICINE LABORATORY
Emergency Laboratory Tests, Process Management and Data Protection

Data Management in Clinical Laboratory Network

Elisabetta Stenner

Clinical Laboratory, Italy

Clinical laboratories are healthcare facilities providing a wide range of laboratory procedures that aid clinicians in diagnosing, treating, and managing patients.

The governmental or private clinical laboratories provide standard diagnostic laboratory tests and, depending on the level of specialization, provide also less commonly used diagnostic and confirmatory tests. Laboratory networks are usually organized as 1. peripheral laboratories that provide routine test within the local community and/or emergency laboratory tests for small local emergency departments, 2. intermediate-level laboratories at the district, provincial, and regional-level facilities and 3. national reference laboratories, also known as the central level, oversee the overall management of the laboratory network and provide a range of routine and specialized laboratory testing, introducing and phasing new diagnostic tests.

Laboratoy networks were of utmost importance to support readiness and response to Sars-CoV-2 and other clinical emergencies as well as to support a high quality level of laboratory service independently from the area where the patients need the service. Laboratoy networks are the base for a real equity of service. Harmonization factor assay-related testing performed in a large laboratory network should be considered and managed appropriately. In this presentation pratical aspects of data management in clinical laboratory network and the North West Tuscany Laboratory Network (Italy) experience will be presented.

SOCIETY ITALIANO PATOLOGICA MEDICINE LABORATORY
Emergency Laboratory Tests, Process Management and Data Protection

Data Management in POCT Network

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In recent years, the field of Medicine has considerably changed. An important, widespread trend is the reorganization of medical laboratories.These facilities made an effort to improve their efficiency through a process of consolidation and decentralization. The pandemic outbreak of SARS-CoV-2 forced to re-think about the use of point-of-care testing (POCT) in settings that are outside of the hospital. The primary care facilities will be the next important field of application for those indispensable tools.

The use of POCT outside of the hospital setting performed by healthcare professionals without formal laboratory education can provide numerous benefifits. However, these benefifits are associated with risks that must be managed, to ensure the provision of reliable test results and minimize harm to the patient. Clinical governance, quality management system and risk assessment are crucial and mandatory.

In this context, the Point-of-care coordinator (POCC) has many responsibilities and one of the most important is operator management. As POCT expands, the coordinator has responsibility for educating, training, and assessing competency for anyone preforming testing outside of the main laboratory. A data manager is essential for the program to successfully track those who are qualified or certified to perform both waived and non-waived testing.

POCT programs can ange in size from a single institution with only glucose devices connected and 50 operators (local network) to a very large integrated delivery network with many institutions, device types, manual tests, and upwards of 5.000 to 10.000 operators (external network).Thus, device connectivity and a robust data manager are imperative for a successful program by providing testing that is safe for patients and helping meet accreditation requirements. Case studies will be prersented.

An other relevant aspect to conside, regards cybersecurity and privacy risks in POCT systems in hospitals and in external networks, thus it is now requested to provide innovative solutions to medical professionals.

POCT systems platforms incorporate devices and applications in order to collect, process and visualize data. Using large amounts of data, with personal health information and sensitive medical data, communicated across various POC systems, backend analytical platforms, user workstations and smartphones, there are multiple threats that may cause data leakages or breach incidents.

In conclusion, POCT plays an increasingly important role in disease management algorithms and in a variety ofsettings inside and outside the hospital. Thus, managing a large POCT network requires a multidimensional approach that addresses challenges related to standardization, quality management, training and competency assessment, data management, and continuing education.
With a robust organizational support system and the implementation of effective strategies, hospitals and decentralized primary care facilities can optimize the quality, reliability, and efficiency of POCT programs, beneficial for patient care and outcomes.

SOCIETY ITALIANO PATOLOGICA MEDICINE LABORATORY
Emergency Laboratory Tests, Process Management and Data Protection

The Laboratory in Clinical Research

Roberto Verna

World Association of Societies of Pathology and Laboratory Medicine

President Elect

Clinical Research is the entire procedure involved into the development of a new drug to its introduction to the market. It starts with the selection of promising candidate molecules then goes on with the pre-clinical or animal studies in order to determine safety, toxicity and efficacy. Pre-clinical studies are conducted by pharmaceutical industries before starting clinical trials on a drug.

Clinical studies are commonly classified into four phases. Each phase of the drug approval process is treated as a separate clinical trial. If the drug successfully passes through Phases I, II, and III, it will be approved by the national regulatory authority for use in the general population. Phase IV is post-approval studies.

The clinical research ecosystem involves a complex network of sites, pharmaceutical companies and academic research institutions. This has led to a growing field of technologies used for managing the data and operational factors of clinical research. Clinical research management is often aided by eClinical systems to help automate the management and conducting of clinical trials.

Of fundamental importance in clinical trials is the assessment of the informed consent. We should not forget that after the approval of a certain drug a great work is due for the market access and, during the clinical trials, the aid of A.I. end of Cybersecurity is relevant. Finally, all the rules for the maintenance of the privacy must be followed and the consulence of appropriate lawyers for the insurance problems..

As until now evidenced, clinical research is a complex world that includes chemists, engineers, biotechnicians, medical doctors, statisticians, but also economists and lawyers.

Every step of the above enumerated points strongly depends on laboratory data. Adverse reactions, deviations from the original protocol are subjected to laboratory results. Every assessment of efficacy or of Adverse reactions, as well as the general behavior of the patient under study, relies on the examination of laboratory data and on their coordination, so that the Clinical Laboratory has a pivotal role in clinical research.

An important example of the role of clinical laboratory is illustrated in the detection of drugs and substances prohibited for doping.

SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Diagnostic Management in Syndromic Multiplex PCR Tests

Evaluation of the Value of Respiratory Syndromic Tests: Experience During the Pandemic

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There are significant improvements in clinical microbiology over the past decade, due to new technologies that have accelerated the diagnosis of infectious diseases. These new technologies include commercial molecular assays that simultaneously detect and identify multiple pathogens associated with clinical syndromes, such as, respiratory, gastrointestinal, bloodstream or central nervous system infections. These multiplex tests enable health care professionals to quickly diagnose infections and decide about hospital admission, isolation, and antimicrobial treatment to be made in a timely manner. Respiratory tract infections are one of the leading causes of morbidity and infectious-disease-related deaths. Among the causative pathogens of pneumonia and respiratory infections, the role of viruses has recently been highlighted by the emergence of syndromic testing.

There are several types of syndromic tests; fully automated tests including extraction and PCR in the same casette/pouch and semi automated tests including manually or in device isolation, master-mix preparation and PCR. Fully automated devices result the tests in two hours, but semi-automated tests result in 4-8 hours. These panels must include most common viruses; influenza A/B viruses, RSV, corona viruses, parainfluenza viruses and include atypical bacteria *Mycoplasma pneumonia* and *Bordetella* spp.

During COVID-19 pandemic respiratory syndromic panels have enabled the management of respiratory infections rapidly and the patients were diagnosed as SARS CoV-2 or other respiratory viruses. Mitigation measures were conducted strictly in the first and second years of pandemic and these measures declined the number of most of the respiratory viruses. Syndromic panels were used for several purposes during the pandemic; diagnosis of respiratory infections, diagnosis and/or confirmation of SARS-CoV-2 cases, epidemiologic data and etc.

Syndromic panel results enables the clinican to decide about the usage of antibiotics and antivirals for the treatment of a respiratory infection. Specific antivirals may be used for treatment of influenza and SARS-CoV-2. Syndromic panels have decreased the usage of antibiotics for the treatment of respiratory infections.

During the pandemics respiratory syndromic tests have enabled to fight against COVID-19 pandemic, determine the epidemiological changes of respiratory viruses, rapid diagnosis and treatment of some viruses and decreased the usage of unnnecessary antibiotics.

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SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Diagnostic Management in Syndromic Multiplex PCR Tests

Antimicrobial Stewardship and Molecular Syndromic Panels

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The ability to simultaneously detect pathogens that cause infectious diseases directly from clinical samples improves patient care, supports hospital infection control, and provides valuable contributions to epidemiological research. Syndromic panels are devices that perform multiplex polymerase chain reaction (PCR) that allows simultaneous detection of multiple pathogens and are becoming increasingly common in the diagnosis of infectious diseases. These panels, which can provide faster and more comprehensive results than traditional diagnostic methods, provide a great advantage in the diagnosis and treatment of diseases such as sepsis, meningitis, respiratory tract infections, and gastrointestinal infections. The speed and accuracy offered by syndromic panels contribute to optimizing antimicrobial management in clinical microbiology.

These panels can simultaneously detect various pathogens such as bacteria, viruses, fungi, and parasites. Multiplex PCRs are generally more sensitive than routine culture or antigen detection. For example, when symptoms of acute respiratory tract infection are observed in a patient, the panel can quickly determine which pathogen is responsible and specific treatment can be started accordingly.

Studies on the effectiveness of syndromic panels show that these panels offer high sensitivity and specificity in the diagnosis of infectious diseases. It has been observed that the syndromic panel used for respiratory tract infections can rapidly detect many viruses such as Influenza A, Influenza B, RSV and Adenovirus, while the panels used in the diagnosis of gastrointestinal infections can identify pathogens such as Rotavirus, Norovirus and Shigella with high accuracy.

Another advantage of syndromic panels is that they do not require the isolation of a specific pathogen, as in traditional diagnostic methods. In traditional methods, a pathogen must first be grown in culture to identify it, and this may not always be possible. However, since syndromic panels directly detect genetic material, they can make a diagnosis without the need for culture. This makes a significant difference, especially in cases requiring rapid intervention.

Despite this, syndromic panels also have some limitations. They are more expensive than traditional culture methods, especially in terms of cost. The pathogens that can be identified are limited; it may not be possible to identify rare pathogens that are not included in the panel. Therefore, additional tests may be required when using syndromic panels.

As a result, syndromic panels offer significant advantages in the diagnosis of infectious diseases. By providing rapid and accurate results, antimicrobial management is optimized and patients are quickly provided with the right treatment. As their clinical use increases, the positive effects of syndromic panels on public health will become even more evident. However, considering the cost and some limitations of these panels, it is important to select the most appropriate diagnostic method in each case.

Heavy Metals and Trace Element Analyses

Critical Health Impacts Analytical Methods and Sample Handling

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Heavy metals and trace elements are widely found in nature and play significant roles in various industrial, agricultural, and health processes. In accordance with the CLSI classification, elements can be categorised as major, trace or ultra-trace. Some have known biochemical functions or beneficial effects in humans, which are classified as 'essential elements'. They play an important role in enzyme activation as coenzymes or prosthetic groups. A deficiency or excess of essential trace elements can alter the concentrations of other elements within the body. To illustrate, low zinc levels result in the accumulation of cadmium within the body, while low selenium levels lead to an increase in the accumulation of arsenic, cadmium and mercury.

Heavy metals, which are relatively dense trace elements, occur naturally in soil, are mined for industrial use and are widely distributed in our environment. Toxic heavy metals can be harmful even at very low concentrations (ppb levels). Arsenic, cadmium, lead and mercury are in the top ten of the CDC's priority list of hazardous substances.

Arsenic exists in two forms: organic and inorganic. Organic arsenic is considered non-toxic and is excreted from the body. The majority of people are exposed to inorganic arsenic through food and drinking water. Arsenic has been linked to a number of health issues, including skin, cardiovascular diseases and type 2 diabetes mellitus. A crucial laboratory test for recent and chronic exposure is urinary arsenic excretion. The primary source of cadmium exposure is smoking. Cadmium is primarily accumulated in the kidneys, and occupational exposure to cadmium can potentially result in renal damage. Mercury has three forms: elemental, inorganic mercury and organic mercury (methylmercury). Most people are exposed to organic mercury compounds in food (such as fish, seafood, rice) or to elemental mercury from dental fillings. Urine specimen is the preferred specimen for elemental mercury. Much of lead comes from human activities including burning fossil fuels, mining, and manufacturing. Lead exposure causes cardiovascular diseases in adults and lost IQ points in children. CDC recommends testing blood for lead exposure in children.

The most common elemental analysis techniques are Flame Atomic Absorption Spectroscopy (FAAS), Graphite AAS (GAAS), Inductively Coupled Plasma (ICP) Optical Emission Spectroscopy and ICP- Mass Spectrometry (ICP-MS). When selecting methods for trace element analysis, we consider factors such as detection limits, analytical range, cost, interferences and ease of use. The advantages of GFAAS are lower instrument cost and reduced maintenance requirements, while ICP-MS is fast analysis speed and the ability to measure a greater number of elements at lower concentrations. Trace element analysis is used in environmental monitoring (soil, seawater, drinking water, air, petroleum) and clinical areas such as occupational exposure, forensic medicine, toxicology and diagnosis of congenital diseases.

Contamination is the main problem in trace element analysis, affecting accuracy and reliability, often due to sample handling, reagents or laboratory equipment. Proper procedures and a clean environment are essential to minimise the risk of contamination.

Heavy Metals and Trace Element Analyses

Environmental Hormone Disruptors and Microplastics

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ABSTRACT
Endocrine Disruptors and Microplastics

The term endocrine disrupting chemicals (EDCs) was first introduced by American biologist Theo Colborn in 1991. In his research on the Great Lakes ecosystem, Colborn found that some chemicals mimicked hormones and disrupted the endocrine system. These findings were discussed at the Wingspread Conference in 1991 and awareness of the effects of endocrine disrupting chemicals increased. Theo Colborn played a pioneering role in understanding the disruptive effects of environmental toxins, particularly on the endocrine system.

An endocrine disruptor is defined by the US Environmental Protection Agency (EPA) as an exogenous agent that interferes with the synthesis, secretion, transport, metabolism, and bioavailability of hormones in the body. EDCs were originally thought to act through nuclear hormone receptors such as estrogen, androgen, thyroid and retinoid receptors. However, further research has shown that these mechanisms are more complex. Endocrine disrupters can act through nuclear and non-nuclear steroid hormone receptors, enzymatic pathways and many other mechanisms. These chemicals can cause human health problems such as reproductive health, obesity, metabolic disorders and thyroid function.

Microplastics are small plastic particles that are formed as a result of the long-term degradation of plastic waste in the environment and spread through various media such as air, water and soil. These particles can enter the human body through water and food and can have potentially toxic effects. Microplastics can carry hormone disrupting compounds on their surface, increasing the exposure of these chemicals to living organisms. Hormone disruptors can cause health problems by interfering with the function of hormones that play critical roles in the body, such as estrogen, testosterone and thyroid hormones. They are particularly associated with reproductive health and metabolic disorders.

Studies have demonstrated the accumulation and inflammatory effects of microplastics in the human body. Animal studies show that microplastics can accumulate in organs and cause

inflammation and oxidative stress. Human studies show that microplastics are found in food sources such as drinking water and seafood, and are passed on to humans in this way. These effects can lead to more serious consequences, especially in children, pregnant women and sensitive individuals.

Microplastics also pose a risk due to their carrier properties; they can adsorb various chemicals (e.g. pesticides and heavy metals) and harm the environment and human health. When ingested by organisms in marine and freshwater ecosystems, these chemicals enter the food chain and eventually reach humans. In particular, in cell culture and animal studies, these compounds have been shown to have estrogenic and antiandrogenic effects and to disrupt hormonal balance.

Analytical detection of microplastics uses different techniques depending on the sample type, particle size and chemical properties.

Filtration and microscopy: Microplastics are separated from water by filtration and analysed by optical microscopy or scanning electron microscopy (SEM).

FTIR and Raman spectroscopy: FTIR and Raman spectroscopy are used to determine the chemical composition of microplastics. Raman is more advantageous for very small particles.

Fluorescence microscopy: Facilitates the detection of microplastics after staining with certain dyes.

Thermal Destruction Analyses (Pyrolysis-GC/MS and TED-GC/MS): Evaluated by gas chromatography using the pyrolysis method to analyse the chemical composition of microplastics.

Density separation: Microplastics are separated and analysed according to their density. These methods are used to determine the quantity, chemical type and structural properties of microplastics.

Environmental and social measures are important to reduce the impact of these chemicals on human health. Reducing plastic consumption, activating recycling processes and controlling the dispersal of microplastics can reduce exposure to these particles. In addition, stricter regulations on the use of endocrine disrupters are also essential to protect public health.

Experts of the Future Discuss – 1
Coagulation Tests

Optical Measurement? Mechanical Measurement?

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Hemostasis takes places in 4 stages when we bleed: 1) Vasoconstriction, 2) Primary hemostasis, 3) Secondary Hemostasis, 4) Fibrinolysis. Coagulation tests are used for the diagnosis of hematologic diseases and monitoring anticoagulant therapies. Due to the increasing demand of routine coagulation parameters such as prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (aPTT) and fibrinogen (Fib), automated coagulation analyzers became an indispensable need for medium and large sized hospital laboratories.

Auto analyzers based on photo-optical and electro-mechanical coagulometer technologies have been widely used in medical laboratories. Analyzers simply activate the coagulation cascade in the plasma by adding reagents and record the clot formation time. Optical method is based on the detection of the change in optical density or turbidity of the sample while the clot formation. Mechanical analysis is based on movement of a metal ball between two magnets or the movement of two probes inserted within the patient sample. Fibrin formation restricts movement of the magnetic ball or movement of the probe, resulting in a finite endpoint, which is recorded as the clotting time. Reaction cuvettes are expensive due to their features and require skillful handling because of the difficulty of loading them into the device.

It has been suggested that mechanical clot detection is not affected by turbid samples and, superior to photo-optical detection which may be affected by turbid samples. However, many comparative studies indicate that results of optical method are equivalent to mechanical method in terms of accuracy, precision and linearity, including turbid samples (1,2). In a comprehensive interference study by Nougier et al. PT and aPTT values were similarly affected by hemolysis and icterus in both optical and mechanical methods (3).

Furthermore, optical measurement provides a clotting wave which enables follow up and interpretion of the reaction. A rare case of dysfibrinogenemia defined by Lefkowitz et al. prolonged PT result only using the optical method while mechanical method gave false negative test result within the normal range. When the researchers examined the clotting waveform, they found that the decrease in aPTT transmittance was also much less than in healthy individuals (4).

A number of studies have been published on prognostic value of critical clinical situations based on clot waveform analysis (CWA) of aPTT test provided by optical method (5). It has been reported that CWA may serve as an inexpensive and easily accessible early marker for sepsis and disseminated intravascular coagulation (DIC) (6,7). In a retrospective study of 214 patients undergoing emergency catheterization due to acute myocardial infarction (AMI), preoperative aPTT based CWA parameters were found to be significantly higher with the development of AMI and its complications compared to the control group (8).

In conclusion, photo-optical coagulation analyzers are cost-effective, highly repeatable, and accurate devices which are poorly affected by interfering factors. It also provides a reaction curve that mechanical devices cannot, which is valuable for reaction monitoring and potential predictor for clinically critical conditions such as DIC, sepsis and AMI.

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URUGUAYAN SOCIETY of CLINICAL PATHOLOGY
The Laboratory as an Ally in the Diagnosis and Monitoring of Frequent Infections in the Transplanted Patient

Role of Fungal Infections in the Transplant Patient

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Fungal infections in transplant patients are a significant concern due to the immunosuppression these patients receive to prevent rejection of the transplant organ. Their high mortality requires special attention.

The epidemiology of fungal infections in transplant patients reveals specific patterns that depend on the type of transplant (Solid Organ Transplant / Hematopoietic Stem Cell Transplant), the patient profile and the characteristics of the hospital environment.

Candida infections are the most common cause of fungal infections in transplant patients, especially Candida albicans and non-albicans species, followed by mold infections of the genus Aspergillus, particularly common in patients with neutropenia and hematopoietic stem cell transplants. Other infections like Cryptococcus are common in this kind of patients.

The laboratory is essential in the management of fungal infections in transplant patients by providing early and accurate diagnostic tools, supporting the selection of appropriate treatment, and monitoring response to treatment. Close collaboration between laboratories and clinical teams is key to the effective management of these infections.

URUGUAYAN SOCIETY of CLINICAL PATHOLOGY
The Laboratory as an Ally in the Diagnosis and Monitoring of Frequent Infections in the Transplanted Patient

Importance of the Laboratory in the Diagnosis and Monitoring of Viral Infections in Kidney Transplanted Patients.

Vania Medina

Hospital de Clinicas - "Dr. Manuel Quintela", Uruguay

This presentation will explore the critical role of laboratory diagnostics in the management of viral infections in kidney transplant patients, focusing on two key pathogens: Cytomegalovirus (CMV) and BK virus. These viruses pose significant risks during the post-transplant period, as immunosuppression increases susceptibility to viral reactivation and infection.

Key topics will include:
- Laboratory methods for the detection and quantification of CMV and BK virus used in Uruguay.
- The importance of early diagnosis and regular monitoring to prevent complications such as graft rejection and organ dysfunction.
- The role of molecular techniques, such as PCR, in tracking viral load and guiding antiviral treatment.

The presentation will highlight how timely and accurate laboratory results are essential for optimizing patient outcomes and reducing long-term risks associated with these infections.

URUGUAYAN SOCIETY of CLINICAL PATHOLOGY
The Laboratory as an Ally in the Diagnosis and Monitoring of Frequent Infections in the Transplanted Patient

Impact of the Antiviral Resistance Study on CMV Infection

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The impact of the antiviral resistance study on CMV infection is a critical topic, especially in the context of immunocompromised patients, such as transplant recipients, where CMV poses a significant threat. The widespread use of antiviral drugs like ganciclovir has led to the emergence of resistant CMV strains, complicating treatment and increasing the risk of morbidity and mortality. This study focused on detecting resistance mutations in CMV, particularly through molecular methods like sequencing the UL97 and UL54 genes. The findings revealed a high prevalence of resistance, with common mutations significantly affecting the efficacy of available antiviral treatments. These results have had a substantial clinical impact, leading to the implementation of preemptive monitoring strategies to detect resistance early and adapt treatment regimens accordingly. By tailoring antiviral therapies to the resistance profiles of individual patients, healthcare providers can significantly improve outcomes and reduce complications. The study underscores the vital role of the laboratory in diagnosing and monitoring infections, guiding personalized treatment, and informing future research on the development of new antiviral agents to combat resistant CMV strains.

SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Antimicrobial Stewardship and Infection Control: Inseparable Trio

The Role of Microbiologist in Antimicrobial Stewardship

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The discovery and commercialization of antibiotics has been one of the most important developments in modern medicine. Antibiotic resistance—the ability of microbes to overcome drugs designed to kill them—is one of the greatest global public health problems of our time.

Overuse and inappropriate use of antibiotics has led to the rapid emergence of multidrug-resistant pathogens, limiting treatment options for serious infections and increasing morbidity, mortality, length of hospital stay, and healthcare costs. Despite advances in healthcare and infection control measures today, healthcare-associated infections (HAIs), formerly known as hospital infections, continue to be relevant.

HAIs are infections that develop directly or indirectly in relation to healthcare. In addition to their incidence, mortality, impact on quality of life, cost, contribution to the development of antibiotic resistance, and additional healthcare burden, they pose a constant threat to the safe and effective operation of healthcare systems and are a global public health problem with their legal dimension. With the shift from the concept of hospital infections to healthcare-associated infections, outpatient healthcare services such as oral and dental health centers, hemodialysis centers, healthcare services provided in nursing homes, and home healthcare services are also included.

Antimicrobial stewardship can be defined as a set of interventions to promote and ensure the optimal use of antimicrobial therapy that "results in the best clinical outcome for the treatment or prevention of infection with minimal toxicity to the patient and minimal impact on subsequent resistance." The antimicrobial stewardship team consists of at least one clinical microbiologist, one infectious disease specialist, and one clinical pharmacist.

The microbiology laboratory plays an important role in antimicrobial stewardship, which aims to optimize antibiotic prescribing to improve patient outcomes, minimize potential toxicity, prevent the emergence of resistance, and reduce healthcare costs.

The microbiology laboratory also plays an important role in the treatment, control, and prevention of HAIs, as well as serving as a surveillance and early warning system. It is involved in the detection and investigation of outbreaks. Unusual events or trends (such as the emergence of clusters or the emergence of multidrug-resistant organisms) are often first detected by the laboratory. Comparison of epidemiologically related isolates ("typing" or "fingerprinting") helps determine whether these organisms are related and is therefore necessary to confirm the existence of an outbreak.

Today, antimicrobial stewardship is one of the three "pillars" of an integrated approach to strengthening health systems. The other two are infection prevention and control and medicine and patient safety. Infection prevention and control is a universally relevant component of all health systems, affecting the health and safety of both those who use services and those who provide them.

The microbiologist is a permanent and active member of the infection control committee and antimicrobial stewardship team. The quality of infection control measures is also an indicator of the quality of healthcare in general. Since most infection control and antimicrobial stewardship programs are based on microbiological results, quality assurance is an important issue.

Consequently, diagnostic management is an integral part of antimicrobial stewardship programs and is also essential for infection prevention and control activities in healthcare facilities. Timely and accurate microbiological results help clinicians select the most appropriate antibiotics or combinations of antibiotics for their patients and also take the necessary precautions to reduce the risk of transmission and prevent pathogen-related outbreaks in healthcare facilities.

SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Antimicrobial Stewardship and Infection Control: Inseparable Trio

Diagnostic stewardship

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The mission of microbiology laboratories is to identify the infection agent and to determine antimicrobial sensitivity1. A quiet microbiological revolution has been taking place over the last 30 years1. While developing new methods, it is aimed to be faster, more accurate, more sensitive, more easily interpretable, more suitable for automation and more efficient1.
Microbiological diagnosis should be made as soon as possible to reduce antibiotic use and initiate targeted treatment rather than empirical1. In the identification process using traditional methods, there must be visible growth, and for antimicrobial susceptibility testing, inhibition of growth must be observed2. However, these processes are time consuming2. In addition, traditional methods are optimized for a limited number of common microorganisms and not all infectious agents can be produced3.
With conventional methods, antimicrobial susceptibility testing in patients with sepsis can be completed after 48 hours at the earliest. However, delay in antimicrobial management in patients with sepsis; reduces survival chance by 7,6% per hour after hour six4.

Antimicrobial susceptibility tests can be classified as follows1;
1. Phenotypic methods; dilution, diffusion, gradient tests, chromogenic media, automated systems
2. Mass spectrometry; Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS)
3. Molecular based methods; genetic methods (amplification-based methods, hybridization-based methods) genomic methods (sequence-based methods)

The advantages and disadvantages of these tests can be listed as follows[1]

Method	Result time	Advantages	Disadvantages
Broth Dilution	48 hours	Well-standardised Commercially available tests are easy to perform	Time-consuming İndividual mistake
Agar Dilution	48 hours	Well-standardised Suitable for testing a large number of isolates	Time-consuming Limited concentration of antimicrobial agents
Disc Diffusion	48 hours	Simple to perform Low cost Simple and fast interpretation The high number of test antibiotics per test High flexibility in antibiotic selection Detection of resistance patterns Mass use and the possibility of automatisation A number of a different use (AST, identification, screening, etc) Detection of heteroresistant population or contamination	Time-consuming No MIC value The inability for some antibiotics to be tested
Gradient tests	48 hours	Convenient and flexible Simple to perform Does not require expertise Detection of resistance patterns	Relatively expensive Relatively long incubation
Automated systems	>20 hours	Simple to perform	Relatively expensive
Chromogenic media	24 hours	Mass use and the possibility automatisation Simple to perform Simple and fast interpretation	Not completely susceptible and specific Time-consuming Limited spectra or single antibiotic Relatively expensive Screening only or required confirmatory identification No MIC value
MALDI-TOF MS	A few hours	Rapid turnaround time Simple to perform Low sample volüme requirements Low per-sample costs	High cost of the MALDI-TOF MS Need further optimisation for each species and antibiotic combination No MIC value
Genetic met hods	24-48 hours	Rapid High accurate Sensitive Reproducible Increased ability to detect slow-growing or non-cultivable organisms	Limited spectra Limited throughput High cost
Genomic methods	A few days	High accurate Sensitive Increased ability to detect slow-growing or non-cultivable organisms	High cost Time-consuming Challenging interpretation of results

The traditional methods used for the detection of carbapenemase presence have a turnaround time of 48 hours, and CarpaNP, Blue-Carba, ß-Carba, Lateral flow and Carbapenem inactivation methods have been developed to shorten the time5.

However, there are two important questions to be asked. These are;
- Does resistance always predict failure?
- Does sensitivity always indicate a positive response to treatment?

Antimicrobial susceptibility tests are performed in vitro. However, many factors called pharmacodynamic (such as concentration-dependent killing, time-dependent killing) and pharmacokinetic (such as absorption, distribution, excretion of the drug) factors in the body may affect the antimicrobial-microorganism relationship6. In addition, the presence/absence of virulence factors of the bacteria may also affect this relationship. Microbiological studies using chip organ technology are promising in this field7.

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CHINESE SOCIETY of LABORATORY MEDICINE
Advancements in Tumor Biomarker Research and Clinical Applications

Applications of DNA Methylation Biomarkers in Tumor Diagnosis

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DNA methylation is the most extensively studied and abundant epigenetic modification within the genome. Frequently observed in various malignant tumors, it often emerges at early stages and is considered an ideal biomarker for early tumor diagnosis. Therefore, the relationship between DNA methylation and tumors was explored, detailing tumor-specific methylation patterns and their mechanisms. Recent advancements in DNA methylation biomarker research were then reviewed, emphasizing four main advantages: characteristic, sample, technical, and application benefits. Additionally, the applications and challenges of DNA methylation biomarker in screening and diagnosing single and multiple cancer types were discussed. Finally, to address current challenges, we explored methylation biomarkers in peripheral blood mononuclear cells (PBMCs) with a focus on changes in immune cells. The methylation arrays were utilized to profile the genome-wide DNA methylation of PBMCs from patients with malignancies like colorectal, breast, lung, and gastric cancers. Multiple machine learning algorithms and methylation detection methods were used to screen and validate methylation biomarkers, constructing a multiplex methylation early diagnosis model. This model was validated in multi-center cohorts, demonstrating application value with sensitivity and specificity both exceeding 90%. Moreover, to tackle clinical challenges like distinguishing benign from malignant lung nodules and assessing early gastric cancer vascular invasion, a PBMCs methylation-based diagnostic model for lung nodule differentiation and a gastric juice-based methylation assessment model for early gastric cancer vascular invasion were established, aiming to provide more precise and effective diagnostic and treatment options for patients.

CHINESE SOCIETY of LABORATORY MEDICINE
Advancements in Tumor Biomarker Research and Clinical Applications

The quality Improvement Effect of EQA/PT On Clinical Applications of Next Generation Sequencing (NGS) of Tumor Gene Mutation

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The detection of tumor gene mutations is crucial for determining appropriate targeted or immunotherapy treatments for cancer patients. Common methods used in clinical laboratories include real-time fluorescence PCR, fluorescence in situ hybridization (FISH), and next-generation sequencing (NGS). NGS technology allows for the simultaneous detection of various mutation types, including SNVs, Indels, CNVs, and SVs. However, the complexity of the NGS process increases the risk of false positives and false negatives. Continuous participation in EQA/PT can help clinical laboratories identify and address the causes of these errors. This study introduces the development of NGS-based EQA/PT programs for detecting tumor gene mutations in tissue and plasma samples, organized by the National Center for Clinical Laboratories (NCCL), and examines their role in improving the quality of clinical laboratory practices in China.

CHINESE SOCIETY of LABORATORY MEDICINE
Advancements in Tumor Biomarker Research and Clinical Applications

Intra-Tumor Microbiome Associated with the Infiltration of Cytotoxic CD8+ T Cells and the Patient's Response to Immunotherapy

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Gut microbiome plays an important role in systemic inflammation and immune response. Microbes can translocate and reside in tumor niches. However, it is unclear how intra-tumor microbiome affects immunity in human cancer. The purpose of the study was to investigate the association between intra-tumor bacteria, infiltrating CD8+ T cells and patient's response to immunotherpy taking cutaneous melanoma as the subject. A TCGA cutaneous melanoma RNA-seq data was using, intra-tumor bacteria and infiltrating CD8+ T cells were determined. Correlation between intra-tumor bacteria and infiltrating CD8+ T cells or chemokine gene expression, and survival analysis of infiltrating CD8+ T cells and *Lachnoclostridium* in cutaneous melanoma were performed. In light of the achievements of other research teams in the recent years, it can be concluded that the intra-tumor-residing gut microbiota could modulate chemokine levels and affect CD8+ T cell infiltration, consequently influencing patient survival in cutaneous melanoma. Manipulating intra-tumor gut microbiome may benefit patient outcome for those with immunotherapy.

Keywords : CD8+ T cells, gut microbiome, intra-tumor bacteria, melanoma

JAPANESE SOCIETY of LABORATORY MEDICINE
Novel Perspective and Recent Progress in Laboratory Medicine

Urinary Biomarker Hunting by LC/MS-omics. Example of Food Allergy

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Urine samples are collected without invasion and easily handled for evaluation of reno-ureter-bladder diseases. Recent advances in molecular biomarker, microRNA, microsome and DNA in the urine are used as biomarkers of non-communicable diseases such as bladder cancer, hypertension and diabetes. However, the evaluation of those markers are expensive and not be able to be performed all over the world. We used LC/MS to screen biomarkers for non-communicable diseases with low cost and found a biomarker for food allergy in children. In this symposium I will discuss the current status of research interests in hunting new biomarkers in urine for non-communicable disease and future direction.

JAPANESE SOCIETY of LABORATORY MEDICINE
Novel Perspective and Recent Progress in Laboratory Medicine

The Application of Mass Spectrometry to Newborn Screening and Diagnosis of Inherited Metabolic Diseases in Japan

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Newborn screening (NBS), introduced nationwide in 1977, is one of Japan's most successful public health programs. A key aspect is the analysis of diagnostic markers in dried blood spots collected on filter paper at birth. In Japan, NBS is a local government program, with the test cost covered by the local government. In Japan, the target conditions included four inborn errors of metabolism (IEM) and two congenital endocrine disorders. NBS using tandem mass spectrometry (TMS) in Japan began as a pilot study in 1997 and was introduced nationwide between 2011 and 2014. TMS is currently being carried out nationwide. The adoption of TMS expanded the number of target conditions of NBS to 20. Although TMS uses the flow injection method without a separation column attached to the tandem mass spectrometer, the LC-MS/MS method with a separation column is employed for diagnostic purposes in TMS-positive cases and for follow- up testing of patients with IEM. Conversely, urine organic acid analysis using gas chromatography/mass spectrometry, which has been used for some time, is necessary for the chemical diagnosis of organic acid disorders, such as methylmalonic acidemia and isovaleric acidemia. Recently, effective treatments and diagnostic methods have been developed for several diseases that were previously difficult to diagnose and treat, and the initiation of NBS as a public health program is anticipated. In Japan, this is referred to as the "newly expanded-newborn screening " (NE-NBS). This test is now being implemented nationwide as an optional fee-paying test. The Tokyo Metropolitan Government and a few local governments have initiated NBS for primary immunodeficiency and spinal muscular atrophy using quantitative polymerase chain reaction as a publicly funded test. Furthermore, we reviewed and restructured the referral and follow-up system for NBS-positive cases in the Tokyo Metropolitan Area and established the "Tokyo Consortium for Newborn Screening," a new initiative to build a more sophisticated system. In NE-NBS, mass spectrometers are used to measure enzyme activity levels and quantify the accumulation of substances for a different set of conditions, including lysosomal storage diseases. This presentation discusses the application of mass spectrometry in NBS.

Keywords: Newborn screening, inborn errors of metabolism, LC-MS/MS, Tokyo Consortium for Newborn Screening

JAPANESE SOCIETY of LABORATORY MEDICINE
Novel Perspective and Recent Progress in Laboratory Medicine

The experience with multi-gene panel testing (MGPT) developed at Chiba University Hospital, JAPAN, and its External Quality Assessment

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Background: Recent cancer gene panel (CGP) testing sometimes reports candidates of pathogenic variants of hereditary cancer genes. In those situations, multi-gene panel testing (MGPT) is beneficial for the diagnosis of hereditary cancers. However, MGPT is not covered by health insurance system in Japan and is generally expensive for clients/patients, so few patients undergo the testing. The germline variants of proband are required for the surveillance of asymptomatic individuals. In order to improve this situation, Chiba University Hospital in Japan (hereafter referred to as our hospital) has been conducting MGPT of 29 genes (coding regions of APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53, and VHL) that cause hereditary tumors since 2018, setting an in-house fee (50,000 yen, approximately 333 dollars, for self-paid testing) and in collaboration with the Department of Clinical Genetics in our institute. Here, we report our experience with this test and the importance of quality control, especially external quality assessment (EQA).

Method: Genetic analysis of hereditary tumors (next-generation sequencing) Panel: AmpliSeq for Illumina On-Demand panel (Illumina). Sequencer: iSeq 100 instrument (Illumina), Data analysis: Local Run Manager (Reference genome: hg19, Illumina), mRNA analysis (RT-PCR/Sanger sequencing). Sequencer: Small capillary electrophoresis sequencer DS3000 (Hitachi-hightech), Data analysis: Mutation Surveyor (SoftGenetics), Base sequence analysis (massively parallel sequencing, reference sequence: hg19) (Our hospital's testing department is ISO 15189 certified, and external quality control is subject to CAP survey. TAT is about 3 weeks). Usage experience: So far, on August 2024, 60 cases have been tested, and 20 cases have been confirmed to have pathogenic variants in germline genes of hereditary tumors. In some cases, CGP testing confirmed pathogenic variants in two germline genes, ATM and BRCA2, in alleles derived from one parent. Variants that affect splicing were confirmed to have changes in peripheral blood-derived mRNA alternative splicing as expected. When conducting MGPT testing as a clinical test, EQA surveys are also important.

Results and discussion: A challenge is to establish a PT/EQA system for gene-related tests, including MGPT, in Japan, that is, a third-party certification organization. Sharing information about at-risk family members of probands is a future challenge. In Japan, MGPT is generally only performed at a limited number of medical institutions with a well-established genetic counseling system. Therefore, in order to make MGPT available to hereditary patients who need it, our facility has begun accepting tests and conducting doctor-to-doctor-with-client remote genetic counseling using Microsoft Teams around Japan. It is expected that MGPT will become a common testing method in cancer genomic medicine in the future as it is covered by public health insurance.

Keywords: cancer gene panel (CGP) testing, MGPT, EQA, hereditary cancer

JAPANESE SOCIETY of LABORATORY MEDICINE
Novel Perspective and Recent Progress in Laboratory Medicine

Early diagnosis and early treatment for neonatal genetic diseases

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Newborn screening (NBS) is one of the healthcare initiatives that identifies conditions that can affect children's lives and prevent the occurrence of failures caused by diseases. The primary goals of NBS are early detection of inborn errors of metabolisms immediately after birth and before occurrence of disabilities by their disease condition. Earlier diagnosis of the disease allows earlier clinical intervention, which leads to preventing disease-related disability. Conventional analyses for newborn screening have been used as tools for some conditions such as phenylketonuria and G6P deficiency. Currently, it is being adapted to other conditions which have been thought that the conditions are not suitable for NBS. Lysosomal storage diseases (LSDs) may be eligible for expanded NBS because effective treatments have been known and could improve prognoses of affected children. Expanded NBS would be made early detection of patients with LSDs. While the importance of expanded NBS has been reported, but the significance and aim of NBS are not known to the parents who have babies, and various concerns have been raised. Analyses of NBS are carried out with the consent of the parents. However, most of the parents have very little knowledge of the conditions of newborns before their perinatal period. Parents must understand the conditions and the necessity of the tests for their newborn child, and they also have to make decisions on tests and learn what the test results mean. Therefore, there is also concern that the results may place a psychological burden on the family. The result of NBS is not mean to final diagnosis of the disease. There are always false positives in screening tests. This is because all of the children with targeted diseases must be found by NBS, no matter how low the specificity of the tests. Families who are receiving reports of suspicion and waiting for the results of diagnostic tests, even if the diagnosis is ultimately rejected, are said to be causing known situations such as Vulnerable Child Syndrome. Many ethical issues are inherent in the selection of target diseases and the implementation of screening tests for them, which careful handling and continued discussion are needed in the future. The development of testing techniques such as LC-MS has increased the accuracy of tests or allowed for more disease-specific studies. I will show some of the initiatives being considered to improve the accuracy of tests and realize expanded newborn screening, including our experiences. We believe that these new methods will help solve the ethical problems of NBS. We are developing several methods as secondary screening tests after NBS.

Keywords: Newborn Screening, lysosomal strage disease

ASSOCIATION of CLINICAL CHEMISTRY & LAB MEDICINE PRACTITIONER

The Laboratory and the Clinic Manning a Single Boat: How to Ensure a Smooth Sailing

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(Clinicians vs Laboratorians : Conflict or Collaboration?)

Both the clinicians and the laboratorians share a common goal, i.e optimal patient care. The benefits of such collaboration are Improved patient outcomes by working together ensuring that, diagnostic tests are accurately interpreted and right treatment is administered promptly to the patients.

Regular interactions by both the parties fosters better understanding and communication, reducing the chance of errors and misunderstandings. Collaborations between two streamlines the processes reducing duplication of efforts and efficient workflow of patient care.

By shared expertise by both, Clinicians can benefit from the technical expertise of labs and at the same time lab professionals can gain insights into clinical implications leading to more informed decision making. Joint efforts can also lead to innovative approaches in diagnostics and treatment, as well as collaborative research opportunities which can enhance medical knowledge.

Most importantly to bring forth an efficient collaboration between the two will definitely reduce unnecessary tests and procedures leading to cost saving healthcare facilities without compromising the quality of treatment.

Effective collaboration between both can be challenging due to several factors like communication barriers leading to misinterpretations, time constraints due to tight schedules, different priorities and approaches of both the groups, lack of understanding between the two with respect to complexities of laboratory processes which are not clearly understood or accepted by clinicians or the laboratorians not being aware of the clinical context and urgency. At times there may be delays and errors in data sharing also due to technological challenges. Ensuring compliance with regulations and standards can also hinder smooth collaboration between the two , last but not the least , resource limitations such as staff and funding can also strain the ability to collaborate effectively.

Hence, outcome based approach for both laboratorians and Clinicians is of paramount importance that links processes to accurate results leading to timely diagnosis, supporting diagnostic excellence.

CONFERENCE
Inherited Metabolic Disease

Laboratory in the Diagnosis and Follow-up of Hereditary Metabolic Diseases

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The increase in the amount of data produced by humans is raising the need for specialization on one hand, while narrowing the scope of specialized fields and deepening them on the other. At this point, one of the important sub-specialties in clinical laboratory medicine is the laboratory diagnosis of "Hereditary Metabolic Diseases" (1).

Esoteric tests are a crucial part of developments in clinical laboratories. Topics such as molecular genetic analyses, advanced immunological tests, pharmacogenetic tests, and specific biomarker analyses for diagnosing rare diseases fall under the scope of esoteric tests. They play a critical role in diagnosing hereditary metabolic disorders, which are challenging to diagnose. Enzyme analyses, biomarkers, drug levels, drug antibodies, and molecular analyses are commonly used tests in the field of hereditary metabolic disorders. To perform these tests, advanced and complex methods are required, including chromatographic systems, fluorimeters, electrophoresis, and blot analyses, which are not available in automated large laboratories today.

The more widely a test is used, the more data and experience accumulate but this is not as easy as it sounds for esoteric tests. These tests typically apply to a limited number of patients and are performed in a limited number of clinical laboratories. Scientific data on analytical and clinical performance is restricted, as is laboratory experience. They require advanced and unusual technology, and clinicians have limited information for ordering these tests. Additionally, both laboratories and clinicians have limited knowledge and experience in evaluating results and making decisions.

Limited information available on the analytical and clinical performance of esoteric tests is another problem. It is challenging to find calibrators, control materials, and external quality control programs for these tests. Scientific data on clinical sensitivity, specificity, positive and negative predictive values, reference ranges, and disease thresholds is limited. Furthermore, the number of laboratories conducting these tests worldwide is also restricted, and harmonization between these laboratories is often insufficient. Regulations such as the IVDR (EU) and FDA-Medical Devices-Laboratory Developed Tests (USA) are expected to fundamentally alter the operations of clinical laboratories and have a significant impact on the use of esoteric tests starting from 2025 and beyond (2, 3)

Even if all these challenges are overcome, achieving full benefit for patients through the implementation of tests may still be difficult. Evaluating the results of esoteric tests is also an important issue. Nowadays, all laboratory guidelines emphasize that test results for hereditary metabolic disorders should be interpreted by clinical laboratories (e.g., Amino acid, organic acid, acylcarnitine profile, etc.) (4, 5, 6, 7).

In conclusion, all current and future tests used in the diagnosis of hereditary metabolic disorders are esoteric tests and are directly related to the points mentioned above. Every day, new diseases, treatments, and tests are defined in the field of metabolic disorders. Indeed, due to the high diagnostic demand and patient needs related to hereditary metabolic disorders, clinical laboratories specializing in these conditions have emerged and become more efficient. Moreover, the demand for these laboratories continues to increase.

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CONFERENCE
Inherited Metabolic Disease

Clinical Approach to Inherited Metabolic Diseases

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Inborn errors of metabolism (IEM) are inherited single gene disorders which result from the deficiency or the abnormality of an enzyme, its cofactor or a transporter resulting in accumulation of a substrate or the deficiency of the product. The first description of these disorders was made by Sir Archibald Garrod in 1908. Although individually rare, IEM collectively constitute a significant proportion of genetic disorders in childhood and are also significant causes of morbidity and mortality in children. There have been significant progress for the diagnosis and treatment of IEM especially during the last decade and early diagnosis is very important to prevent mortality and morbidity in untreated cases.

IEM are generally inherited as autosomal recessive although dominant and X-linked types of inheritance are also possible. A group of IEM that involve biochemical pathways in mitochondria also results from the mutations in mitochondrial DNA. The genetic defects include point mutations, deletions, insertions, or chromosomal rearrangements that result in loss- or gain-of-function of mutant enzymes or transporters. The blockage of the relevant biochemical pathway resulting from the genetic defect causes accumulation of the substrate which may directly show toxic effects or indirectly by the diversion of the metabolic flux to other pathways, as well as the deficiency of the product There have been various classifications for IEM based on the substrate accumulated, the group of enzymes affected, and the organelle that has the defective pathway or the clinical presentation.

A widely accepted classification has been put forward by the Society for the Study of Inborn Errors of Metabolism (SSIEM) that focuses on the main substrate which is effected Inborn errors can present with a wide spectrum of clinical signs and symptoms at any age. Mainly the signs and symptoms resulting from IEM can be divided into the early-onset and late-onset forms. Disorders such as non-ketotic hyperglycinemia, glutaric aciduria type II, and some of the mitochondrial diseases, cobalamin and urea cycle defects (UCDs) are known to begin during the prenatal period. Also, some of the LSD may present as nonimmune hydrops fetalis. The patients who have higher residual enzyme activity generally present later in life. The presentation may be seen in childhood, adolescence, or even during adulthood. The clinical picture is generally made up of recurrent attacks during the chronic progressive course. Initiation of the attacks may be triggered by fever, excessive intake of the substrate by the diet, excessive fasting, and by concomitant medications.

In general, a series of special investigations are needed for the definite diagnosis of IEM. A stepwise approach is preferred especially in patients presenting with nonspecific signs and symptoms, where as a more targeted approach can be used for the patients presenting with a more specific clinical picture.

CONFERENCE
Laboratory in Substance Analyses

The New Era of Psychedelics and the Role of Laboratory Medicine

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Psychedelics—derived from the Greek words *psychē* (mind) and *dēlos* (manifesting)—are consciousness-altering substances. They can be categorized into naturally occurring and synthetic compounds, each with unique chemical structures, such as ergoline alkaloids and tryptamines. While naturally-occurring-psychedelics have been used for healing and spiritual purposes for centuries, modern scientific interest began with the synthesis of LSD and Albert Hofmann's description of its effects in 1943.

The first phase of psychedelic research in the 1950s and 60s faced criticism regarding scientific rigor and coincided with the counterculture movement, leading to restrictions under the United Nations' 1971 treaty, "The Convention on Psychotropic Substances." After a 30-year hiatus, a new wave of research emerged, spearheaded by reputable academic institutions like Johns Hopkins University and Imperial College, along with non-profit organizations such as the Multidisciplinary Association for Psychedelic Studies (MAPS). Today, numerous research centers and over 50 scientific groups globally are investigating psychedelics.

Current research explores the healing potential of psychedelics across various diseases, particularly mental health disorders. Since 2000, the number of scientific papers published on this topic has increased significantly, with a 5-fold rise in publications and a 20-fold increase in clinical trials over the past two decades. Some clinical trials have reached Phase 3, suggesting that regulatory approval from organizations such as the FDA and EMA for treating conditions like treatment-resistant depression and post-traumatic stress disorder may soon be within reach.

The mechanisms of action of psychedelics have garnered considerable interest, though they remain only partially understood. Key findings indicate that 5HT-2A receptors play a crucial role in the effects of tryptamine psychedelics. Research is ongoing into downstream signaling pathways (biased agonism) and the spatial location of 5HT-2A receptors (location bias) to dissect the various effects of psychedelics, such as their psychedelic, psychoplastogenic, and therapeutic properties. Additionally, alterations in the default mode network and other brain regions under the influence of psychedelics are being studied, with researchers exploring whether these changes contribute to the effects of psychedelics or are merely byproducts.

Another promising theory posits that psychedelics may reopen developmental critical periods for a limited time, creating a window of opportunities to reshape unhealthy neural patterns.

Despite the growing interest in psychedelics, a formal role for clinical laboratories in this field has yet to be established. However, the integration of psychedelics into medical practice presents both challenges and opportunities for medical laboratories. Currently, some countries screen for psychedelics in drug abuse testing, which may require reevaluation. The medical use of psychedelics also raises questions about potential interferences with existing laboratory testing methods. Furthermore, the unpredictable nature of psychedelic experiences and their varying effects between individuals highlight the need for personalized treatment protocols. Identifying and utilizing predictive biomarkers related to the effects of psychedelics could help tailor these treatments.

In conclusion, a group of psychoactive substances known for their mind-altering effects is emerging as potential tools for treating a range of mental health disorders within modern medicine. As in all areas of healthcare, clinical laboratories will be creating value for patients by actively engaging in this evolving field.

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Drug and Toxicology

Preanalytic Factors Affecting Immunosuppressive Drug Monitoring

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Introduction

Immunosuppressive drugs (ISD) are critical in managing organ transplants, autoimmune diseases, and severe allergic reactions by reducing the immune system's activity. These drugs are also sometimes used in cancer treatment. The main classes include corticosteroids, calcineurin inhibitors, antiproliferative agents, mTOR inhibitors, and biologics. Immunosuppressant drugs typically include cyclosporine (Cys), tacrolimus (TAC), mycophenolic acid (MPA), sirolimus, and everolimus. While highly effective these medications significantly increase the risk of infections, making ongoing medical supervision essential.

Treatment with ISD requires careful consideration of several important factors. Precise dosing and timing are crucial to achieving effective immunosuppression while minimizing the risk of toxicity. Additionally, patient-specific factors such as age, weight, genetic profile, and organ function can influence how these drugs are metabolized, often necessitating dose adjustments. Interactions with other medications and foods can also impact the safety and efficacy of the treatment, highlighting the importance of following specific guidelines. Patient adherence to the prescribed regimen is vital, as missed doses can lead to serious complications such as organ rejection or uncontrolled autoimmune activity. Due to the increased risk of infections caused by immune suppression, regular monitoring and preventive care are necessary. Long-term use of immunosuppressive drugs can result in severe side effects, including infections, cancer, and organ damage, which underscores the importance of continuous monitoring throughout the treatment process. Therefore, an individualized treatment plan combined with regular laboratory assessments is essential for optimizing patient outcomes and minimizing potential risks. This chapter discusses the preanalytical effects of immunosuppressive drug assays.

The mechanism of action of immunosuppressive drugs

TAC, formerly known as FK-506, is a macrolide antibiotic isolated from the species Streptomyces tsukubaensis and has significant immunosuppressive properties. Cyclosporine is a cyclic peptide composed of 11 amino acids isolated from the fungus Trichoderma polysporum. Although TAC and Cys are chemically different, their mechanisms of action are much the same. Both drugs bind to cytoplasmic peptidyl-prolyl isomerases that are abundant in all tissues. While Cys binds to cyclophilin, TAC binds to the immunophilin FK-binding protein (FKBP). Both complexes, Cys-cyclophilin A and TAC– FK506 binding protein, inhibit calcineurin, which is necessary for the Nuclear Factor of Activated T cells (NFAT) activation. Tacrolimus is used for the same indications as cyclosporine.

Sirolimus and everolimus are drugs that share similar mechanisms of action and are primarily used to suppress the immune system. Both are mTOR (mammalian target of rapamycin) inhibitors, which means they inhibit the protein mTOR that plays a crucial role in cell growth, proliferation, and survival. Although the drugs have the same mechanism of action, their pharmacokinetic properties are different. for example, everolimus has a shorter half-life than sirolimus. Everolimus is used not only to prevent organ rejection but also in the treatment of certain cancers, such as renal cell carcinoma and breast cancer.

MPA is the active form of the immunosuppressive drug mycophenolate mofetil, and it works by inhibiting an important enzyme involved in the proliferation of immune cells. MPA selectively inhibits the enzyme inosine monophosphate dehydrogenase (IMPDH), which is crucial for the de novo synthesis of guanine nucleotides. Guanine nucleotides are essential for DNA and RNA synthesis, and thus for the proliferation of cells, particularly lymphocytes (T and B cells).

Preanalytical conditions

The accuracy and reliability of (ISD) measurements can be significantly affected by preanalytical conditions. The timing of sample collection is crucial because drug levels fluctuate throughout the day, and improper timing can lead to inaccurate results Understanding pharmacokinetics is particularly important for drugs with a narrow therapeutic range, as the timing of blood sample collection can significantly impact results. Sampling during steady-state, usually after more than four doses, is also critical for accurate monitoring. The type of sample collected, such as whole blood, serum, or plasma, also impacts measurement outcomes, with whole blood often preferred for immunosuppressive drugs. Proper sample storage is essential to maintain drug stability, as incorrect storage temperatures can cause erroneous results. Transportation conditions further influence drug levels, especially if samples are exposed to extreme temperatures. Hemolysis, the breakdown of red blood cells, can falsely elevate drug levels, while lipemia and icterus can interfere with certain analytical methods. Different devices and methods (such as immunoassays or mass spectrometry) may produce varying results. Mass spectrometry is more precise, but it can still be affected by preanalytical errors.

Dried blood samples(DBSs) could allow patients to prepare their samples at home. Blood spot volume, blood spot inhomogeneity, stability of analytes in DBS, and hematocrit (Hct) are considered important DBS-related preanalytical factors.

Patient conditions, including underlying health issues and concurrent medications, can significantly affect drug metabolism and should be considered when interpreting results. Pregnancy is another important factor affecting ISD concentrations. Plasma protein levels decrease during pregnancy. Accordingly, the free form of the drugs may increase. The recommended frequency of follow-up is before pregnancy, at the beginning of each trimester, and every month in the 3rd trimester. In the postpartum period, analysis is performed once a week or every two weeks.

Tacrolimus and preanalytical factors

The timing of sample collection is crucial for accurate TAC level measurement, as the drug's blood levels vary depending on the time since the last dose. It is recommended to take the blood sample 12 hours after the last dose or just before the next dose (trough levels). Incorrect timing can lead to falsely high or low Tacrolimus levels. Tube type is critical in Tacrolimus measurement, with EDTA tubes being recommended. Using incorrect tubes (such as those with citrate or heparin) can affect the accuracy of the results. Additionally, errors during blood collection, like insufficient filling of the tube or prolonged use of a tourniquet, can impact the quality of the sample and thus affect TAC levels. Using serum or plasma instead of whole blood may produce different results, increasing the risk of measurement errors. Additionally, since TAC is found in red blood cells, the patient's hematocrit level can influence the measured levels. Abnormal hematocrit levels, whether too low or too high, can lead to inaccurate results. The choice of laboratory equipment and methodology also plays a significant role. For example, cross-reactivity assessments for the most abundant metabolites of tacrolimus have shown that the M2 (31-desmethyl tacrolimus) and M3 (15-desmethyl tacrolimus) metabolites exhibit up to 80% cross-reactivity in some immunoassays. Patient-specific factors are equally important. TAC is metabolized by the CYP3A4 enzyme system, so other medications that influence this enzyme can alter TAC levels. CYP3A4 inhibitors can increase TAC levels, while CYP3A4 inducers can decrease them. Liver and kidney functions also affect TAC metabolism; impaired liver function can lead to higher drug levels. Taking tacrolimus with antacids containing aluminum or magnesium hydroxide can increase serum levels and pose a risk of toxicity. Additionally, genetic factors, such as polymorphisms in the CYP3A5 enzyme, can cause variations in how patients metabolize TAC, leading to differences in blood levels.

Sirolimus and preanalytical factors

Patient conditions can significantly impact Sirolimus levels. Kidney function, although not directly responsible for excreting Sirolimus, can influence drug levels, especially in patients with impaired renal function where Sirolimus metabolites may accumulate. Liver function plays a crucial role, as Sirolimus is metabolized in the liver; liver disease can extend the drug's half-life, increasing blood levels. Age is another important factor, with older patients experiencing slower drug metabolism due to decreased liver and kidney functions, leading to higher Sirolimus levels. Body weight and fat percentage also affect Sirolimus distribution, as it is a lipophilic drug that can accumulate in fat tissue, potentially altering blood levels. Concomitant medications, particularly those affecting the CYP3A4 enzyme system, can either increase or decrease Sirolimus levels, with drugs like ketoconazole and rifampin being key examples. Genetic factors, including variations in CYP3A4 and CYP3A5 enzymes, can cause individual differences in drug metabolism. Nutritional status also influences drug absorption, with high-fat meals potentially increasing Sirolimus levels, while poor nutrition may affect its metabolism. Finally, the patient's overall health, including acute conditions such as infection or dehydration, can alter the pharmacokinetics of Sirolimus, further impacting its levels in the blood.

Everolimus and preanalytical factors

Pre-analytical factors can significantly impact the accuracy of Everolimus-level measurements. The timing of sample collection is crucial, with blood samples ideally taken 24 hours after dosing to capture trough levels. Incorrect timing can result in inaccurate readings, either too high or too low. The type of collection tube used is also important, with EDTA tubes being recommended. Using the wrong type of tube can alter the results. How samples are handled and stored post-collection can affect the stability of Everolimus; they should be stored at appropriate temperatures, typically between 2-8°C, to maintain drug concentration integrity. Additionally, physiological variables such as serum albumin levels and kidney function can influence Everolimus pharmacokinetics, leading to variations in drug levels. Hemolysis, or the breakdown of red blood cells in the sample, can release intracellular components that may interfere with the measurement of Everolimus. Similarly, high levels of lipids in the blood can interfere with assay methods, leading to inaccurate results. Specifically, a high-fat meal can alter the absorption of Everolimus. The absorption of Everolimus can vary depending on whether it is taken before or after meals. Therefore, it is crucial to follow the doctor's instructions regarding whether to take the medication with or without food. When taken with food, the absorption rate of Everolimus may decrease, which can lead to lower drug levels in the blood. Thus, taking the medication under the same conditions each day (with or without food) helps maintain consistent blood levels.

Cyclosporine and preanalytical factors

Preanalytical factors that influence Cys levels include the timing of sample collection, which is crucial since Cys is usually measured as a trough level, requiring blood samples to be taken just before the next dose. Incorrect timing can lead to inaccurately high or low readings. The type of sample, with whole blood preferred due to Cys's distribution in erythrocytes, also impacts measurements. EDTA tubes are recommended for collection to avoid interference from other additives. Blood should not be collected from the same line where drugs or other fluids go, as contamination with infusion fluid can lead to erroneous results in such a practice. Cys is a hydrophobic drug. When intravenously administering cyclosporine, it tends to bind to the hydrophobic surface of some IV catheter tubing. When blood is drawn from the catheter line, cyclosporine concentrations are increased by direct venous inflow. Proper storage at 2-8°C is vital for maintaining Cys stability, as prolonged storage at room temperature can cause degradation. Handling and processing errors, such as delayed processing or improper mixing, may result in hemolysis, which can skew drug levels. Hemolysis itself, along with high lipid (lipemia) or bilirubin (icterus) levels in the blood, can interfere with assay methods, leading to inaccurate results. Additionally, physiological factors such as hematocrit variations, liver function, and drug interactions, especially with CYP3A4-affecting medications, can affect Cys pharmacokinetics and its levels in the blood. Managing these factors is essential for accurate and reliable Cys monitoring. Many drugs alter the distribution of Cys. Ketoconazole, erythromycin, melphalan, amphotericin B, and aminoglycoside antibiotics sufficiently prolong the metabolism of

Cys, increasing the risk of nephrotoxicity. Phenytoin, phenobarbital, carbamazepine, and rifampin induce cytochrome P450 enzymes, increasing the rate of Cys metabolism when co-administered. Intravenous administration of sulfadimidine and trimethoprim reduces Cys concentrations.

MPA and preanalytical factors

Important differences for MPA include (1) the measurement of the drug is in plasma rather than whole blood; and (2) a peak or C0 measurement is not sufficiently correlated with the area under the curve (AUC), so a single measurement is not sufficient. It is recommended that AUC with limited sampling be used for this compound. Plasma or serum samples are required for MPA analysis. EDTA is used as an anticoagulant. Plastic or gel-barrier tubes are not used. Plastic gel-barrier tubes may cause falsely lower concentrations for certain drugs due to absorption by the gel.

The time and speed of centrifugation and temperature changes affect absorption.

Free MPA concentrations are increased in hypoalbuminemia, hyperbilirubinemia, and uremia. In chronic renal failure, the free concentration of MPA can increase indicating over-immunosuppression when the total MPA concentration is within the therapeutic range. For solid organ transplant patients with normal renal and liver function, monitoring total MPA is sufficient. However, in transplant recipients with renal insufficiency, hypoalbuminemia, or liver disease, the free fraction of mycophenolic acid may be significantly elevated, making it strongly recommended to monitor both free mycophenolic acid and the traditionally monitored total MPA. Few clinically significant drug–mPA interactions (salicylate, fenofibrate*)* that lead to elevated free MPA levels have been described.

In conclusion, the accurate monitoring of immunosuppressive drugs is heavily dependent on controlling preanalytical factors. Ensuring proper timing, sample collection, handling, storage, and considering physiological and interference factors are all essential to obtaining reliable drug level measurements. By carefully managing these preanalytical factors, healthcare providers can optimize immunosuppressive therapy, ensuring effective treatment while minimizing the risks of adverse outcomes.

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SOCIETY of CLINICAL BIOCHEMISTRY SPECIALISTS
Drug and Toxicology

The Role of Clinical Laboratories in Drug Abuse Treatment and New Legislations

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Drug-related issues have become a global concern, affecting societies everywhere. Psychoactive substances frequently enter the market, often mislabeled or as part of mixtures, making it difficult for consumers to know what they are using. This widespread social issue affects individuals directly or indirectly.

Clinical laboratories have played a key role in drug control programs over the past five decades, working closely with healthcare providers, forensic scientists, and law enforcement agencies to combat substance abuse. Despite the growing global issue of substance abuse and the longstanding practice of drug testing, both clinicians and laboratory professionals continue to face analytical and interpretative challenges.

Clinicians rely on drug tests to diagnose, identify, and guide treatment for individuals at risk of substance use disorders. These tests are crucial for early detection and help to monitor treatment and recovery progress. Clinical laboratories also support workplace drug testing, driving under the influence (DUI) screenings, and other social programs aimed at substance detection.

Aim of Drug Abuse Test Request

Clinical toxicology laboratories serve two main purposes in the context of drug addiction: diagnostic testing for treatment and testing for administrative and forensic reasons. Since the interpretation of test results and cutoff values can vary depending on the context (clinical or forensic), it is essential to understand the test's objective, the sample type, and the conditions surrounding sample collection (e.g., time of collection). Typically, cutoff values are used in clinical and workplace settings, while forensic samples require the limit of quantitation and are usually analyzed through chromatographic methods.

Matrix Selection

Urine is the most common biological sample for drug testing, although blood, saliva, and hair can also be used in certain cases. While urine remains the primary focus, exploring other biological matrices can provide valuable insights in clinical, forensic, and social situations. It is critical to collect appropriate samples at the right time, under the correct conditions, and with sufficient background information to ensure accurate analysis. Detection periods for urine, blood, and hair, along with cut-off values, must be considered when interpreting the results.

Analytical Methods

Significant advancements have been made in analytical toxicology, particularly in the past century, allowing for more sensitive and comprehensive detection across a wide range of substances and matrices.

In clinical settings, presumptive drug screening is the most commonly performed toxicological test. These tests, usually conducted via immunoassay, detect the presence or absence of a specific drug or drug class in urine. The cross-reactivity of immunoassays allows for the detection of various drugs within the same category. However, these tests are subject to interference, which can be physiological (related to the individual's biology) or analytical (related to the testing method).

Confirmatory drug testing, conducted using chromatographic techniques, provides more specific information by identifying particular drugs, their metabolites, and their quantities within a sample. It is

also used to detect newer psychoactive substances (NPS) which may not be detected by immunoassay technique. Both screening and confirmatory tests are vital for patient care and safety.

Quality Assurance

A laboratory responsible for drug testing must ensure the reliability of its services through a robust quality management system. Quality control should begin in the pre-analytical phase and continue through reporting and interpretation. This includes proper sample handling, validity testing, and attention to sample storage and stability. During the analytical phase properties of calibration, internal and external quality samples, and validation or verification in the case of instrument or method change are critical for maintaining accuracy and reliability.

Screening Panels

When assessing drug abuse or administering treatment, determining which substances to include in the screening panel can be challenging, especially in terms of balancing thoroughness with cost-efficiency. Beyond the administratively recommended five-test panel, laboratory specialists and clinicians need to stay informed about emerging substances of abuse and the detection capabilities of their chosen methods. It is crucial to consider regional variations in drug use and monitor the rise of new psychoactive substances (NPS). For instance, in Turkey, substances like pregabalin, gabapentin, ADB-BUTINACA, MDMB-4en-PINACA, and xylazine are increasingly prevalent.

NPS are defined as narcotic or psychotropic drugs that are not yet regulated by national drug laws but pose similar risks to public health. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) actively monitors and assesses these substances as they appear on the market.

Interpreting Test Results

Accurate interpretation of drug test results requires consideration of clinical factors such as age, sex, body mass index, health conditions, prescribed medications, and dietary supplements. Laboratory specialists and clinicians also need a well understanding of drug metabolism.

In treatment programs for substance abuse, a decrease in drug concentration within the sample matrix can indicate compliance, even if levels are still above the cutoff value. However, in forensic cases, any detectable amount of the substance may be deemed a violation, regardless of whether it falls below the cutoff threshold.

Other key factors in result interpretation include pharmacogenomics, drug-drug interactions, and individual tolerance. In the future, more detailed analysis of a person’s metabolic profile may allow for personalized medical and judicial responses.

New Legislation in Turkey

In Turkey, drug use and possession offenses, as well as the associated probation process, are governed by Article 191 of the Turkish Penal Code. According to recent legislation, probation for these offenses is set at a minimum of one year and a maximum of five years. If individuals meet their obligations during this time, they can avoid prosecution and complete the process without punishment. Under new 2024 regulations, all positive screening results must be confirmed by an official confirmation laboratory.

Additionally, Turkey's hemp cultivation policy has undergone changes, allowing for controlled scientific research under the supervision of the Turkish Ministry of Agriculture. These regulations pave the way for the production of medical products and cannabidiol (CBD) based food and supplements. Accurate measurement of tetrahydrocannabinol (THC) and CBD levels will become a new challenge for clinical laboratories in Turkey.

Conclusion

Clinical biochemistry laboratories play a pivotal role in both drug abuse treatment programs and forensic cases, as well as contributing to preventive medicine through screening programs. As a key component of evidence-based medicine, laboratory professionals must engage in continuous education

related to clinical, pharmacological, and analytical aspects of drug testing, while remaining mindful of the broader social implications of their work.

Keywords: laboratory professional; drug abuse; screening and confirmation; legislation

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SOCIETY of CLINICAL BIOCHEMISTRY SPECIALISTS
Drug and Toxicology

Ethanol and Its Metabolites: Metabolism, Measurement Methods, Interferences, and Case Studies

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Introduction

Alcohol has been utilized for its stimulant, sedative, narcotic, and medicinal properties since antiquity. It is one of the oldest known psychoactive substances and one of the most commonly used substances in United States (1). However, alcohol consumption has significant negative consequences, making it one of the most pressing public health concerns of modern times.

Alcoholism contributes to a range of psychosocial issues, including health problems, traffic accidents, suicides, criminal behavior, family breakdown, economic instability, and workplace disruptions (2).

Absorption and Metabolism

Orally ingested alcohol is rapidly absorbed through passive diffusion, with approximately 10% absorbed in the stomach and 90% in the intestines (3). Alcohol can be detected in the bloodstream within five minutes of ingestion, and peak blood alcohol concentration (BAC) occurs within 30–60 minutes in a healthy adult following a single dose.

Although the liver is primarily responsible for the metabolism and oxidation of ethanol (approximately 90%), some alcohol metabolism also occurs in the gastrointestinal (GI) tract. Alcohol dehydrogenase (ADH) activity in the GI mucosa contributes to the first-pass metabolism of ethanol, which occurs via gastric ADH. Approximately 10% of ingested alcohol is excreted unchanged through urine, breath, sweat, saliva, and tears (4).

Elimination and Forensic Considerations

The rate of alcohol elimination is a critical parameter in forensic and legal assessments of BAC. The average rate of alcohol metabolism is about 15 mg/dL per hour, though this rate can vary between 10 and 34 mg/dL per hour. For forensic purposes, an elimination rate of at least 10 mg/dL per hour is typically applied (5-6).

Several factors, such as the time of the offense, time of alcohol intake, measurement time, BAC test results, gender, weight, height, food consumption, number and size of drinks, alcohol concentration in drinks, previous alcohol use, concomitant medications, body temperature, and the gastric first-pass effect, are important in evaluating BAC levels. The amount of ethanol consumed can be estimated in grams using the Widmark formula(6).

Methods for Alcohol Measurement

Clinical laboratories require fast and accurate methods for detecting and quantifying ethanol in biological fluids such as plasma, serum, and urine. Failure to recognize interfering substances may lead to clinical and legal consequences. Several methods are used for alcohol measurement in biological samples, including:

- Chromogenic methods
- Enzymatic methods utilizing ADH or alcohol oxidase (AOD)
- Gas chromatography

Among enzymatic methods, ADH-based techniques are commonly used due to their stability in solutions and ease of automation, primarily relying on the spectrophotometric determination of NADH. Automated test systems often analyze multiple types of body fluids and are available for serum/plasma and urine testing. Commercial control materials for blood, serum, saliva, and urine are used to meet legal requirements for matrix-compatible controls. In addition to internal quality controls, external quality control programs, such as those offered by the College of American Pathologists (CAP), ensure the accuracy of laboratory measurements (7). For ethanol measurements, CAP guidelines under the Clinical Laboratory Improvement Amendments (CLIA'24) allow an acceptable performance range of ±20% for all methods (8)

Units of Measurement

There is variation in the units of measurement used for ethanol quantification. International toxicology journals generally prefer grams per liter (g/L), whereas clinical chemists favor millimoles per liter (mmol/L). In the United States, milligrams per deciliter (mg/dL) is commonly used, while legal documents often employ percentage units, complicating interpretation. To avoid confusion, good laboratory practices recommend that all test results be reported with a reference range, along with unit conversions. The following conversions are commonly used for ethanol measurements:

- To convert mg/dL to g/L, divide by 100.
- To convert g/L to mg/dL, multiply by 100.
- To convert mg/dL to mmol/L, multiply by 0.217.
- To convert mmol/L to mg/dL, multiply by 4.6.
- To convert percentage (%) to mg/dL, multiply by 1000.

Alcohol and Biochemical Markers

While acute alcohol consumption can be easily measured in blood and breath, these measurements do not provide insight into chronic alcohol use patterns, alcohol abuse, or dependence. Few markers are available for retrospective evaluation of alcohol consumption over recent days or weeks, which is important for monitoring alcohol-related diseases.

Tests indicating acute alcohol consumption include the measurement of ethanol in body fluids or breath, as well as the detection of ethanol metabolites such as ethyl glucuronide (EtG), ethyl sulfate (EtS), fatty acid ethyl esters (FAEE), and phosphatidylethanol (PEth). Serotonin metabolites like 5-hydroxytryptophan may also be relevant. For chronic alcohol consumption, biomarkers such as gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), mean corpuscular volume (MCV), carbohydrate-deficient transferrin (CDT), acetaldehyde compounds (e.g., acetaldehyde-bound hemoglobin), dolichol, and hyaluronic acid can be used (9).

The use of these biochemical markers in clinical practice aids in the diagnosis, treatment, and monitoring of alcohol-related disorders, helping to prevent alcohol-induced problems, such as traffic accidents.

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BRAZILIAN SOCIETY of PATHOLOGY/LABORATORY MEDICINE
A Glimpse on Brazilian Experience in Clinical Laboratories: Mixing Public and Private Health Systems

BRCAST The Brazilian Microbiology Sensitivity Test Program

Andre Mario Doi
MD, PhD

Scientific Director of Brazilian Clinical Pathology/Laboratory Medicine Society
Coordinator of Molecular Biology Section – Clinical Laboratory – Hospital Israelita Albert Einstein
Ex-coordinator of Brazilian Committee on Antimicrobial Susceptibility Testing

My lecture will address the implementation of European Committee on Susceptibility Testing (EUCAST) in Brazil – a national experience. The lecture will cover "where we are and where we are going" in terms of bacterial resistance and standardization and interpretation of antimicrobial susceptibility testing in Brazil.

BRAZILIAN SOCIETY of PATHOLOGY/LABORATORY MEDICINE
A Glimpse on Brazilian Experience in Clinical Laboratories: Mixing Public and Private Health Systems

Insights into the Brazilian Quality Indicators Program

Luciana Franco

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The "Indicators Program" of the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML) is an initiative aimed at improving the quality of laboratory services in Brazil. This program collects and analyzes data from several associated laboratories, creating indicators that help monitor and enhance the performance and quality of the services provided.

These indicators include metrics related to accuracy, efficiency, response time, and patient satisfaction, among other factors relevant to clinical laboratories. The goal is to establish a standard of excellence and ensure that laboratory processes adhere to best practices, providing reliable results for both patients and physicians.

This program also enables comparisons between laboratories, fostering a culture of continuous improvement and performance benchmarking.

The harmonization of quality indicators between the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML) and international organizations, such as the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), is essential to ensure that Brazilian laboratories are aligned with global standards. This harmonization allows performance and quality indicators adopted in Brazil to be internationally comparable, promoting effective benchmarking and driving continuous improvement. Collaboration with international organizations enables the sharing of new technologies, practices, and scientific advancements, fostering innovation in the field of laboratory medicine.

BRAZILIAN SOCIETY OF PATHOLOGY/LABORATORY MEDICINE
A Glimpse on Brazilian Experience in Clinical Laboratories: Mixing Public and Private Health Systems

HPV A Front Line Molecular Assay in the Cervical Cancer War

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The lecture will describe HPV (Papilomavírus) biology, clinical importance in cancer, and role of HPV testing in early diagnose of cancer precursor lesions, in cervical cancer and also other cancer types, as well.

Specifically, it will be mentioned on the several comercial assays for HPV and on the assays using HPV Genotyping, and how HPV genotype these add on prognosis and risk stratification.

SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Interpretive Reporting in Clinical Virology, and New Approaches in Diagnostic Management

Serological Tests

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Interpretive reporting is performed to ensure post-analytical standardization and to prevent possible misinterpretation of laboratory results. Laboratory experts are responsible for reviewing analysis results and providing the necessary information for reliable transfer of results and accurate evaluation by the clinician. As stated in the quality standards in healthcare, it is recommended that the report format in medical laboratories be designed in a dynamic way that the laboratory specialist's comments can be added and that the opinions and suggestions of the relevant clinicians be taken into account when designing the reports (1,2).

In Clinical Virology, the routine difficulties of isolating viruses with conventional methods have required diagnosis mostly with serological tests for many years. On the other hand, there may be false positives, false negatives, cross-reactions, differences in sensitivity, and cut-off values between kits in serological tests. The most important serological tests that may require interpretative reporting in tests for viral agents are antibody tests for TORCH group agents, including cytomegalovirus (CMV), antigen-antibody tests for viral hepatitis agents, and rapid antigen tests.

In clinical virology, the routine difficulties of isolating viruses with conventional methods have required diagnosis mostly with serological tests for many years. On the other hand, there may be false positives, false negatives, cross-reactions, differences in sensitivity, and cut-off values of the kits in serological tests. The most important serological tests that may require interpretative reporting in tests for viral agents are antibody tests for TORCH group agents, including cytomegalovirus (CMV), antigen-antibody tests for viral hepatitis agents, and rapid antigen tests.

In terms of congenital CMV infection, especially in societies where CMV seroprevalence is over 90%, such as our country, recurrent infection, which may be reactivation of latent virus or reinfection with a new CMV strain, rather than primary infection, can be seen more frequently in pregnant women (3). Therefore, CMV serology tested in pregnant women should be interpreted carefully, and CMV DNA should be investigated in amniotic fluid in cases of suspected congenital CMV infection.

When evaluating the situation in terms of hepatitis C virus (HCV) serology, which is one of the viral hepatitis agents, it should be taken into account that antibodies may not be detected in cases where the immune system is suppressed. Current new-generation immunological tests, namely fourth-generation tests, have reduced the window period in HCV detection to 17 days compared to existing tests (4). However, in laboratories where these tests or HCV RNA analysis are not available, the serological result should be evaluated together with the patient's immune status, risk factors, and clinical findings, and if necessary, it should be recommended to repeat the anti-HCV test after two weeks in terms of seroconversion. In hepatitis B virus (HBV), another viral hepatitis agent, clinical situations with atypical serological profiles can be observed due to HBsAg mutation or different kit sensitivities, where the test cannot detect HBsAg despite its presence and potentially false negative or low measurement results occur (5,6). In such cases, in addition to repeating the test with the same sample, repeating the test with a different kit and repeating it with a new sample should be recommended.

The results of rapid antigen tests used in the diagnosis of many viral agents, especially respiratory viruses, should also be interpreted carefully. Rapid antigen tests have high specificity and are unlikely to give false positive results when applied according to the manufacturer's instructions. The positive predictive value (PPV) and negative predictive value (NPV) of in vitro diagnostic tests vary depending on the pre-test probability, i.e., the prevalence of infection in the tested population (7). If the prevalence is high, the pre-test probability is generally considered high. Accordingly, if the prevalence

of infection in the community is high, PPD increases, NPD decreases, and false negativity increases. In this case, if the rapid antigen test is applied in high-prevalence populations, it is important to confirm the negative antigen test result (in terms of false negativity), and if it is applied in low-prevalence populations, it is important to evaluate the positive antigen test result (in terms of false positivity) with molecular tests and to evaluate it together with the clinic.

Due to the increase in the number of health centers and patients and the increase in the number of laboratories and clinical samples, automation and standardization are inevitable. We believe that the greatest contribution that we, laboratory experts, can make to interpretative reporting in clinical virology can be in the form of establishing a common language by establishing working groups with effective communication with clinicians, creating a guide with standard algorithms for the rational use of serological tests together with molecular tests, and developing artificial intelligence-supported and digital interactive platforms.

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SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Interpretive Reporting in Clinical Virology, and New Approaches in Diagnostic Management

Molecular Tests

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Molecular methods have become essential to the clinical microbiology laboratory. These tests are used for medical testing to diagnose symptomatic individuals, screen asymptomatic individuals, monitor disease, provide prognosis in diseased patients, detect risk, and select patients for specific therapies. The advantages of these tests are rapid turnaround time and high sensitivity and specificity. Molecular diagnostic tests require both analytical and clinical evaluations. Today molecular testing procedures and analysis are becoming increasingly complex, even though qualitative and quantitative molecular technology can provide uniquely valuable information about patient conditions, clinicians may not gain maximal benefit from test results without clear and adequate details about test methods and limitations, and in-depth interpretation. Individualized clinical interpretation of the results is needed, especially with further molecular tests, reports also must be written clearly and understandable to non-microbiologist professionals.

Steps in the Analysis, Reporting, and Interpretation Process Included

- **The intent of use, testing plan, and pre-analytics:** Confirming appropriate test orders, evaluating sample collection methods, and consulting with ordering physicians on alternative tests.
  The intent of use describes the clinical purpose, the type of test, the criteria it measures, the specimen it measures (specimen type), the site of measurement, and the population for which the test is intended. Many variables can influence the performance of a test, such as population characteristics, the prevalence of the target condition of interest, the setting, and the type of test, among others. In general, it is important to evaluate the following: the clinical purpose (eg, screening, diagnosis, prognosis, risk prediction, therapy, or treatment selection for patients), target condition (eg, disease, disease stage, or any other condition of interest), target population, and the environment (eg, clinical laboratory, point of care, home use). Other important things to consider are anatomical location (eg, finger stick, venous) or specimen type from which the measurement is taken (eg, whole blood, plasma, serum, tissue).
- **Simple Analysis:** Performing molecular testing procedures. Clinical test development and validation and evaluation of analytical and clinical performance characteristics.
- **Quality Control:** Confirm that the test is within parameters, appropriate control results, and any other steps needed to QC initial test data.
- **Complex Analysis:** Identifying relevant clinical literature, researching potential treatment options, etc. Exploring case studies from internal and external laboratories on how existing analysis burdens impact laboratory function and how this will increase with anticipated changes.
- **Reporting:** Combining multiple test results, considering clinical history with testing results, and writing/reviewing the final testing report.
- **Ongoing Dialogue:** It is the final and most important step. Explaining test results to ordering physicians, discussing potential follow-on tests, discussing clinical literature, etc. Engaging with physician and patient groups to better define negative outcomes from slow, expensive, or insufficient testing.

Medical microbiology specialists are heavily involved in each step of molecular testing analysis, interpretation, and reporting. Clinical interpretation, additional research requirements, and technical complexity were the major drivers of the effort.

Detection of viral infections and antiviral resistance

Molecular testing methods allow laboratories and healthcare professionals to detect the presence of viruses causing infectious diseases both qualitatively and quantitatively. A qualitative measure of the infectious agent is often sufficient for the detection of many infectious diseases. There are several clinical conditions where the quantification of certain viruses is required. Specimens suited ideally for viral quantification include EDTA whole blood, plasma, serum, CSF, and urine. However, specimens such as swabs, secretions, lavages, seminal fluids, stool, and biopsies should be preferably used for the qualitative detection of viruses.

Of particular importance for immunosuppressed patients, viral load can be determined using quantitative molecular tests to identify the number of certain viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and polyomavirus BK (BKV). Many people have been infected with these viruses and present with latent infection with low levels of viral DNA. Unless a patient becomes immunosuppressed, such as after a transplant or due to an immunosuppressive disease, these viruses tend to be clinically insignificant. The virus can reactivate in an immunosuppressed patient with dire consequences. In immunosuppressed patients, healthcare providers can monitor the virus quantitatively using molecular testing to determine when the viral load rises to critical levels.

Furthermore, quantitative molecular tests are used to monitor the response to therapy against certain viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Quantitative detection of infectious agents can be of particular importance when measuring tumor burden. For example, if EBV is present within a tumor, clinicians can gather important information concerning the size of a tumor by measuring the amount of EBV present in a patient sample using a quantitative molecular test.

Antiviral resistance tests are usually requested following suboptimal antiviral response or viral rebound. Both can be measured objectively by quantitative viral load tests for viral infections such as HIV, HBV, or CMV. Lack of clinical improvement can also be an indication of suboptimal antiviral response, for example, influenza or herpetic ulcers. Routine baseline antiviral resistance tests are also used to identify transmitted drug resistance before starting therapy when the prevalence of circulating resistant strains is high enough to warrant screening, for example, HIV and influenza. Clinical guidelines on managing HIV, CMV, HBV, and influenza resistance advise on when to test for antiviral resistance, what sample type as well, and how to interpret the absence of drug resistance mutations.

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SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Interpretive Reporting in Clinical Virology, and New Approaches in Diagnostic Management

Stay Up to Date with the Latest Information on HIV Diagnosis

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With the UNAIDS 95–95–95 targets to end HIV/AIDS by 2030, much effort has been spent and new HIV infections in 2022 were estimated to be 1.3 million, the fewest in decades, with the declines especially in sub-Saharan Africa having the highest HIV burden. However new HIV infections continued to increase in eastern Europe and central Asia since 2010 (49%) and the Middle East and North Africa (61%). In 2022 globally approximately 86 % knew their HIV status. This marked improvement is unknown in Turkey.

Appropriate HIV testing algorithms that enhance the detection of individuals including newly acquired infection and improved access to accurate and rapid HIV testing with wright interpretation and with appropriate counceling and link to medical care and treatment lead to increased access to antiretroviral therapy (ART), which can decrease the advanced immunocompromised patients and also reduce transmission to others

Serological diagnosis of human immunodeficiency virus (HIV) infection became available first in 1985, with the rapid increase in sensitivity and specificity of enzyme-linked immunosorbent assays (ELISAs) with new generations and the supplement tests. Molecular tests for detection of HIV in the diagnosis of HIV infection in special settings and monitoring of HIV-1 infection followed this.

In the diagnosis of HIV infection in adults and children older than 18 months serological assays are used followed by NAT. To have the greatest sensitivity and specificity a reactive ELISA confirmed by WB algoritm and in acute HIV infection suspicion follow up for 2-4 weeks by serology or NAT was used. Diagnosis of HIV infection in babies born to HIV infected mothers is complicated by the presence of HIV-specific IgG maternal antibodies. Therefore for the diagnosis of pediatric HIV infection, NAT is used. World Health Organization stated that in adults equivalent sensitivity and specificity may be obtained if diagnosis is made using consecutive ELISAs that contain different antigens and rely on different testing principles. ELISA's in time became more sensitive and specific starting from first generation to fourth generation assays which detect both antigen and antibody. Fifth generation assays even could detect HIV-1/2 antibodies seperately. WB a time consuming test which is difficult to interpret in time was replaced by a rapid HIV-1/2 differentiation assay in 2015 by CDC to have desantralization, shorting the turn around time of the test result. This new algorithm is also applied in Turkey since 2018 but desantralization have not been managed by now yet. CDC by the new proposed algorithm also published detailed interpretation of the test results which also have been underlined in National 2018-9 Guide and also in more detail in HIV/AIDS book 2024 for turkish specialists.

To maximize public health impact, accurate and timely diagnostic HIV testing should be combined with clear result reporting and prompt linkage to medical care and services for infected persons. Laboratory reports should state each test that was performed, the result of each test, and the laboratory algorithm interpretation for the specimen.

While it is currently not recommended as the standard laboratory algorithm, in certain circumstances such as symptoms coinciding with a known or suspected recent exposure, an alternative testing sequence in which NAT with a diagnostic claim is applied in the second step may be ordered by a health care provider. This may accelerate detection of acute HIV infection and be beneficial to timely clinical decision-making. Any changes to the recommended algorithm require taking the countries seroprevalence into consideration and validation prior to updating testing recommendations. And every country should be checking their algoritms and propose if availale new better algoritms and validate

them. The aim should be to present the rapid and accurate result to the applicant as soon as possible and. provide person tested with appropriate counselling and link to medical care and treatment.

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PORTUGUESE SOCIETY of CLINICAL PATHOLOGY

From Lab to Clinical Visit: The Laboratory Physicians Handling Red Cell Disorders

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Anemia is an important public health problem worldwide, which can occur in any age group and can have different etiologies, often being multifactorial. The Portuguese Society of Clinical Pathology (SPPC) intend to position Anemia as a clinical entity, due to the impact it assumes in the context of other pathologies, with a view to establishing a group of doctors with competence to study this important Public Health problem in its various aspects. A disease that in Portugal affects one in five adults (1) and which has been considered as an interfering factor in the prognosis of various pathologies, directly interfering in the quality of life of patients cannot be ignored and must be evaluated and treated correctly according to its etiology.

Taking into account that there are no population screening programs for anemia or red blood cell disorders, the creation of a “Consultation of Anemia” is of great importance, with a view to studying and guiding patients and detecting asymptomatic carriers, in order to implement the most appropriate early treatment and carry out family counseling. That consultation should be implemented routinely, carried out by experienced Clinical Pathologists, with diagnostic skills and vast knowledge of laboratory methods and execution and interpretation of tests in the field of Hematology. In all suspicions of red blood cell disorder, blood count with reticulocyte evaluation and observation of erythrocyte morphology in the peripheral blood smear are the initial fundamental steps to begin the diagnostic journey.

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PORTUGUESE SOCIETY of CLINICAL PATHOLOGY

From Lab to Bedside: Integrating Clinical Microbiology Into Multidisciplinary Medical Teams

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Clinical microbiology directly impacts all other medical specialties by providing critical support for infectious disease diagnosis and management. In addition to their laboratory duties, modern clinical microbiologists must embrace their role beyond the laboratory walls, including real-time clinical consultancy in medical/surgical wards and, particularly, in critical care wards. This should be a multidisciplinary team-based approach and rely on collaborative efforts to integrate different backgrounds and perspectives aiming to improve patient management and outcomes. Interventions by clinical microbiologists should be focused on the tenets of diagnostic stewardship (right test, right time, right patient, right interpretation, and right treatment) and antimicrobial stewardship (right drug, right dose, right drug-route, right therapy adjustment, and right duration).
Attendees of this session will not only gain practical insights into how to leverage microbiological expertise to actively engage in clinical-decision making, manage complex clinical scenarios, optimize antimicrobial therapy, and develop institutional protocols, but also understand the benefits of a team-based approach to patient care.

Artificial Intelligence

Technological Applications for the Diagnosis and Treatment of Bloodstream Infections

MD, MBA, Juan Carlos Gómez de la Torre Pretell*

Bacteremias are infections that can trigger a severe immunological cascade and targeted organ damage (sepsis) with risk of death in patients. Knowing the type of germ and resistance mechanisms as soon as possible makes it possible to administer optimal and personalized treatments, avoiding the initiation of broad-spectrum therapies, that can increase the global problem of antimicrobial resistance. Likewise, from the laboratory it is possible to provide therapeutic recommendations based on these findings, whether molecular or phenotypic, considering the complexity of the knowledge that exists today.

The work we have been doing begins with correct sampling of blood cultures using techniques that minimize contamination of the bottles by skin-colonizing germs through training of phlebotomy personnel and materials necessary for sample collection. Once the sample has been taken, it enters incubation equipment with automated reading of bacterial growth and generation of alerts that allows removing the bottle and performing gram staining with the objective of defining whether it is: 1) Gram negative vs. positive bacillus, 2) yeast , 3) gram positive coccus in chains or 4) in clusters, with which we proceed to perform a molecular panel by FilmArray® Blood Culture Identification Panel (https://www.biofiredx.com/products/the-filmarray-panels/filmarraybcid/) in options 1,2 and 3, and molecular detection of Staphylococcus aureus and MecA gene with Xpert® MRSA/SA Blood Culture (https://www.cepheid.com/es-ES/tests/hai-other-infectious-diseases/xpert-mrsa-sa-blood-culture.html) in option 4, but that also meets the criterion that the alert has occurred before 16 hours of incubation (predictive of pathogenic germ).

Once the germ is identified (if they match the molecular targets), it is automatically reported and the information is processed by a software (https://arkstonemedical.com) that uses tools based on machine learning and artificial intelligence to generate therapeutic recommendations based on the molecular result and some clinical characteristics of the patient (pregnancy, age, allergies, etc.), recommendations that are based on FDA indications, IDSA recommendations, meta-analysis, scientific publications, local reality, etc. Both results (molecular and therapeutic recommendation) are communicated to the treating physician so that therapy can be initiated. If the treating physician wants to interact with the therapeutic indication report, he or she may do so through a QR code located next to the indication. With this, the treating doctor will be able to make adjustments depending on the microbiological map, pregnancy not previously reported to the laboratories, calculated creatinine clearance, etc.

At the same time, the sample is seeded on solid media in order to subsequently identify it with MLADI-TOF technology(https://www.biomerieux.es/diagnostico-clinico/productos/vitekr-ms) and antimicrobial resistance is investigated with commercial panels (https://www.biomerieux-industry.com/es/products/vitek-2-compact-identificacion-microbiana-de-rutina-para-aplicaciones-alimentarias). If it is necessary to extend studies with phenotioic vs immunochromarigraphic vs molecular methods, this will be done on a case-by-case basis.

Having the pathogen together with the antibiogram and resistance mechanism, this information is sent again to the software described above where a new and definitive therapeutic recommendation report is generated considering the molecular, genotypic and phenotypic variables of the isolated pathogen.

With this, we have considerably reduced the pathogen detection time in 100% of positive blood cultures, as well as validating that the 16 hours described above are correct for resource optimization. The agreement between molecular and conventional methods is 100% when bacteria or yeasts are present in the molecular targets. Likewise, therapeutic recommendations are provided with absolute certainty and based on clinical and epidemiological evidence, considering the possibility of dose adjustments according to the pharmacokinetic conditions of the antibiotics in each patient, bridging the gap that exists between the types of bacteria and molecular or phenotioic mechanisms of resistance

with therapeutic indications. With the information generated, an ambiseptive study was designed to evaluate the impact of artificial intelligence and machine learning on correct therapeutic indications consistent with the pathogens as well as staging, length of hospital stay and mortality in patients who had blood cultures performed with pathogens detected before and after being accompanied with the report based on AI and ML. The study has started following approval by the ethics committee with IRB and the hospital institution. Preliminary results from the study are expected to be presented in Q3 of this year.

Part of the information described is published in my book Molecular Dx of Infectious Diseases, second edition (www.dxmoleculariid.com).

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Artificial Intelligence

Model and Applications of Artificial Intelligence in the Clinical Laboratory

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Artificial intelligence (AI) has emerged as a powerful creative and transformative tool in laboratory medicine and promises significant advances in healthcare delivery. Some of the most important concepts that I must begin to master are the following: Artificial Intelligence: Branch of computer science that teaches or trains machines to act or perform like a human brain, Machine Learning: Algorithms and models that are trained with the available data make a prediction, Deep Learning: Uses artificial neural networks to work with data sets and perform tasks, Neural Networks: Interconnected artificial neurons organized in layers. The use of neural networks applied to deep learning allows the interpretation of images in reading units that are pixels and these, after going through a simplification process using computer filters called Kernels, generate the prediction of an image in terms of probability. , said prediction is validated by one or a group of experts depending on the type of "Learning used by the algorithm", and may use metadata to achieve a better request. In general terms, this Artificial Intelligence tool can be used to interpret Immunofluorescence patterns in the antinuclear antibody test, in the identification of hematological cells in different stages of maturation. But its use is of primary importance in gynecological cytopathology, where its many benefits among others that can be cited the following: Less investment of time per evaluated slide, greater productivity with less observer fatigue, better response in massive campaigns, less hiring of personnel, greater reliability, lower rate of False Positives ALPHA ERROR, lower rate of False Negatives BETA ERROR, fewer complaints, have material for training, teaching, training resources (Cytology Education Learning Lab), International leadership for the publication of scientific articles, most robust software on the international market, software self-maintenance and own development, new versions and likelihood of commercialization In a world where computer advances are taking place at a dizzying pace, we must recognize the profound impact of AI in laboratory medicine. We must also dare to take on the challenges represented by the availability and interpretation of data with digital technology that is not fully coupled to clinical laboratory technology and that we must promote taking into consideration the ethical and regulatory codes in each country, particularly in developing countries.

Keywords: Artificial intelligence, Laboratory, Deep Learning

Artificial Intelligence

Applications of Human Intelligence in the Clinical Laboratory

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We witness the rise of the artificial intelligence (AI) in all fields of life sciences and clinical pathology is no exception (1). Clinical laboratories welcome new applications of AI which are the topics of previous two presentations.

AUTOVERIFICATION

The increase in workload requires medical laboratory specialists to devote a significant portion of their working hours to report approval. Confirmation support systems will enable medical laboratory specialists to spend more time on good laboratory practices (reflex and reflective testing), more complex cases, stronger communication with clinics, consultation and other essential duties, especially through the rapid and effective evaluation of relatively less complex test results by autoverification.

This paragraph is adapted from the official website of Turkish Ministry of Health, in which the duties of laboratory specialists are defined (2) (google translate). Accordingly: we are expected to perform good laboratory practices including reflex and reflective testing; we are expected to build stronger connections with the clinicians; we are expected to deal with complex cases and finally, we are expected to perform consultation. Meanwhile, the AI will verify the relatively simple test results. I believe it is just a matter of time before AI finds all of our test results simple and verifies almost all of our test results. From this point of view, laboratory specialists must focus on the duties defined by our government which I call ‘applications of human intelligence’.

Second part of my presentation includes examples of cases on applications of human intelligence in the clinical laboratory:

Reflex testing is the most widely used good laboratory practice in Türkiye. Direct LDL and free PSA applications are the most known. It is a simple but highly effective good laboratory practice (3).

Reflective testing is a high function of human intelligence. It has some routine applications like adding fT4 and/or fT3 to a TSH test result when it is out of the normal range in stepwise thyroid function screening. Any stepwise testing protocol needs reflective testing (4). However, reflective testing is highly case specific in general.

Putting comments or notes to test results is a high function of human intelligence. Complex tests like Lupus antigen testing must be verified with a comment. Some test results may need special notes as a warning or an aid to the clinician.

Finally, consultation is a very high function of the human intelligence. It needs time, effort and motivation (5).

We must prepare ourselves for the post-artificial intelligence era.

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CONFERENCE
Innovations in Solid Organ Transplantation

Immunosuppressive Drug Use in Kidney Transplantation and the Role of Laboratory

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Therapeutic drug monitoring (TDM) is necessary because some of the immunosuppressive drugs used after transplantation to prevent organ rejection have a narrow therapeutic index. These are the calcineurin inhibitors cyclosporine and tacrolimus and the mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus. It is critical for the patient to maintain blood concentrations of these drugs within the recommended target ranges. Because the use of immunosuppressive drugs well above the recommended therapeutic ranges may cause significant side effects such as infection, malignancy, cardiovascular disease, diabetes, proteinuria, hyperlipidemia and peripheral edema, while low therapeutic concentrations may lead to graft loss, acute or chronic rejection (1). Reliable, accurate and precise testing methods are therefore essential for effective monitoring of levels and appropriate dose adjustments.

Although TDM significantly helps individualize drug doses, it is insufficient on its own and dose adjustment based on trial and error delays the drug reaching target blood levels in many patients. Until a stable level is reached, the patient is exposed to high or low doses of the drug, resulting in undesirable side effects. Therefore, studies on the pharmacogenetics of immunosuppressive drugs have gained importance in recent years. In addition to CYP3A enzymes and P-glycoprotein, which are effective in immunosuppressive drug metabolism and excretion, ethnicity, age, hepatic function, concomitant corticosteroid dose, diarrhea and time after transplantation also affect drug pharmacokinetics and lead to variations in drug blood levels (2).

For an effective TDM, the dose and timing of the drug administered to the patient must first be known. Also, the relationship between some drug formulations and concentration may be poor. The time of sample collection should correspond to the target time, which is usually just before the last dose is taken (C0). Catheters used for blood collection (if sampling is done through a catheter) and some tubes may cause incorrect measurements as the drug adsorbs to them. Therefore, preanalytical factors for TDM are crucial and require effective collaboration between the laboratory and the clinic (3).

Since these drugs are largely contained in erythrocytes, the analysis starts with cell lysis and protein precipitation, so that it is possible to measure the total drug level in the whole blood. Lysis is often performed manually using solutions such as methanol containing zinc sulfate. It is critical that this step is done correctly, as it is one of the potential sources of error if not done correctly. At present, immunosuppressive drugs are measured in central laboratories by analytical methods such as high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), fluorescence-based immunoassays and electrochemiluminescence. HPLC-MS/MS stands out due to its high specificity and sensitivity compared to other available technologies. However, careful selection and preparation of internal standards, calibrators, solvents used for column elution are crucial for reliable test performance(4). Immunoassays are integrated into automated instruments to measure around the clock in a standardized way that is easy, fast and often less prone to technical errors. However, immunoassays have disadvantages such as cross-reactivity to structurally similar molecules, lack of concordance between different lots, Hook effect. Antibodies against tacrolimus applied to immunoassays display similar reactivity with two of its metabolites, 31-desmethyl-tacrolimus and 15-desmethyl-tacrolimus.The immunosuppressive effects of the two metabolites are identical; the 31-desmethyl metabolite is similar to tacrolimus in its action, while the 15-desmethyl metabolite reveals minimal immunosuppressive activity. While most people have low amounts of these metabolites, liver failure-related alterations in metabolism may cause them to accumulate and produce a systematic error that complicates test interpretation. Selectivity is a critical performance attribute that must be understood when evaluating test results, especially when various laboratories are utilizing different

testing methods (e.g., immunoassay versus HPLC-MS/MS). This is because selectivity differs greatly throughout analytical systems (5).

Although the measurement of these drugs for TDM is usually performed from whole blood collected in tubes containing anticoagulants, alternative sampling methods such as paper-impregnated dry blood samples, volumetric absorptive microsampling and intracellular drug measurement are also being developed. Monitoring of the pharmacokinetics of these drugs is often performed by pre-dose sampling, but for special populations and specific clinical situations, such as during the period when immunosuppression is minimized or immediately after transplantation, it is recommended to determine the area under the curve by determining the concentration-time curve. Analysis of pharmacogenomic, pharmacodynamic and immunologic biomarkers such as CYP3A4, CYP3A5 genotyping, T-cell IFN-γ determination, cell-free DNA measurement are not in routine practice but are promising for minimally effective immunosuppression (2).

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Experts of the Future Discuss - 2
Ideal Nutrition

With Gluten? Gluten Free?
With Gluten

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What is gluten?
A protein found in grains. This protein's duty is to provide necessary nutrition for the optimal growth of wheat plant. In the recent years, consumption of gluten has been a very debatable subject. There has been misinterpretation on that there are unhealthy effects of gluten even in individuals who are not diagnosed with celiac disease.

Is gluten actually harmful?
People with celiac disease experience an extreme allergic reaction in their metabolic and immune system to the gliadin in grains, especially in patients with HLA gene. Therefore, people with this disease should follow a gluten-free diet. On the other hand, in an article published in Harvard University, emphasized that gluten does not have any harmful effects on individuals without celiac disease. Indeed, it has been shown that as a result of continuing a gluten free diet for a long time led to the reduction of beneficial probiotic bacteria in the intestines, thus, damaging the digestive system (1). If gluten diet was known to be this dangerous for humanity, everyone who consumes grains should experience complaints such as abdominal pain, indigestion and bloating which are common in celiac disease.

What is gluten sensitivity?
In the last years, there has been a significant increase in the number of people who complain of uncomfortableness and symptoms similar to celiac disease, when they consume gluten containing foods. As the number of these individuals increases, a new term has emerged in the medical world, it is known as ''non-celiac gluten sensitivity''. This recently developed terminology has been quite popular; however, no biochemical, immunological or genetic test has been found to confirm it (2). A double-blind, placebo-controlled, gluten challenge has been proposed to confirm a diagnosis of nonceliac gluten sensitivity (NCGS) in patients without celiac disease who respond to a gluten-free diet. To determine the accuracy of this approach, analysis was conducted from the data of 10 double-blind, placebo-controlled and gluten-challenge trials. According to the results, only half of the subjects in both groups reported that they were uncomfortable with what they ate. Interestingly, those who consumed gluten-free also reported that they were uncomfortable. After one week, they swapped the groups vice-versa. In the results, some subjects who ate gluten-free also noted that they were uncomfortable, subjects who consumed gluten mentioned no uncomfortableness, %16 of subjects were comfortable when they eat gluten-free and uncomfortable when they eat gluten. In conclusion, many researches demonstrate that the true non celiac gluten sensitivity ratio is between %10-%20. As its can be seen that it is not valid as exaggerated (3).

Wheat allergies and FODMAP
There is a term wheat allergy and FODMAP, which causes a picture similar to non-celiac gluten sensitivity complaints. However, its situation has no absolute relation with gluten, but share a similar condition with gluten sensitivity. It may also cause problems such as spastic colon and irritable bowel syndrome (4). When a person describes having bloating issues, stomach pain or diarrhea, it is not easy to know whether it is gluten-related or not. Blaming on gluten containing diet is amplified way too much than it deserves.

Conclusion

Lately, in addition to the increase in the number of people reporting gluten sensitivity diseases, autoimmune disease, diabetes, blood pressure disease, obesity, childhood allergies and autism have increased drastically. Whether these diseases are prominently increasing universally, or they have recently been recognized are unknown. What is known is that the immunity of the human race has been impaired. Due to this reason, antibodies become mistargeted and awaken disorders. Financially and commercially, there is an industry which thrives to brag on gluten-free diet. In my opinion, instead of spending catastrophic amount of money on gluten-free diet, improving lifestyle and choosing genetically healthier grains for consumption which may also ameliorate our microbiome, resulting in stronger immune systems, is a distinctive solution.

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Experts of the Future Discuss - 2
Ideal Nutrition

With Gluten? Gluten Free? Gluten Free?

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Of Course Gluten Free,

In recent years, the effects of gluten on health have been widely debated. With the increase in conditions such as celiac disease and gluten sensitivity, there has been significant awareness regarding the health benefits of a gluten-free diet. In light of these discussions, the notion that a gluten-free diet could be a healthy lifestyle choice not only for individuals with these conditions but also for a broader audience has gained traction.

Gluten is a group of proteins found in grains such as wheat, barley, and rye (1). These proteins can be quite difficult for the body to digest and may trigger adverse immune responses in some individuals. Particularly, individuals with celiac disease can experience severe intestinal damage when they consume gluten. For these individuals, gluten-containing foods damage the intestinal mucosa, hindering nutrient absorption and leading to chronic health issues over time. However, research indicates that gluten may affect a broader population beyond those with celiac disease (2). These studies have shown that gluten can increase intestinal permeability, leading to inflammation and negatively impacting the digestive system.

Another adverse effect of gluten is its potential to harm the gut microbiota. A healthy gut microbiota is vital for immune system functions, nutrient absorption, and mental health. However, gluten consumption can disrupt the balance of beneficial bacteria in the gut, leading to a condition known as ‘leaky gut’ (3). This condition increases the permeability of the intestinal wall, allowing toxins, undigested food particles, and pathogens to leak into the bloodstream. Consequently, this process can activate the immune system, triggering inflammation and contributing to the development of chronic diseases. Given these detrimental effects of gluten on gut health, which can lead to systemic health issues in the long term, limiting or completely eliminating gluten consumption may be an important step in maintaining overall health.

The adverse effects of gluten are not limited to the digestive system; it is also thought to negatively impact the brain and nervous system. Research has shown that gluten can lead to neurological disorders, headaches, migraines, and even movement disorders known as ‘gluten ataxia’ in some individuals (4). Gluten can adversely affect the gut-brain axis, triggering symptoms such as mental fog, attention deficit, and anxiety. Additionally, inflammatory processes associated with gluten consumption can disrupt the body’s immune response, causing long-term damage to both the brain and other organ systems. Therefore, gluten should be considered a risk factor that threatens not only the digestive system but overall health (5).

There is evidence suggesting that the risk of metabolic syndrome, a major public health issue worldwide, can be reduced through a gluten-free diet. One of the main reasons for this is that a gluten-free diet often promotes the consumption of healthier and less processed foods. When fresh fruits, vegetables, healthy fats, and lean proteins are consumed, a gluten-free diet has been observed to help regulate blood sugar levels, improve insulin resistance, and aid in weight control. Additionally, individuals who follow a gluten-free diet tend to avoid packaged, processed, and overly refined carbohydrates, which is noted to have positive effects on cardiovascular health (6).

The benefits of a gluten-free diet are not limited to gut health alone. Many individuals report having more energy, experiencing fewer skin problems, and maintaining a more balanced mood when following this diet (7). Additionally, there is evidence that a gluten-free diet can reduce inflammation levels in the body. Chronic inflammation plays a significant role in the development of diabetes, heart disease, and certain types of cancer. Therefore, considering that gluten can trigger inflammation in the body, a gluten-free diet is thought to help prevent such chronic diseases.

In conclusion, a gluten-free diet is beneficial not only for individuals with celiac disease but also for those with gluten sensitivity and those seeking to improve their overall health. Considering the adverse effects of gluten on gut health, inflammation, and metabolic syndrome, it is plausible to argue that a gluten-free diet can yield positive outcomes for both the digestive system and general health. Today, a gluten-free diet stands as the most robust alternative for those aiming to lead a healthier, more energetic, and disease-free life.

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SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY
Antifungal Susceptibility Testing of Candida in Routine Microbiology Laboratory: Current Practices

Why do I need to Perform Antifungal Susceptibility Tests for *Candida*?

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The genus *Candida* is the most common causative agent of opportunistic invasive fungal infections worldwide. Factors related to the host, the infecting fungus, and the antifungal drug in use have impact on clinical outcome in invasive *Candida* infections. Antifungal resistance of the infecting *Candida* strain stands as one of the major factors in this multifactorial setting and antifungal susceptibility testing has proven to be beneficial for guidance of therapy and prediction of clinical outcome. While the most commonly isolated *Candida* spp. globally are *C. albicans*, *C. glabrata* (*Nakaseomyces glabratus*), *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, many other *Candida* species, including rare or emerging ones that display reduced susceptibility or resistance to antifungal drug(s) are also being increasingly observed as etiological agents of candidiasis. Emergence of antifungal resistant species may be driven by new immunosuppressed populations, climate change, and antifungal exposure. The recent remarkable example regarding the emergence of resistant species is *Candida auris*, which is included in WHO Fungal Priority Pathogens List and may lead to hospital outbreaks. *C. auris* displays high rates of resistance to fluconazole, moderate rates of resistance to amphotericin B, and low rates of resistance to echinocandins. Pan-resistant *C. auris* strains have also been reported. In addition to emerging resistant species, increase in secondary antifungal resistance rates for some common species of *Candida* also renders the implementation of antifungal susceptibility tests mandatory in routine laboratory practice for guidance of treatment and patient management. Examples of the latter include increasing fluconazole resistance in *C. parapsilosis* strains in some centers and areas and emergence of echinocandin resistance (in addition to fluconazole resistance) in *C. glabrata*. Not all clinical *Candida* strains need to be tested for in vitro antifungal susceptibility. For patient management, antifungal susceptibility testing is particularly recommended for all *Candida* strains isolated from blood, body fluids and sterile samples. Also, it is useful to perform antifungal susceptibility testing for strains isolated from patients exposed to antifungal drugs or from cases with clinical failure. In addition, it is beneficial to test strains that belong to rare and emerging species or species that are known to be resistant or less susceptible to antifungal drugs. Regarding isolates of superficial infections, antifungal susceptibility testing can be recommended in cases who have failed to respond to antifungal therapy or has relapsing infection. Awareness of antifungal resistance in *Candida* and utility of reliable methods for determination of antifungal susceptibility profiles are crucial for rational patient management. Access to Mycology Laboratory and antifungal susceptibility testing expertise remain as a challenge in this respect in many centers and areas and need to be improved for good laboratory and clinical practice.

SOCIETY FOR CLINICAL MICROBIOLOGISTS of TURKEY

Antifungal Susceptibility Testing of Candida in Routine Microbiology Laboratory: Current Practices

Reference Methods vs. Commercial Systems: Principles and Challenges

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Medical mycology is gaining traction given to the increasing number of patients at risk of invasive fungal infections. The increase in the number of vulnerable patients goes along with an increase in antifungal resistance rates. Given the limited number of antifungal agents available for the treatment of patients with severe mycoses, antifungal resistance is a real threat. Considering the current threats, detection of antifungal resistance in the clinical microbiology laboratory is a must. Standard methods developed by CLSI or EUCAST are appropriate to detect antifungal resistance, however they are time consuming and require skilled staff. Alternatively, commercial methods are widely used in the clinical setting. The current presentation will give an overview of the pros and cons of standard procedures and commercial methods. Pitfalls and strengths of abovementioned methods will be also discussed in a practical way for clinical mycologists.

AZERBAIJAN CLINICAL LABORATORY SPECIALISTS PUBLIC UNION
Clinical Use of Tumour Markers

Quality Requirements in the Clinical use of Tumor Markers

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Tumor markers are crucial in oncology for cancer screening, diagnosis, prognosis, and monitoring treatment responses. To ensure their clinical effectiveness, stringent quality requirements must be met across all phases of their application: pre-preanalytical, preanalytical, analytical, and postanalytical.

Preanalytical Quality: Firstly in pre-preanalytical phase requestors should be aware of the lack of sensitivity of most tumor markers, particularly for early-stage disease, their lack of specifity for a particular cancer, and the numerous non-malignant conditions in which they may be increased. Unfocused requests such as “tumor marker screen” or “malignancy?” from emergency departments and other receiving units should be actively discouraged and met with offers of educational support.

The preanalytical phase covers specimen collection, handling, transport, and storage. Standardized procedures ensure the integrity of the sample, with careful attention to factors such as sample type, timing, and proper labeling to avoid errors.

Analytical Quality: The analytical phase requires validated assays with high sensitivity, specificity, and reproducibility. Methods like immunoassays, mass spectrometry, or PCR are used depending on the tumor marker. Regular calibration, quality control, and proficiency testing are essential to maintain accuracy and reliability.

Postanalytical Quality: This phase focuses on interpreting and reporting tumor marker results. Interpretation must consider patient-specific factors, tumor biology, and clinical context. Reports should include numerical values, reference ranges, assay methods, and interpretative comments, ensuring that results are actionable and relevant to patient care.

Conclusion: Adherence to rigorous quality standards across all phases—from pre-preanalytical to postanalytical reporting—ensures the accurate and reliable use of tumor markers in clinical practice. This comprehensive approach enhances patient care, enabling early detection of recurrence and tailored treatment strategies.

AZERBAIJAN CLINICAL LABORATORY SPECIALISTS PUBLIC UNION
Clinical Use of Tumour Markers

Tumor Markers in Gynecological Oncology

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Tumor markers, also known as oncomarkers, are specific molecules synthesized directly by tumor cells or by non-tumor cells in response to the presence of a tumor. These markers can be detected in the blood and other body fluids. Based on their chemical composition, tumor markers can be proteins and their fragments, enzymes, carbohydrates, or hormones. Gynecological malignancies, such as ovarian, cervical, vulvar, and endometrial cancers, account for 20% of the 5.1 million new cancer cases in women each year, with 2.9 million cancer-related deaths. Serum tumor markers play a crucial role in cancer diagnosis, treatment monitoring, follow-up, and the detection of metastasis and relapse. An ideal tumor marker should: 1. Be specific to only one type of cancer and be synthesized by all cells within that cancer. 2. Be secreted only during cancer, distinguishing the malignant state from the benign state. 3. Be synthesized in measurable amounts in body fluids. 4. Provide information about tumor localization. 5. Have levels that correlate with tumor size. 6. Assist in determining the therapy and prognosis of the disease.

Although several tumor markers are used in gynecological oncology, CA125, HE4, SCC, and beta-HCG remain particularly relevant. The CA125 tumor marker (Carbohydrate Antigen, Mucin 16, MUC16) is a glycoprotein tumor marker. Normally, it is found in the epithelium of the fallopian tubes, endometrium, endocervix, and occasionally in the healthy tissues of the eye, pleura, pericardium, and peritoneum. CA125 has excellent diagnostic value for ovarian carcinoma recurrence HE4 (Human Epididymal Protein) is an oncomarker used in the evaluation of ovarian and endometrial cancer. This marker is synthesized in small amounts in the epithelium of reproductive organs, the upper respiratory tract, glandular cells of the mammary gland, distal renal tubules, and salivary glands. The combined use of HE4 and CA125 oncomarkers enhances monitoring. The ROMA index is used to assess the risk of developing ovarian cancer. There is no specific tumor marker for cervical cancer; however, the Pap smear test is used to detect cervical cancer at an early or preinvasive stage. Tumor markers that may be elevated in cervical cancer include SCC (squamous cell carcinoma antigen) and VEGF (vascular endothelial growth factor). Conclusion: Serum tumor markers play a significant role in the late stages of gynecological cancers, but their use in the early stages is limited due to their low sensitivity and specificity. Oncomarkers alone are not sufficient; they should be used in conjunction with clinical and other instrumental examination methods in the diagnosis, follow-up, and treatment of cancer.

Keywords: Tumor, gynecological cancer, tumor markers.

Experts of the Future Discuss - 3
Screening for Risk of Coronary Artery Disease

Non HDL Cholesterol / Apo B Which is More Guiding?

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Calculated by subtracting HDL cholesterol from Total Cholesterol (TC), non-HDL-C represents the cholesterol in all particles causing cardiovascular disease, that is, LDL, VLDL, IDL, and Lp(a); in the nonfasting state this additionally includes the cholesterol in chylomicrons and their remnant particles.

In general, LDL-C, non-HDL-C, and ApoB concentrations are very highly correlated. As a result, under most circumstances, they provide very similar information about Atherosclerotic Cardiovascular Disease (ASCVD) risk. However, under certain circumstances—including among people with elevated triglyceride(TG) levels, diabetes mellitus(DM), obesity, or very low achieved LDL-C levels—the calculated or directly measured LDL-C level may underestimate the total concentration of cholesterol carried by LDL and, thus underestimating the risk of ASCVD. Considering the potential inaccuracy of LDL-C in dyslipidaemia, among patients with DM or high TG levels, and in patients with very low LDL-C levels, measurement of both ApoB and non-HDL-C is recommended as part of routine lipid analysis.

The relationship between non-HDL-C and CV risk is at least as strong as the relationship with LDL-C. Non-HDLC provides a more comprehensive risk assessment than LDLC in certain individuals with hypertriglyceridemia because it adds RemnantC to LDLC. Therefore, takes into account the atherogenic potential of remnant lipoproteins. Non-HDL-C levels contain, in essence, the same information as a measurement of apo-B plasma concentration.

The 2021 European Society of Cardiology (ESC) guidelines on cardiovascular disease prevention highlight the importance of non-HDL-C by replacing total cholesterol with non-HDL-C in the risk chart. Non-HDL-C is used as an input in the Systemic Coronary Risk Estimation 2 (SCORE2) risk algorithm, not apoB. The new guidelines also state that non-HDL-C is a reasonable alternative treatment goal for all patients, particularly for those with hypertriglyceridemia or diabetes.

The clinical effectiveness of LDLC-guided management of cardiovascular risk is most strongly evidence-based. All guidelines concur that LDLC remains the primary target of lipid-lowering strategies to prevent ASCVD. Implementation of apoB assays in follow-up of lipid lowering therapies would impose healthcare systems and patients with yearly extra cost. At this point of time, there is insufficient evidence of benefit from outcome studies to support the option to replace the standard lipid profile (with calculation of cLDLC and non-HDLC) by single follow-up measurement of apoB to guide lipid-lowering therapies.

Result: Recent evidence indicates that both non-HDL-C and apoB are more accurate indices of cardiovascular risk than LDL-C, but there are no conclusive results for comparing apoB and non-HDL-C as risk markers. Non-HDL-C and apoB are similarly effective in predicting cardiovascular events. On autoanalyzers apoB assays perform using the immunoturbidimetric method. Immunoturbidimetric method can sometimes lead to non-specific binding or cross-reactivity, which may negatively affect the accuracy of the results. Non-HDL-C is easily calculated from the existing standard lipid profile, whereas apoB measurement would result in additional complexity and expense. Non-HDL-C may more accurately reveal risk in type III hyperlipoproteinemia (dysbetalipoproteinemia)

than ApoB. Dysbetalipoproteinemia is a critical exception to the rule that the lipoprotein-related risk of cardiovascular disease is directly related to plasma apoB. Our recommendation is: Laboratories should automatically calculate and report non-HDLC on all lipid profiles.

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Experts of the Future Discuss - 3

Screening for Risk of Coronary Artery Disease

Non HDL Cholesterol / Apo B Which is More Guiding

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The rising incidence of cardiovascular diseases highlights the need for effective lipid assessment tools in clinical practice. Non-HDL cholesterol (non-HDL-C) has long been used as a reliable marker for evaluating atherogenic risk due to its straightforward calculation from standard lipid panels. In contrast, Apolipoprotein B (ApoB), has emerged as a potentially more accurate predictor of cardiovascular risk. This presentation examines the comparative effectiveness of non-HDL-C and ApoB in guiding clinical decisions and screening targets of treatment.

Current research that compares the predictive capabilities of these two markers across diverse populations will be analyzed, emphasizing their relationship with cardiovascular events. While non-HDL-C offers simplicity and ease of use in clinical settings, ApoB may provide enhanced specificity, especially in individuals with conditions such as metabolic syndrome, diabetes or chronic kidney disease, where traditional lipid metrics may fall short.

Key studies will be discussed that illustrate the scenarios in which ApoB offers distinct advantages and the implications of these findings for treatment approaches will also be addressed, focusing on how emerging therapies can leverage these insights to reduce cardiovascular risk more effectively.

This presentation aims to elucidate the clinical relevance of non-HDL-C versus ApoB, advocating for a more tailored approach to lipid management. Attendees will gain insights into which parameter may be more beneficial for patient care in diverse clinical scenarios.

CONFERENCE
Rare Disease: Autism

Autism and New Pathways, Biochemical Markers, Laboratory Approach

Assos. Prof. Özlem Doğan

Ankara University School of Medicine

Autism Spectrum Disorder (ASD) is increasingly understood through its biochemical and molecular underpinnings. Several key biochemical disruptions are implicated in ASD, including oxidative stress, neuroinflammation, methylation cycle dysregulation, and hormonal imbalances. These processes affect cellular function, gene expression, and contribute to the clinical features observed in individuals with autism.

The role of biochemistry in autism is central to understanding the molecular basis of the disorder. Biochemical research allows for the identification of biomarkers that are essential for early diagnosis, better understanding of disease mechanisms, and development of targeted therapies. By studying metabolic abnormalities, such as oxidative stress or immune dysregulation, researchers can pinpoint specific pathways to target for intervention.

Oxidative stress is a significant biochemical abnormality in ASD. It involves an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these harmful molecules with antioxidants. This oxidative imbalance leads to cellular damage, particularly in mitochondrial function, which is essential for energy production in the brain. Individuals with ASD often exhibit elevated levels of oxidative stress markers and reduced levels of key antioxidants like glutathione.

Neuroinflammation also plays a critical role in ASD's biochemical profile. Studies have shown that immune cells in the brain, such as microglia and astrocytes, are chronically activated in individuals with ASD, contributing to persistent neuroinflammation. Elevated levels of pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1β, are found in the blood and cerebrospinal fluid of individuals with autism. This neuroinflammation is believed to interfere with normal brain development and ASD.

The methylation cycle is a crucial biochemical pathway that regulates gene expression and overall cellular health. In ASD, disruptions in the methylation cycle are frequently observed, with abnormal levels of key metabolites like homocysteine and S-adenosylmethionine (SAM). This cycle controls the addition of methyl groups to DNA, proteins, and other molecules, a process essential for regulating gene expression. Folate and Vitamin B12 are critical to the proper functioning of the methylation cycle.

Hormonal imbalances are also central to the biochemical profile of autism. Key hormones such as oxytocin, vasopressin, cortisol, and melatonin are frequently disrupted in individuals with ASD. Oxytocin and vasopressin regulate social behavior, emotional responses, and stress management, all of which are areas of difficulty for individuals with autism. Intranasal oxytocin therapy is being researched as a possible treatment to improve social functioning in ASD.

Liquid biopsies and the analysis of circulating exosomes provide non-invasive ways to monitor biochemical changes in autism. Next-generation sequencing (NGS) is also revolutionizing the genetic understanding of autism, enabling comprehensive analysis of genetic mutations and alterations.

In conclusion, biochemistry plays a crucial role in identifying the molecular basis of autism and offers new pathways for targeted interventions. The application of advanced techniques in biochemical research holds the promise of improving diagnosis and personalized treatments for individuals with autism.

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CONFERENCE
Rare Disease: Autism

Early Diagnosis and Treatment Options in Autism

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Autism Spectrum Disorders (ASD) is a neurodevelopmental disorder characterized by challenges in social interaction, communication, and repetitive behaviors. The spectrum nature of ASD implies a wide range of symptoms and severity, influencing individuals differently.
The etiology of ASD is multifactorial, involving a complex interplay of genetic and environmental factors.
Genetic Factors (multiple candidate genes associated with ASD, gene variants affecting synaptic function and neural connectivity), Environmental Factors (prenatal exposures- maternal infections during pregnancy, certain medications, advanced paternal age, postnatal exposures of environmental toxins (e.g., heavy metals), air pollution), Neurobiological Factors (differences in neurotransmitter systems, dysregulation of serotonin, dopamine, and glutamate pathways, abnormalities in brain structure and connectivity), Epigenetic Mechanisms (environmental influences altering gene expression without changing the DNA sequence, the interaction between genetic predispositions and environmental factors), GIS-Brain Axis (microbiome imbalances (dysbiosis) potentially affecting behavior and metabolism, gastrointestinal issues commonly reported in individuals with ASD).
Individuals with ASD often exhibit differences in neurotransmitter systems, particularly those involving serotonin, dopamine, and glutamate. Alterations in serotonin levels have been consistently noted in individuals with ASD, which may affect mood regulation and social behaviors. Similarly, glutamate, as the principal excitatory neurotransmitter, is crucial for synaptic plasticity and cognitive function, and its dysregulation may contribute to the neurodevelopmental anomalies seen in ASD.

Clinical Features
Social Communication and Interaction Difficulties
Stereotypes and Repetetive Motor Movements
Sensory Sensitivities
Restrictive Interests
Insistence on Sameness
Comorbid Conditions

Treatment Options
Treatment for ASD is typically multidisciplinary and tailored to the individual's needs. Interventions may include:
Behavioral Therapies: Applied Behavior Analysis (ABA)
Speech and Language Therapy
Occupational Therapy
Pharmacotherapy
Family Support and Psychoeducation

Conclusions
As the understanding of Autism Spectrum Disorders continues to evolve, it is imperative for medical professionals to remain informed about the multifaceted nature of ASD, including its heterogeneous etiology, biochemical underpinnings, and comprehensive treatment approaches. A collaborative, individualized approach that addresses both the clinical and social dimensions of ASD can significantly improve outcomes and quality of life for affected individuals and their families. Ongoing research into the genetic and environmental contributions to ASD, as well as innovative therapeutic strategies, holds promise for enhancing our understanding and treatment of this complex disorder.

Tests to Guide the Pregnancy Process

The Current Approach to Noninvasive Prenatal Screening

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Fetal malformations can be caused by genetic problems resulting from chromosomal abnormalities, monogenic disorders, or teratogens. One in every 150 live newborns has a chromosomal abnormality. The likelihood of a pregnancy being genetically impacted is increased by numerous factors, including the age of the biological mother and father, the maintenance of a shared autosomal recessive resistance, and genetic abnormalities in the family (1). The most prevalent chromosomal defect, trisomy 21, affects 1 in 800 live births. Although it is far less often, trisomies 13 and 18 can also result in a live birth (2).

In Europe, increasing gestational age over the years has increased the number of pregnancies affected by trisomies 21, 18 and 13. Despite this, the number of live births with trisomies has remained stable since 1990 due to widespread prenatal screening and termination of affected pregnancies (3).

Multiple screening tests, including biochemical screening in maternal serum, DNA testing, and ultrasound for chromosomal abnormalities, single gene defects, and structural congenital defects, are recommended for pregnant women (4). Prenatal screening can be performed using a single marker or several markers together. In some cases, pre-test risk factors are also included in the calculation. In addition to Down syndrome, the majority of screening programs cover additional prevalent trisomies like Edwards and Patau. The most commonly used markers for Down syndrome are human chorionic gonadotropin (hCG, fβ-hCG), pregnancy-associated plasma protein A (PAPP-A), unconjugated estriol (uE3), alpha fetoprotein (AFP), inhibin A, and ultrasonographic nuchal translucency (NT) measurement. Multiple marker Down syndrome screening was initially performed in the second trimester; options such as hCG (or fβ-hCG) and AFP (double test), with the addition of uE3 (triple test), and with the addition of inhibin A (quadruple test) have emerged. In recent years, many developed countries have extended prenatal screening to the first trimester using PAPP-A, hCG (or fβ-hCG), and NT parameters. The advantages of first trimester screening include early diagnosis, better screening performance, and, if pregnancy termination is necessary, being safer and less traumatic (5).

Non-invasive prenatal testing (NIPT), which was first used in clinical trials in the United States and China in 2011, dethroned established screening methods and spread rapidly around the world, becoming available in over 60 countries (6).

There is a fundamental difference between screening tests and diagnostic tests; screening tests separate the high-risk group for a certain disease among pregnant women and then direct them to either a definitive diagnostic test or a second screening test in the second stage. Down syndrome screening tests do not diagnose the disorder, but rather direct pregnant women to invasive and expensive diagnostic procedures such as amniocentesis and chorionic villus sampling (5).

When evaluating screening tests, test characteristics should be taken into account; first of all, the test should be well defined, safe and acceptable to patients. The ideal screening test should be sensitive to detect the disease and specific enough to reduce the false-positive rate. Finally, it is important that the test is cost-effective (7).

Carrier screening is ideally performed before pregnancy, and patients may be offered ethnic or extended carrier screening. If the mother is a carrier, screening is also recommended for her partner. Women with negative screening tests should not be offered further screening tests, as this increases the likelihood of false positives. Regardless of the screening method, diagnostic testing is universally recommended in the event of a positive result (8).

FIRST TRIMESTER SCREENING TESTS

First trimester tests are screening tests performed between the 10th and 14th weeks of pregnancy using maternal serum markers and fetal NT measurement. A risk calculation is performed using free β-hCG and PAPP-A markers studied in venous blood, as well as values such as maternal age and weight, number of fetuses, history of previous pregnancy, and NT. The 1/300 rate is generally used as a limit for high risk, but this limit is determined by laboratories. The detection rate of trisomy 21 varies between 82% and 87%, with a false-positive rate of 5% (2).

In pregnancies with trisomy 21, maternal serum β-hCG level doubles compared to euploid pregnancies (2 MoM), while PAPP-A levels decrease by half (0.5 MoM). In trisomies 18 and 13, maternal serum free β-hCG and PAPP-A levels decrease. In sex chromosome anomalies, maternal serum free β-hCG levels are normal and PAPP-A levels are low (9).

SECOND TRIMESTER SCREENING TESTS

The first prenatal screening test emerged in the 1970s when maternal serum AFP levels were used to determine neural tube defects. Aneuploidy tests using maternal serum markers began in the 1980s, and the complexity of the parameters and tests used has continued to increase to the present day (8).

In the following years, other markers such as uE3, hCG, and inhibin A were added to the second trimester screening test panel as a result of research. The second trimester quadruple screening test provides a risk report on trisomy 21, trisomy 18, and NTD. It cannot provide a reliable risk assessment for trisomy 13. While uE3 and AFP are 25-30% lower in pregnancies with DS fetuses, hCG and inhibin A have a median value that is twice that of normal pregnancies. The DS detection rate with quadruple screening is 80%, and the false positive rate is 5%. In pregnancies with fetuses affected by trisomy 18, all AFP, uE3, and hCG values are reduced (10).

NON-INVASIVE PRENATAL TEST (NIPT)

Traditional methods used in screening for trisomy 21 have a detection rate of 80-90% and a false positive rate of 5%, leading to significant missed diagnoses and unnecessary invasive diagnostic tests with false positive results. Therefore, research in the last 20 years has focused on non-invasive screening tests with high detection rates and low false positive rates. Following the discovery of feto-placental cfDNA in 1997, this screening test became clinically available as a result of significant advances in DNA sequencing technology. In non-pregnant individuals, the genomic profile in plasma cfDNA provides information about the individual's karyotype and the size of each chromosome, while in the event of pregnancy, fetal cfDNA (cffDNA) is released from the placenta into the maternal plasma and can be used to detect fetal chromosomal and genetic anomalies. From the 5th week of pregnancy onwards, cffDNA, which can be detected in maternal plasma, constitutes 10-15% of the total cfDNA. The amount of cffDNA gradually increases as the weeks of pregnancy increase and is cleared from the maternal blood after birth, so there is no possibility of it remaining from previous pregnancies (6).

Following its launch in 2011, the cell-free DNA test used for NIPT swiftly gained traction in prenatal diagnostics. CffDNA is the most specific and sensitive test for common chromosomal abnormalities (chromosomes 13, 18, 21). There were no large-scale randomized, controlled trials to demonstrate the clinical validity of this test prior to its implementation in clinical practice. Later, large-scale studies in singleton, high-risk pregnancies determined the sensitivity and specificity of this test (11). Although CffDNA has a high diagnostic efficiency for trisomy 21 (99% sensitivity, 0.1% false positive), its most important disadvantage is its high economic cost (12).

In the last decade, fetal aneuploidy screening with NIPT has significantly reduced the use of invasive diagnostic methods such as amniocentesis and CVS (13). While NIPT is widely accepted as a screening test for trisomies 21, 18 and 13 in both low- and high-risk pregnancies, NIPT is not considered a diagnostic test due to the rare occurrence of false-positive results. Therefore, NIPT is not used as a diagnostic test for trisomies. Aneuploidy detection is performed with different NIPT

applications, some of which are random whole genome or massively parallel sequencing (MPS), targeted or chromosome selective sequencing (CSS) and single nucleotide polymorphism (SNP) (6).

NIPT, which provides more accurate results than traditional screening methods, also provides incidental information about genome-wide or segmental chromosome imbalances, microdeletions, microduplications, and even the maternal genome. The use and interpretation of data obtained as a result of this rapidly advancing technology can lead to complicated results for mothers and clinicians. NIPT has been included in screening programs in some countries such as Belgium, the Netherlands, the United Kingdom, and Denmark, and is covered by insurance in the United States, but is still patient-financed in most countries (14).

As a result, prenatal screening tests have evolved and expanded in availability since 1970. All pregnant women should be provided with detailed information about prenatal screening test options, the risks and benefits of each test, and how to provide the report. The cffDNA test, which has been used in recent years due to its high sensitivity and specificity rates, is not a diagnostic test; positive results must be confirmed with diagnostic tests. After being informed, the decision to have the test or not should be left to the family. In addition, families should be informed about the diagnostic tests, as prenatal screening tests are not diagnostic and are only used to select pregnant women who will be referred for diagnostic tests.

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Tests to Guide the Pregnancy Process

Prenatal Screening Tests for Adverse Pregnancy Outcomes

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The early identification of patients at increased risk of adverse pregnancy outcomes and the reduction of maternal and fetal complications by providing more intensive prenatal care are two of the main obstetric goals. Many studies have identified a correlation between circulating biochemical markers and the risk of adverse pregnancy outcomes, such as preeclampsia, spontaneous preterm birth, fetal growth restriction (FGR), and miscarriage.

Excessively high or low serum biomarkers (such as low pregnancy-associated plasma protein A (PAPP-A) and high human chorionic gonadotropin, alpha-fetoprotein, and inhibin levels) measured in prenatal chromosomal aneuploidy screening tests should be evaluated due to their association with placentation problems and an increased risk of adverse outcomes.

In pregnant women at risk for preeclampsia, the balance between angiogenic placental growth factor (PIGF) and antiangiogenic soluble FMS-like tyrosine kinase-1 (sFlt-1) is associated with placental dysfunction and is disrupted in favor of antiangiogenic factors. These molecules are measured as biochemical markers for predicting the risk of preeclampsia and are associated with FGR. The sFlt-1:PlGF ratio alone can be used as a “rule-out” test for preeclampsia.

Preterm preeclampsia can only be prevented by the prophylactic use of aspirin when started before the 16th week of gestation. Therefore, it is important to determine the risk in the first trimester. To that end, an algorithm can be employed that uses maternal factors, mean arterial blood pressure, the uterine pulsatility index (assessed by US Doppler), and serum biochemical markers for early risk stratification of preeclampsia. This model achieves a high detection rate to predict preterm preeclampsia. However, the detection rate for predicting term preeclampsia is less satisfactory.

There is no universal consensus regarding screening for adverse pregnancy outcomes as part of routine examinations for pregnant women. However, it is high time a contemporary, evidence-based approach to prenatal screening be universally adopted.

Tests to Guide the Pregnancy Process

Considerations in the Use and Evaluation of Prenatal Screening Tests

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Prenatal screening tests should be offered to all women in each pregnancy. The most common tests used are first trimester combined test and cell-free DNA screening. Before testing counseling is vital, advantages and limitations of the tests should be discussed with the parents. Prenatal diagnostic tests are usually performed in patients with fetal anomalies detected with ultrasound. Serum screening tests with some ultrasound markers like nuchal translucency have been used for decades with high sensitivity but low positive predictive value. First trimester combined screening and second trimester serum screening can be used as either integrated, sequential or contingent screening providing increased screen positive rates. However, increased false positive rates cause emotional distress and unnecessary invasive procedures with a risk of fetal loss. Noninvasive prenatal screening with cell-free DNA has been used for more than a decade with extremely high sensitivity and low false positive tests, especially in trisomy 21. This test can be done from nine weeks of gestation and can detect many anomalies other than common trisomies such as microdeletions and single gene disorders.

Primary cell-free DNA screening is offering this test as the first screening test. In some countries, this approach is being used with high sensitivity and positive predictive value. However, there are some important concerns including cost, inappropriate pretest counseling and pregnancy termination without a diagnostic test. Secondary cell-free DNA screening involves offering this test to high and moderate risk groups after a serum screening. A major advantage of this approach is decreased unnecessary invasive tests while it relies on the sensitivity and specificity of the initial screening test which is lower than the cell free-DNA test.

Laboratory in Hemophilia in the Light of Current Treatments

Laboratory Diagnosis and Monitoring of Hemophilia

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Hemophilia is an X-linked congenital bleeding disorder characterized by a deficiency of coagulation factors VIII and IX, which are collectively referred to as hemophilia A and B, respectively. These deficiencies result from pathogenic variants of clotting factor genes. Individuals with hemophilia experience prolonged bleeding following injuries, tooth extraction, and surgical procedures (1, 2).

The diagnosis and frequency of bleeding episodes were correlated with factor clotting activity. In severe hemophilia, clotting factor levels are below 1 IU/dL, in moderate hemophilia, between 1 and 5 IU/dL, and in mild hemophilia, between 5 and 40 IU/dL (1, 2).

Hemophilia is characterized by prolonged bleeding caused by insufficient levels of coagulation factors VIII or IX, which are involved in the intrinsic pathway. Consequently, a deficiency in coagulation factors leads to an extension of the intrinsic pathway, as evidenced by the prolongation of activated partial thromboplastin time (aPTT) (2, 3).

The diagnosis of hemophilia A in male patients is based on reduced factor VIII clotting activity and normal functional von Willebrand factor levels. In hemophilia B, diagnosis is based on reduced factor IX clotting activity. Laboratory findings typically show a normal platelet count, prolonged aPTT, and normal prothrombin time (PT) (2). aPTT is a clot-based assay used to assess the activity of intrinsic and common coagulation pathways. It is initiated by the activation of contact factors (e.g., high molecular weight kininogen, prekallikrein, and factors XII and XI) through the addition of inorganic or organic activators (e.g., kaolin, micronized silica, ellagic acid, or vegetable phosphatides). Prolonged aPTT suggests insufficiency in the intrinsic pathway, possibly due to deficiencies in factors XII, XI, IX, or VIII. Additionally, prolonged aPTT can indicate deficiencies in the common pathway (factors V, X, II, or fibrinogen) or the presence of inhibitors or anticoagulant medications like heparin [3, 4].

The impact of the measurement techniques on the aPTT was also considered. The availability of reagents with varying sensitivities, the use of different manufacturer-recommended incubation times, and the use of disparate coagulometers, each with distinct calibrators, activators, measurement techniques, and phospholipid sources (3, 4), can also influence the outcome.

Factor assays

Factor assays are essential tools for determining the severity of hemophilia, assessing coagulation status before surgery, and optimizing prophylactic treatment. A decrease in factor activity during treatment can indicate the development of inhibitors. Factor assays can be performed using one-stage, two-stage, or chromogenic methods (4).

To obtain accurate factor activity results, proper handling, processing, and storage of samples are crucial. Samples should be collected within 4 hours after collection or frozen for later analysis. An alternative option would be to store the samples at a temperature of ≤ −24°C for a period of no longer than two months or at a temperature of −74°C for up to 18 months. Implementing appropriate quality assurance procedures ensures reliable results, proper documentation, and effective communication with clinicians.

The one-stage clot-based assay, which is primarily based on aPTT, is the most frequently used method in clinical laboratories. One-stage assays can be based on aPTT or PT, depending on the specific factor being evaluated. In this assay, dilutions of patient plasma are mixed at a 1:1 ratio with factor-deficient

plasma. Factor-deficient plasma lacks only the specific factor being measured but contains approximately 100 IU/dL of all other coagulation factors. This mixture ensured that the clotting time is dependent on the level of the deficient factor in the patient's plasma. APTT-based one-stage assays are used to evaluate the activity of intrinsic factors, whereas PT-based assays were primarily employed to assess factor VII and factors involved in the common coagulation pathway. The factor activity was subsequently determined using a calibration curve [5].

Several aPTT reagents exhibit varying sensitivities for detecting coagulation factor deficiencies, estimating lupus anticoagulants, and assessing heparin sensitivities. Although most aPTT reagents produce abnormal results in patients with moderate or severe hemophilia, not all reagents are capable of accurately identifying mild hemophilia. Therefore, selecting an aPTT reagent that can detect all types of hemophilia, ranging from mild to severe, is crucial for accurate diagnosis [4].

The two-stage assay is another method for evaluating factor activity. In the first stage, activated factor X (FXa) is generated, corresponding to the number of clotting factors present in the patient's plasma. The second stage measures the number of FXa produced, with the clotting time proportional to the factor concentration in the sample. Two-stage assay is less affected by interference from heparin or direct thrombin inhibitors than the one-stage assay. However, the presence of direct FXa inhibitors can influence the results. The chromogenic factor activity assay operates on a similar principle with two stage assay. Chromogenic factor VIII assays are generally more expensive than one-stage clot-based factor VIII activity assays [6].

Inhibitors

A higher prevalence of inhibitor is observed among individuals with hemophilia of Black and Hispanic ethnicity. Approximately 30% of patients with severe hemophilia A develop alloimmune inhibitors against factor VIII, typically within the first 20 exposures to the infused factor. In a smaller subset of cases, this phenomenon has been reported in individuals with more than 50 exposures [1, 7].

Acquired hemophilia (AH)

AH is a rare disorder characterized by the presence of autoantibodies that inhibit factor VIII activity. However, cases involving other coagulation factors, such as V, VII, IX, X, XII, and XIII, have also been reported. Diagnosis of AH should be considered in patients presenting with new-onset bleeding and an isolated prolongation of aPTT. The diagnosis of acquired hemophilia A (AHA) is based on decreased factor VIII activity and the presence of neutralizing anti- factor VIII antibodies, also referred to as inhibitors. In approximately 50% of AHA cases, the cause remains unknown, whereas the remaining cases are often associated with underlying malignancies, infections, drug therapies, or autoimmune diseases, such as rheumatoid arthritis [1, 7, 8].

A mixing study should be conducted on samples exhibiting prolonged aPTT to determine the underlying cause. This involves mixing the patient's plasma with normal pooled plasma (NPP) at a 1:1 volume/volume ratio. According to the CLSI guidelines, NPPs should consist of a pool of at least 20 donors with a coagulation factor profile containing >80% of all factors, and it may include either fresh frozen or lyophilized plasma with a platelet count of <10,000/uL. After mixing, the aPTT was repeated, and the result approached the reference range, indicating clotting factor deficiency. The presence of coagulation inhibitors, such as anti- factor VIII inhibitors, lupus anticoagulants, or pharmacological anticoagulants like heparin, can prevent the correction typically observed in mixing studies [7, 10].

To enhance the detection sensitivity of specific factor inhibitors, such as factor VIII inhibitors, the mixed sample should be incubated at 37°C for 1–2 h. Some studies have shown that the percent correction in a 4:1 (patient plasma: NPP) mixture provides adequate sensitivity and specificity for identifying anticoagulant and factor deficiencies [11].

Inhibitors are neutralizing antibodies, primarily of the IgG class, that bind to coagulation factors in the plasma of patients with hemophilia or healthy individuals, thereby disrupting the function of factor VIII in the circulation. The detection and quantification of these inhibitors can be performed using antigen-antibody assays, such as ELISA, or functional and coagulation-based methods, including the Nijmegen and Bethesda assays. Inhibitor levels are expressed in Bethesda units (BU), with one BU

representing the amount of factor VIII inhibitor necessary to neutralize 50% of the factor VIII activity in plasma after a 2-hour incubation at 37°C.

Bethesda Assay

The patient's plasma was serially diluted in buffered pooled plasma (PP), and a control sample was prepared by mixing PP with factor-deficient plasma at a 1:1 ratio. After incubation, residual factor activities were evaluated and compared with those of the control. For factor VIII inhibitor testing, the incubation period lasted 2 h at 37°C; however, prolonged incubation was not necessary for factor IX inhibitor testing. The range of valid residual activities (RAs) was limited to 75%–25%, which is necessary for testing sample dilutions when inhibitor titers exceed 2 BU/mL [12].

Nijmegen-modified Bethesda assay

Nijmegen modification improves sensitivity and specificity by buffering normal plasma and substituting the diluent buffer with inhibitor-free factor VIII-deficient plasma [7].

The World Federation of Hemophilia Recommendations

In the laboratory evaluation of patients with clinical suspicion of hemophilia A, the World Federation of Hemophilia (WFH) recommends using both the one-stage factor VIII assay and the chromogenic factor VIII assay as part of the primary diagnostic workup. Both methods should be used even if one of the assays indicates normal factor VIII activity.

The WFH also advocates the use of multiple factor VIII assays to assess the full spectrum of mild hemophilia A. In genetically confirmed mild hemophilia A cases, a one-stage method may yield a normal factor VIII activity result. However, this may not reflect the complete clinical picture, as reduced activity may be detected using chromogenic or two-stage clotting assays. The reverse scenario is also possible.

The one-stage factor VIII method requires the use of factor VIII-deficient plasma, containing less than 1 IU/dL (<1%) factor VIII activity while maintaining normal levels of other clotting factors that can affect aPTT (such as fibrinogen, factors II, V, IX, X, XI, XII).

Additionally, the WFH recommends that aPTT results within the normal range should not be used to exclude the possibility of mild hemophilia A or B in patients with clinical suspicion of the disorder. It is important to recognize that the levels of factor VIII and von Willebrand factor may temporarily increase owing to strenuous exercise, stress, or inflammation, potentially affecting the accuracy of diagnosis. Moreover, factor VIII and von Willebrand factor levels increase during pregnancy.

Significant inter-pool variability exists in the concentration of clotting factors in pooled normal plasma. To maintain continuity and traceability, the system of international units (IUs) has been established. Factor levels are expressed in international units per milliliter or deciliter (IU/dL), which are not directly interchangeable with percentages of pooled normal plasma [2].

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FEDERATION of TURKISH PATHOLOGY SOCIETIES
Pathology Practice: Current Applications and Future Perspectives

From Morphology to Molecular Diagnostics

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Pathology is positioned between basic and clinical sciences in medicine. Diagnostic pathology is a science that is a continuation of morbid anatomy, with the use of the microscope and the foundations laid on morbid histology, and is a science that examines the etiology, pathogenesis and prognosis of diseases. The examination of tissues, organs, body fluids and autopsies under the microscope to diagnose diseases continued for about 150 years. During this period, the practice of pathology did not change much and morphology remained the most important diagnostic guide. Later, with the discovery of histochemical methods and antibodies, the use of immunohistochemical techniques entered a new phase. Today, with the widespread use of digital technologies, artificial intelligence applications, -omics technologies, better understanding of the pathogenesis of diseases, and the introduction of targeted therapies into the clinic, biomarker-based molecular methods have caused the routine pathology practice to evolve appropriately and transform into today's needs.

FEDERATION of TURKISH PATHOLOGY SOCIETIES
Pathology Practice: Current Applications and Future Perspectives

How Artificial Intelligence transforms the world of pathology

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The field of pathology is undergoing a revolutionary transformation driven by advancements in digitalization, computational methods, and AI. This presentation will explore how these technologies enhance, extend, and transform the traditional pathology processes to improve diagnostic accuracy, efficiency, and patient outcomes.

We will begin by discussing how AI and computational methods enhance existing pathology workflows. Key topics will include improved image analysis, automated biomarker quantification, data integration, standardization, workflow optimization, enhanced reporting, quality control, training and education for pathologists.

Next, we will explore the completely new capabilities that AI and computational methods are adding to pathology. This section will cover digital pathology platforms, AI-assisted triage, virtual microscopy, telepathology, AI-driven research, predictive modeling, and automated documentation.

We will then examine how traditional pathology processes are being replaced with more efficient and accurate methods. Topics will include transition from manual to automated image analysis, traditional microscopy to digital pathology, subjective assessment to quantitative analysis, conventional staining to multiplex imaging, AI-generated reports, multi-omics integration, AI-based tumor grading, and AI predictive models.

Finally, we will discuss how AI and computational methods are bypassing the existing processes to save time, reduce costs, improve effectiveness and quality.

The presentation will conclude with a discussion on the future directions of AI and computational methods in pathology, emphasizing the potential for continued innovation and improved patient care. Attendees will gain a comprehensive understanding of how these are reshaping the field and the practical implications for clinical practice and research.

Keywords: Pathology, Artificial Intelligence, Computational methods, Digital Pathology

CONFERENCE
Nutrition and Metabolism

Protein Energy Malnutrition and Refeeding Syndrome with Laboratory Test

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The global nutrition crisis we faced even before Covid-19 has worsened, with worrying trends across every form of malnutrition, from hunger to obesity. There has been an increasing prevalence of nutritional issues, particularly protein-energy malnutrition (PEM) and micronutrient deficiencies. The World Health Organization defines malnutrition as "the cellular imbalance between the supply of energy and nutrients and the body's demand for them to maintain, grow, and perform specific functions." Loss of body stores and protein mass are the cardinal features of the group of syndromes collectively referred to as protein-energy malnutrition or PEM. During periods of negative energy balance, endogenous energy sources are consumed to provide fuel for metabolic reactions.

As the body is depleted of glycogen, fat, and protein, body weight declines andlosses of protein-related cellular functions occur. Protein deficit may be due to absolute lack of protein, lack of sufficiently complete proteins, and/or imbalance between carbohydrates and proteins.The term protein–energy malnutrition pertains to a group of allied disorders that includes kwashiorkor, marasmus, and intermediate states of kwashiorkor-marasmus. No clear distinction has yet been made between growth failure (marasmus and undernutrition) and edematous malnutrition (kwashiorkor and marasmic kwashiorkor) in terms of pathology and clinical conditions.

Clinical Syndrome:
- Kwashiorkor (predominantly protein deficit)
- Marasmus (predominantly energy deficit)

Pathogenesis of PEM; decreased secretory function of digestive glands, failure of cavitary and parietal digestion, dysbiosisdevelopment, changes in protein metabolism and degradation of tissue proteins, hypoproteinemia, abnormal protein fraction ratios, increased excretion of aminoacids with urine, negative protein balance, exhaustion of glycogen, fat, mineral substance storage andultimately changes in main metabolism, leading to exhaustion.Main clinical symptoms in children are retardation or arrest of body weight gaining.PEM diagnosis is made in a multidisciplinary manner. The team includes a nurse, a dietitian, a clinician, and a clinical biochemist.Diagnosis of PEM involves anthropometry, clinical evaluations, biochemical examinations, and dietary assessment. The most helpful laboratory studies in assessing malnutrition in a child are hematological analyses and laboratory parameters evaluating protein status. Hematological studiesshould include totallymphocytecount with erythrocyte indices and peripheral smear. Measures of protein nutritional statusincludeserum albumin, prealbumin, retinol-binding protein, transferrin, creatinine, and BUN levels. Laboratory markers of malnutrition other than visceral proteins areurinary creatinine,urinary 3-Methylhistidine, and delayed hypersensitivity.

Refeeding syndrome describes the metabolic and clinical changes attributed to aggressive rehabilitation of malnourished subjects. The metabolic changes of refeeding are related to hypophosphatemia, hypokalemia, hypomagnesemia, sodium retention and hyperglycemia, and these are believed to be mainly the result of increased insulin secretion following high carbohydrate intake. In the past few decades, increased consumption of processed food lowered the intake of several macrominerals (mainly phosphorus, potassium and magnesium). This seems to have compromised the postprandial status of these macrominerals, in a manner that mimics low-grade refeeding syndrome status. At the pathophysiological level, this condition favored the development of the different

components of the metabolic syndrome. Thus, it is reasonable to postulate that metabolic syndrome is the result of long-term exposure to a mild refeeding syndrome.
Management of high-risk patients involves starting nutrition slowly, restoring circulatory volume, providing thiamine and multivitamins, and supplementing electrolytes. Close monitoring is essential for effective management.It is very important to recognize and take steps to avoid it.