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**Congress Abstracts**

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# Response and Mitigation Strategies for the Ransomware Attack on the Modena Local Health Authority

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**Introduction**

This abstract examines the measures adopted by the Modena Local Health Authority (Azienda USL di Modena) following the ransomware attack on 28 November 2023. The organisation, located in Emilia-Romagna, Italy, with over 5,400 employees, serves a large regional area with a population of 690,000 inhabitants. The increasing incidence of ransomware attacks, which block access to essential data until a ransom is paid, is severely impacting healthcare facilities, compromising both patient data security and service efficiency, with serious consequences for public health.

**Incident Identification and Response**

On 28 November 2023, at 10:00 pm, a report regarding access issues with the Radiology Information System/Picture Archiving and Communication System (RIS/PACS) in the radiology department allowed the detection of the ongoing ransomware attack. Cybersecurity technicians from the Security Operation Center, in collaboration with company technicians, managed to limit the malware's spread.

To maintain operations, the organisation adopted measures such as the use of paper documentation, manual management of patient identification wristbands, and the use of word processing systems on PCs.

The attack affected various facilities due to the extensive integration of company networks, including the Local Health Authority, the Hospital-University Company, and the Sassuolo Spa Hospital, all interconnected within the Modena healthcare network.

The following day, support arrived from the Computer Security Incident Response Team (CSIRT) of the National Cybersecurity Agency (ACN). A team was formed consisting of technicians from the affected companies, cybersecurity experts, and CSIRT members, operating under ACN directives.

The reactivation prioritised central systems and those critical for clinical and automated processes, following strict security procedures and minimising recovery times. Concurrently, a forensic analysis was conducted to reconstruct the events and collect evidence.

On a dark web portal, a ransomware gang claimed the exfiltration of 1,202,175 files (954.7 GB) from the Modena Local Health Authority, demanding a ransom of 3 million euros to avoid disclosure. The criminal organisation followed through on the threat by publishing the data. Throughout the incident, the public was continuously updated via press releases, and the criminal actions were promptly reported to the competent authorities and the Privacy Guarantor.

**Conclusions**

The ransomware attack on Modena's healthcare systems highlights the growing global threat of cyberattacks on healthcare systems. Investigations into the incident are ongoing, but it is clear that the event has accentuated the risk of attacks in a modern context. This situation underscores the critical importance of developing advanced security systems to protect citizens and ensure the confidentiality of their sensitive data.

**Acknowledgements**

All authors contributed equally to this work.

We sincerely thank all those who participated in mitigating the impact and restoring our healthcare systems, ensuring the efficient restoration of public service.

# Management of a laboratory during a cyberattack

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Cyberattacks on healthcare facilities are increasing exponentially and can result in a kaleidoscope of serious consequences, potentially impacting patient care, compromising sensitive medical data and disrupting essential healthcare operations.

Operating a laboratory during a cyberattack is particularly challenging as it requires a combination of preparedness, resilience and rapid response. Some steps can be identified to mitigate the impact of a cyberattack on laboratory operations. First, the laboratory needs to develop a cybersecurity incident response plan that includes a number of well-documented activities outlining the steps to take in the event of such an incident. This plan should include employee roles and responsibilities, communication protocols, and steps to contain and mitigate the attack. All employees must then be trained in cybersecurity best practices and know how to recognize and respond to potential threats. Regular training can help to reinforce good cybersecurity habits. The proactive implementation of strong security measures such as firewalls, intrusion detection systems, anti-virus software and data encryption would then allow the laboratory's systems and data to be protected. Critical data and systems must be regularly backed up to offline or cloud storage to ensure that important data can be restored at any time. Continuous monitoring of laboratory systems for unusual activity or anomalies can draw timely attention to a potential cyberattack (early detection can help minimize the impact of the attack). Contingency plans also need to be developed for maintaining essential laboratory operations, mostly based on manual processes or alternative systems that can be used when primary systems are compromised. Rapid resumption of service by available staff may be required to cope with the exponentially increasing burden of manual sample processing and alternative transmission of test results to requesting physicians (i.e., by fax). The availability of emergency modules for requesting laboratory tests (preferably with unique identification number and barcode) and for the transmission of test results by paper sheet must be prepared before the crisis occurs. Open communication channels with employees, stakeholders and relevant authorities must be ensured to communicate the situation, any impact on laboratory operations and measures to counter the attack. Last but not least, once the cyber-attack has been dealt with, a thorough review of the incident must be conducted to draw lessons learned and identify areas where the lab's cybersecurity posture can be improved. The policies could be updated accordingly to be better prepared for possible future incidents.

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# Secrets of successful publication of a scientific article in high-ranking journals: Data analysis

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Over the past decade, immunological studies have advanced significantly in single-cell technologies, generating extensive datasets with multiple parameters. As a result, new and sophisticated data analysis methods have been developed to facilitate precise investigation. One of the main challenges has been identifying the primary phenotypes present, which allows for an efficient and meaningful profiling of the tissue and assessing whether their frequencies correlate with clinical outcomes. Dimensionality reduction techniques such as principal component analysis (PCA), t-distributed stochastic neighbor embedding (t-SNE), and uniform manifold approximation and projection (UMAP) aid in data visualization but do not explicitly identify and categorize cells into subpopulations[1]. Additionally, not all subpopulations are visually distinct when high-dimensional data is rendered in only two dimensions. Therefore, non-parametric clustering methods like Phenograph and FlowSOM have been introduced to enable researchers to robustly identify subpopulations in high-dimensional single-cell data. Furthermore, new supervised learning methods, such as PENCIL, enable the prediction of subpopulations associated with clinical outcomes. Single-cell data allow the exploration of dynamic processes such as differentiation, disease progression, or vaccine efficacy. As a result, trajectory inference methods have been developed to map out the developmental and temporal changes that cells experience. Here, we demonstrate how the application of state-of-the-art data analysis methods can lead to insights into how disease-modifying therapies (DMTs) administered to patients with multiple sclerosis (MS) impact immune responses to SARS-CoV-2 and vaccine efficacy[2,3,4]. Despite numerous

publications in the field, there is still limited detailed data on the phenotypic, functional, and metabolic characteristics of antigen (Ag)-specific cells following the third dose of the mRNA vaccine. Using flow cytometry and 45-parameter mass cytometry, we comprehensively investigate the phenotype, function, and single-cell metabolic profile of SARS-CoV-2-specific T and B cells up to 8 months after the third mRNA vaccine dose in a cohort of 94 MS patients treated with various DMTs, including cladribine, dimethyl fumarate, fingolimod, interferon, natalizumab, teriflunomide, rituximab, and ocrelizumab[2]. By applying a high-dimensional data analysis approach, we found that almost all patients exhibit a functional immune response to SARS-CoV-2. Significantly, fingolimod- and natalizumab-treated patients exhibit distinct metabolic profiles in their antigen-specific T and B cell responses, contrasting with those observed in patients receiving other MS treatments. Lastly, employing PENCIL, a novel supervised learning approach for predictive analysis, we pinpointed SARS-CoV-2-specific $Ag^+$ T and B cells linked to symptomatic infection following the third vaccine dose[2].

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## Prerequisites for A.I. modelling or application – Expectations vs. Reality

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Artificial Intelligence (AI) has changed the way we live, maybe even without us noticing (1). These innovations are being adopted more slowly in healthcare due to strict regulations like the *General Data Protection Regulation* or the *In Vitro Diagnostic Medical Device Regulation,* and several others. However, especially in diagnostic processes using image recognition, AI is increasingly being implemented, with radiology leading the field with the most FDA approved software solutions (2).

With the recent hype of large language models such as ChatGPT, Claude, Gemini and others, expectations on revolutionizing healthcare are high. But there are many obstacles along the way from conception to implementation. At the beginning, and maybe most importantly, a large amount of high quality and “FAIR” data is needed (3). This data should be accurate, reliable, and representative of the real-world scenario for the intended use and ideally should be labelled correctly (e.g. with the correct diagnosis).

Due to the afore-mentioned data protection regulations, but also because of the data being distributed across many different IT-systems and its quality mostly relying on the correct input by medical personnel, the task of gathering the data is probably the most burdensome and the most common reason for failure. After approval by ethical committees, and data collection, the data then needs to be cleaned and pre-processed to remove duplicates, inconsistencies, and irrelevant information, while also handling missing information. Thereafter, this data can be used to adapt a pre-existing model to the intended tasks (fine-tuning), which requires a certain technical infrastructure, such as secure and fast storage solutions, reliable and secure network solutions with the necessary bandwidth if cloud services are being used, as well as an adequate software and programming environment.

Even with all of these prerequisites in place, data scientists or technically advanced IT personnel are needed to handle the data processing and fine-tuning of the selected model, who sadly are part of “a scarce breed” (4). Of course, healthcare

facilities do not necessarily need to train their own models or finetune pre-existing ones and may purchase commercially available solutions, but these, similarly to the above-mentioned prerequisites, come with a certain price tag and considering the current financial situation of healthcare systems in many countries, the path to functioning AI-based clinical decision support systems (CDSS) is quite a long and cumbersome one.

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# Experience of the SIBioC GdS in the development and validation of a multicenter model based on hematological parameters for sepsis screening

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Sepsis is a condition characterized by an abnormal reaction to infection: its early identification is critical for improving patient outcomes. Laboratory medicine is essential in detecting biomarker alterations before clinical signs and symptoms appear. In the last decade, the role of Monocyte Distribution Width (MDW) as a screening sepsis biomarker has emerged. However, MDW has low sensitivity and positive predictive value compared to other biomarkers.

Machine Learning (ML) approaches can improve the performance of traditional biomarkers by integrating several factors and, therefore, enhancing sepsis detection accuracy. Making ML models work in clinical practice, on the other hand, can be challenging. Indeed, even widely used commercially available models have been shown to generalize poorly. We performed a multi-centric study to construct and externally validate ML models for early identification and screening of sepsis using MDW and other Complete Blood Count parameters. Five patient cohorts (5344 patients) from five different Italian hospitals were used to develop six ML models. The cohorts were selected to evaluate the models' performance in various settings, including the emergency department and ICU, using different diagnostic criteria (Sepsis-2 vs Sepsis 3), inclusion criteria, and data availability. To ensure generalizability and robustness in these markedly different settings, the developed ML models adopt advanced techniques inspired by controllable AI, namely: cautious classification, which allows the ML models to avoid making predictions; and explainable AI, which provides clinicians and health operators with helpful information about how the models work.

The developed models achieved good diagnostic performance on internal validation (AUC between 0.91 and 0.98) and consistent generalization performance across external validation datasets (AUC between 0.75 and 0.95), outperforming baseline biomarkers and cutting-edge ML models for sepsis detection. Controllable AI approaches were also employed to boost performance and provide a basic, understandable set of diagnostic guidelines. Our findings show how controllable AI techniques based on CBC and MDW may be useful for early identification of sepsis, as well as how the suggested methodology can be used to create ML models that are more transportable and generalizable to different clinical settings.

## Direct-to-consumer laboratory testing (DTCT): Challenges and implications

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In paternalistic medicine, the attending physician determines the indication of medical tests only by the medical need and the rules of marketplace, the major triggers of decisions outside of healthcare, should have only negligible influence. Recently, technological progress, the self-empowerment of patients (a.k.a. “4P-medicine”), and the widespread experiences with Covid self-testing are strong triggers for Direct-To-Consumer Testing (DTCT). In DTCT, the consumer himself initiates and pays for the testing which is performed either by the consumer himself or self-collected samples are analyzed in nonmedical or medical laboratories, the latter is named DAT (direct access testing). The clear differentiation between the setting of the testing can be obscured but will affect the quality of the test results and therefore their potential use for medical decision making or for “lifestyle modification”.

Healthcare is highly regulated and numerous laws, regulations and restrictions are being enforced by the authorities to protect the patients.

Aggressive marketing including promises to optimize the own health by IVD testing draws much attention of the consumers to DTCT. It is not obvious whether the increased interest and demand in DTCT testing might have beneficial effects on laboratory testing in healthcare – or whether DTCT might be a burden for healthcare or even the society as a whole.

The intended use of IVD testing should be claimed when offering an IVD test but in DTCT this description should not contain the term healthcare. Vendors of DTCT tests achieve this by either using confusion, by negligence, or by false claims.

The user of DTCT is hardly aware of the shortcomings of DTCT. Reasons for the often-inferior quality are the selection of unsuited tests, non-scientific methods for testing (such as sink testing or bogus/quacksalver technologies), unsuited preanalytics, lack of quality control, and poor interpretation of the test results not considering the medical history and pretest probabilities.

DTCT has a higher rate of false positive results which often trigger extensive medical studies to calm down the scared consumer. One can assume that the resources spent for DTCT (from the consumer as well as from the national healthcare budget for the follow-up procedures) are not always used efficiently and that the medicalization by DTCT challenges the optimized use of the limited resources in healthcare.

Data protection is often hampered in DTCT and the customers are not aware of the obvious risks of this for themselves and – in particular in genetic testing – even for their families. Medical health records even might become worthless if hampered by unreliable DTCT data.

In summary, ethical risks of DTCT are manyfold such as by overmedicalization, wasting healthcare resources, infiltration of medical records by bogus data, and hampering the trust of patients and healthcare professionals in real laboratory test results.

## Response and Mitigation Strategies for the Ransomware Attack on the Modena Local Health Authority

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Artificial Intelligence (AI) has revolutionized and transformed the medical field, changing how healthcare is delivered and managed. AI can be involved in many different tasks. Among others, AI systems can analyze vast amounts of medical data with unprecedented speed and accuracy, offering remarkable opportunities in healthcare, especially valuable from an ethical perspective. For instance, AI can bridge gaps by providing critical assistance and prevention programs in regions with limited resources and a shortage of trained professionals, ensuring more equitable access to healthcare. AI also holds promise in research, potentially reducing the time needed to develop new treatments and advancing cutting-edge surgical techniques.

However, the application of AI comes with relevant risks, making it essential to ensure its responsible and ethical use, particularly in healthcare and other areas where AI directly impacts human lives.

Among others, a significant risk is related to informed consent and data protection. Users are often unaware of their interactions with AI tools and may not know how their personal data is used or shared with third parties.

Additionally, there is a dangerous misconception that AI outputs are neutral and flawless, contrasting with the acknowledged fallibility of human beings. While automation is captivating, it's crucial to remember that AI can contain and propagate errors and biases. These biases, absorbed and amplified by AI algorithms, can become entrenched, exacerbating existing inequalities. Moreover, the issue of bias in AI is further complicated by AI opacity (non-explainability).

Given the risks and benefits of AI, it is imperative to maintain a critical perspective and encourage its ethical, responsible, and inclusive use. This approach is vital to ensure that the advantages of AI, particularly in healthcare, benefit everyone rather than just a select few.

## The Coalition for Advancing Research Assessment (CoARA): first outcomes of a global initiative for a systemic change of research assessment

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There is a broad consensus among research communities worldwide that the existing tools of academic rewards and recognition criteria, such as h-indexes or the weight of publisher prestige, in particular if determined on the basic of indicators such as the journal impact factor, have ceased to accurately reflect what we most value in, and need from, research. A wide range of innovative, born-digital scholarship such as databases, visualisations, software development, or contributions to research infrastructures, are still invisible from formal research administration and assessment. Therefore it is essential that a qualitative-centered approach and not a quantitative one, characterizes research assessment activities. Besides, beyond focussing solely on the end products of research it is also clear that it is the integrity and transparency of research processes that lead to truly innovative, open and high-quality research. Building on progress made so far (DORA, Leiden Manifesto, Hong Kong Principles), over 700 research organisations, funders, assessment authorities, professional societies, and their associations have agreed on a common direction and principles for reforming the assessment of research, researchers and research organisations, outlined in the Agreement on Reforming Research Assessment (ARRA). They commit to a common vision, which is that the assessment of research, researchers and research organisations recognises the diverse outputs, practices and activities that maximise the quality and impact of research. This requires basing assessment primarily on qualitative judgement, for which peer-review is central, supported by responsible use of quantitative indicators. Based on these principles and committements a Coalition for Reforming Research Assessment (COARA) has been implemented offering a platform for member organisations for collaboration and mutual learning that, initially born in Europe, is now expangin globally. CoARA, founded in December 2022 currently supports 13 Working Groups and 16 National Chapters to facilitate exchange and develop resources that member organisations can rely on, for their reform journeys. Working groups, jointly collaborated by several Institutions who joined CoARA, cover a variety of topic of relevance for research assessement, e.g, are "Ethics and Research Integrity Policy in Responsible Research Assessment for Data and Artificial Intelligence" , "Towards an Inclusive Evaluation of Research", "Supporting the alignment of research assessment systems with CoARA in biomedical disciplines through administrative reforms and governance".

## Requirements needed for the implementation of an emergency urgency diagnostic network in the territory: suggestions for tender specifications

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In order to achieve the *Azienda*'s goal of decreasing improper patient access to the emergency room with hospitalization, anticipating emergency/urgency treatment in the territory, and making the best use of available human resources, the use

of portable *Point of Care Testing* (POCT) blood gas analyzers was evaluated. Such instruments can provide results of both blood gases and basic metabolites in less than a minute using room temperature CARDs. They operate via a POCT technology connected via Wi-Fi or Bluetooth to the emergency/urgency information system (EMUR), without requiring manual operations to connect, for rapid data communication between operators on duty and the 118 dispatch center. Based on the geolocation of 118 centers and a needs analysis, 51 devices were delivered to medical and demedicalized cars (named "India").

In the implementation phase of the initiative, a critical issue was addressed, related to the lack of a definition of the technical execution modalities in the tender specifications, which stated only the general objectives. This resulted in a slowdown of the processes, which could have been overcome with the inclusion of a professional figure from the IT sector in the drafting committee.

Collaboration between the corporate ICT department, which ensured the connection between networks and compliance with privacy legislation, the entity responsible for managing the tender procedures, and the supplier, which was willing to adapt the systems to the territorial typicality, proved decisive.

The informatization of the POCT processes made it possible to a) govern the entire blood gas process by the Analysis Laboratory, b) minimize possible sources of error for patient safety, and ensure maximum operator safety at all stages of the analytical process from sampling to maintenance, c) ensure traceability of the entire process from request to reporting phases, to the Health Record d) improve critical patient management and the outcome through Turn-around-time (TAT) reduction, and e) aspire to accreditation according to current regulations.

## Comparative Analysis of organizing Point-of-Care Networks in Wales (UK), France, Switzerland, and Norway

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**Background:** Point-of-care (POC) networks are critical in enhancing healthcare delivery by providing timely diagnostics and treatment options directly to patients, especially in rural and underserved areas. This study aims to compare the POC networks in four European countries—Wales, France, Switzerland, and Norway—to identify best practices and areas for improvement.

**ACCESSIBILITY:** Wales: POC networks are integrated with the National Health Service (NHS), ensuring broad accessibility. However, rural areas face challenges due to lack of health facilities. France: A centralized healthcare system allows regional customization of POC services. Accessibility is generally high, but there are strong disparities between urban and rural regions. Switzerland: A robust POC network supported by a well-funded healthcare system. High accessibility is facilitated by strong public-private partnerships. Norway: Extensive coverage due to government initiatives targeting rural healthcare. Telemedicine is widely used to enhance accessibility in remote areas.

**INFRASTRUCTURE:** Wales: Infrastructure is improving, with recent investments in mobile POC units. However, there is a need for further development in digital health records integration. Dr Annette Thomas, POCT National Lead wrote.: "*Building on the success of the informal network, one of the key actions in the National Pathology Programme Statement of intent, published in 2019, was to establish a more formal structured arrangement to deliver Point of Care Testing services in NHS Wales. A National Strategy Group of mPOCT clinical leads and POCT Managers from each Hub Board, stakeholders and government representatives was established with the aim of setting the strategy and standards, with the existing National POCT Delivery Group supporting the delivery of the service.*" France: widespread availability of POC devices in primary care settings is needed. Continuous innovation in telehealth and mobile health units.Switzerland: Highly developed infrastructure with seamless integration of digital health records and POC devices. Strong emphasis on data security and patient privacy. Norway: Comprehensive infrastructure supported by government funding. Advanced telehealth services and POC units are standard in both urban and rural areas.

**TECHNOLOGICAL INTEGRATION:** Wales: Efforts are underway to improve technological integration. Current systems are fragmented, impacting efficiency.France: High level of technological integration, supported by national e-health initiatives.

Strong focus on interoperability and data sharing. Switzerland: Leading in technological integration with extensive use of electronic health records (EHRs) and common regulatory rules. Norway: Advanced technological integration, with widespread use of EHRs and telemedicine platforms.

**CONCLUSION:** Switzerland and Norway lead in terms of infrastructure, technological integration, and patient outcomes, with Norway excelling in rural healthcare delivery. France has a robust regulatory system in public health, ensuring high standards of care and patient safety through stringent policies and oversight, while Wales is improving through recent investments and reforms. Lessons from these countries highlight the importance of government support, technological integration, and tailored approaches to rural healthcare in developing robust POC networks. Future research should focus on longitudinal studies to assess the impact of ongoing reforms and innovations in POC networks across these countries.

## Unlocking the Future of mTBI Management: Innovative Biomarkers and Value-Based Approaches

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Mild traumatic brain injury (mTBI) is a prevalent concern, both in clinical-surgical contexts (neurology, neurosurgery and emergency room) and in sports-related and military contexts, due to its subtle clinical presentation and potential long-term consequences [1]. Laboratory biomarkers have emerged as crucial tools in diagnosing, prognosis, and managing mTBI. This abstract synthesizes findings from recent publications and evaluates the introduction of innovative diagnostic technologies to provide an overview of the current state of mTBI biomarkers, highlighting their clinical utility, challenges, and the role of value-based medicine in optimizing outcomes.

Recent studies emphasize the importance of the neurofilament light (NfL) chain, tau protein, S100 calcium-binding protein B (S100B), glial fibrillary acidic protein (GFAP), and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) as potential biomarkers for mTBI. NfL and tau proteins indicate axonal damage, while S100B and GFAP are markers of glial injury and blood-brain barrier disruption. UCH-L1, an enzyme expressed in neurons, has shown promise as a biomarker for neuronal injury. Elevated levels of these biomarkers in cerebrospinal fluid (CSF) and blood correlate with the severity of brain injury and provide critical information on neural damage.

The utility of these biomarkers in sports settings is significant, given the high incidence of mTBI among athletes [2]. Biomarker analysis can aid in timely diagnosis, reducing the risk of repeated injuries and facilitating appropriate management decisions. NfL and UCH-L1, for instance, have shown promise in monitoring recovery and predicting long-term outcomes, which is essential for safe return-to-play decisions. However, clinical application faces challenges like variability in biomarker levels due to individual differences (age, sex, genetics) and timing of sample collection. Standardization of assays and normative data is crucial to enhance reliability and utility.

The Abbott i-STATTM TBI Plasma Test, approved by the FDA and CE-marked, is a significant advancement in mTBI management [2]. This technology quantifies GFAP and UCH-L1 levels in peripheral blood, providing a rapid, reliable tool for diagnosing mTBI in emergency settings. Studies indicate that this test can reduce unnecessary CT scans, decrease radiation exposure and shorten patient management time in emergency departments. The ALERT-TBI trial reported a sensitivity of 95.8% and a negative predictive value of 99.3%, suggesting a substantial reduction in CT scans among mTBI patients with negative biomarker results.

Incorporating value-based medicine into mTBI management is essential for optimizing outcomes. Value-based medicine focuses on achieving the best health outcomes relative to costs, ensuring medical practices benefit patients [3]. In mTBI, this approach emphasizes the judicious use of biomarkers to guide decisions, reduce unnecessary interventions, and allocate resources efficiently. Integrating biomarker data with clinical assessments and neuroimaging findings allows for personalized, cost-effective care.

Health technology assessment (HTA) plays a crucial role by systematically evaluating the medical, social, economic, and ethical implications of health technologies [4]. The new EU HTA regulation, from January 2025, aims to ensure inclusion, transparency, and predictability in evaluating health technologies, thereby improving access to innovative

diagnostics [4], like the Abbott i-STATTM TBI Plasma Test. HTA helps in assessing the value of these biomarkers in clinical practice.

Furthermore, integrating biomarker data with clinical assessments and neuroimaging is crucial for comprehensive mTBI evaluation. This multimodal approach improves diagnostic accuracy and stratifies patients based on long-term sequelae risk.

In conclusion, laboratory biomarkers are promising for advancing mTBI diagnosis and management, particularly in sports-related contexts. The Abbott i-STATTM TBI Plasma Test exemplifies how innovative technology can enhance clinical practice by reducing unnecessary imaging and improving patient flow in emergency settings. Incorporating value-based medicine and HTA principles can further enhance these biomarkers' impact by ensuring effective and efficient clinical practices. Continued research is needed to address current limitations and validate clinical applicability. By enhancing our understanding and utilization of mTBI biomarkers within a value-based framework, we can improve patient outcomes and ensure safer practices in sports and other high-risk activities.

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# The role of the new analytical research parameters of the haematology analyzers BC 6800 plus (Mindray), DxH 900 (Beckman Coulter) and XN-2000 (Sysmex) in the differential diagnosis of peripheral lymphocytosis.

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**Background and Aim:** The presence of lymphocytosis in peripheral blood, even in asymptomatic individuals, is a frequent occurrence in clinical practice. This poses a challenge for laboratory professionals who must differentiate between morphologically reactive lymphocytes of benign origin and atypical lymphocytes indicative of lymphoproliferative disease. Modern automated hematology analyzers offer various research parameters that describe the activity status of the blood cells in detail. These parameters are available without additional reagents and have the same turnaround time (TAT) as the complete blood count (CBC).

The aim of this study was to evaluate the diagnostic utility of these parameters in differentiating between reactive and chronic clonal lymphocytosis.

**Materials and Methods:** The retrospective study analysed traditional and research parameters of 823 subjects admitted to the emergency room with lymphocytosis. The analysis was performed with different platforms: 230 subjects with the BC 6800 plus (Mindray), 255 with the DxH 900 (Beckman Coulter) and 338 with the XN-2000 (Sysmex). Predictors of clonal disease vs reactive lymphocytosis were evaluated by multivariate logistic regression. Multivariate models were built considering only non-collinear predictors found significantly associated at the univariate analysis.

**Results**: [Mindray]: the multivariate model included NEU# ($p=0.019$), MON% ($p<0.001$), RDW-SD ($p=0.003$), HFC ($p<0.001$) and LY-Y ($p=0.012$) [Nagelkerke r2=0.735]; the percentage of corrected predictions increased from 56.1% for the model without predictors to 90.1% for the multivariate model. [Sysmex]: the multivariate model included RBC ($p<0.001$), Ht ($p=0.009$), MCHC ($p=0.001$), LYM% ($p<0.001$), BAS% ($p<0.001$) and LY-WY ($p<0.001$) [Nagelkerke r2=0.628]; the percentage of corrected predictions increased from 60.2% for the model without predictors to 83.1% for the multivariate model. [Beckman]: the multivariate model included Hb ($p<0.001$), MCH ($p=0.001$), NEU% ($p=0.002$), MN-SU-LI ($p<0.001$), SD-SU-LI ($p=0.012$), MN-SA-LI ($p=0.019$), MN-SU-MO ($p<0.001$) and SD-LMALS-NRBC ($p=0.001$) [Nagelkerke r2=0.733]; the percentage of corrected predictions increased from 50.4% for the model without predictors to 90.1% for the multivariate model.

**Conclusion:** The multivariate logistic regression models for each analysis platform showed significant improvements in predictive accuracy. For the BC 6800 plus (Mindray), the multivariate model increased the percentage of correct predictions from 56.1% to 90.1%, for the XN-2000 (Sysmex) the prediction accuracy increased from 60.2% to 83.1% and for the DxH 900 (Beckman) the accuracy of the model improved from 50.4% to 90.1%.

The study shows that modern automated hematology analyzers can significantly improve diagnostic accuracy in distinguishing between reactive and chronic clonal lymphocytosis in patients with lymphocytosis. By incorporating various research parameters, these analyzers provide detailed insights into the activity status of blood cells without the need for additional reagents and with the same turnaround time as CBC.

## The clinical laboratory in the evaluation and monitoring of the patient with monoclonal gammopathy of renal significance

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Monoclonal gammopathy of renal significance (MGRS) is a clonal proliferative disorder that produces a nephrotoxic monoclonal (M) immunoglobulin and does not meet haematological criteria for treatment of a specific malignancy. The nephrotoxic activity of the noxious M-immunoglobulin, often synthesized by a dangerous small B-cell–derived clone, is independent from the clonal mass. Despite kidney biopsy is essential to make diagnosis of MGRS, once an MGRS-associated lesion has been confirmed, the presence of serum or urine M-protein pathophysiologically linked to MGRS should be defined. Serum protein electrophoresis (SPE), quantitative, rapid and easy to perform on automated

analyzers. is the first test performed. However, its poor sensitivity is an issue in searching for the MGRS related M-protein, often small, reflecting the low tumor burden, and made up of free light chains, difficult to find when comigrate with other proteins. Urine protein electrophoresis (UPE) is less sensitive than SPE for M-protein detection, but in a 24 h urine specimen, provided total protein assay, permits quantitation of Bence Jones protein and albumin needed for diagnosis, risk stratification and response assessment of MGRS. Serum and urine immunofixation (IFE) is more sensitive and specific than SPE and UPE, and is performed to detect and type M-protein as well as in treatment monitoring to define a complete response. Every laboratory should be aware of the analytical sensitivity and limitations of all the techniques used to find M protein in MGRS diagnosed patients. Serum κ and λ free light chain (sFLC) assays and κ to λ ratio estimation can increase the sensitivity of M-protein detection even more, except for MGRS associated with an intact M-protein. However, sFLC are cleared by the kidney, and that affects their serum concentration in patients with renal failure, since the moderate stages. To measure sFLC concentration, two major assays and some other are available, and their results cannot be compared. Moreover, the effects of renal impairment differ between the different assays. Thus, the same assay must be used to monitor a patient with MGRS from the diagnosis to treatment monitoring. In MGRS patients, laboratory should perform all available tests to reach the highest sensitivity in detecting the M-protein inducing disease. However, despite all laboratory efforts, a MGRS without detectable circulating M-protein may occur, tipically the proliferative glomerulonephritis with monoclonal immunoglobulin deposits. Novel techniques, such as mass spectrometry, greatly improving the sensitivity of M-protein detection are now available and will be discussed in a dedicated speech.

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# The bioanalysis of New Psychoactive Substances in biological matrices in the field of laboratory medicine

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The most recent data reported over 1200 compounds detected all over the World, divided into different pharmacological classes exerting similar effects to those provoked by the classical drugs of abuse. Indeed, the NPS market is fluctuant and adaptable to the general socio-economic conditions, with a variable panel of new substances emerging and others disappearing from year to year, increasing the phenomenon complexity. Over the last two years, the European Drug Agency (EUDA) reported record quantities of NPS seized in Europe, especially cathinone stimulants and ketamine. Accordingly, the NPS related risks for the public health have also grown during the last decade, with threats increased by the continual flow of new, potent products, distributed on ever easier ways to buy and use the NPS. In this scenario, the laboratory medicine plays a crucial role in containing the NPS related issues and to identifying the new emerging molecules on the illegal markets. Therefore, the development of new approaches is fundamental, based on multi-method and multi-analytical techniques analyses to identify and characterize the unknown compounds in the biological matrices. Considering the classical systematic toxicological analyses, new comprehensive extraction protocols should be implemented for the biological samples pretreatment in order to extract a larger panel of molecules with different physicochemical properties. To this concern, the pharmaco-toxicology laboratory of the National Center of Addiction and Doping of the National Institute of Health developed different analytical methods in high-pressure liquid chromatography coupled to High-resolution mass spectrometry (HPLC-HRMS/MS) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) to

characterize, detect and quantify drugs of abuse, NPS or their adulterant in biological matrices. In particular, a suspected opioid related death, occurred in Italy, raised the attention of the EUDA which requested to further investigate the fatality. A semi-unknown approach was applied to the analytical investigation of the available biological matrices through a comprehensive HPLC-HRMS/MS screening method. Therefore, the analytical screening of whole blood and urine revealed the presence heroin biomarkers and xylazine, a veterinary drug used as an opioid adulterant. Then, a quantitative method for xylazine was developed and validated in HPLC-HRMS/MS to confirm the analytical results and evaluate the possible role of xylazine in the determinism of death [1]. A similar approach was applied in the detection of levamisole in biological matrices which was identified as the principal cause of extensive ulcers on the limbs of a cocaine user. Whereas, a multi-analytical approach was applied to study the excretion profile in different biological matrices, and the metabolism of the semi-synthetic cannabinoid hexahydrocannabinol and cathinones, such as a-PVP[2]. In particular, the NPS and metabolites were quantified in GC-MS/MS, while the unknown metabolites identification was conducted by HPLC-HRMS/MS unknown method analysis supported by the Compound Discoverer™ assisted data-mining for the structure elucidation.

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# I*n silico* and *in vitro* metabolism studies for the identification of novel psychoactive substances’ biomarkers

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Novel psychoactive substances (NPS) have flooded the illicit drug market for almost two decades as alternatives or adulterants for traditional drugs of abuse, in an attempt to reduce manufacturing and processing costs and evade legal controls and analytical detection. NPS pose an important threat to public health as they have caused thousands of intoxications and deaths worldwide with societal and legislative implications. The turnover also is high: new NPS regularly emerge on the drug market to replace newly banned substances or produce ever-more-potent psychotropic drugs.

Little to no pharmacological data are available on NPS when they first surface on the market, which is a concerning matter since users are unaware of the potential risks associated with their consumption. From an analytical point of view, in toxicology, the topic is also problematic: the methods must be constantly updated to adapt to the market dynamics, but NPS are often challenging to detect as they may be active at trace concentrations in biological matrices and may undergo substantial metabolic degradation. Assessing NPS pharmacokinetics is therefore essential to be able to document consumption in clinical and forensic casework. Particularly, the metabolic profiling of NPS is a major step to identify specific metabolite biomarkers of consumption that may be more easily detected than the corresponding parent drug.

Clinical studies with NPS are time-consuming and hardly feasible due to health, ethical, and legal considerations. Positive biological samples from authentic cases of NPS consumption may also be difficult to obtain due to the high turnover rate and analytical challenges. Researchers have therefore relied on *in silico*, *in vitro*, and *in vivo* prediction tools to simulate NPS human metabolism. *In silico* models typically identify the molecules’ functional groups and predict the sites of metabolism and the metabolic transformations based on machine-learning algorithms. *In vitro* models usually include incubations with recombinant enzymes or animal or human liver microsomes or human hepatocytes to simulate metabolism at the liver, the main site of metabolization in humans. Undeniably, *in silico* and *in vitro* models provide quick results, which is essential to keep up with the NPS market dynamics. However, they cannot totally account for interindividual variations and all *in vivo* post-metabolism processes such as reabsorption, extrahepatic metabolism,

enterohepatic circulation, and elimination. *In vivo* animal models with controlled drug administrations to rats, mice, or zebrafish have been used to circumvent these difficulties. However, they are more time-consuming, and inter-species variations may be a problematic limitation.

Only through comprehensive research and adaptative methodologies can we hope to stay ahead of the evolving NPS landscape, ensuring public safety and informed legislative responses. Continuous advancements in metabolite prediction through various models are essential to provide suitable biomarkers of consumption to update analytical methods in clinical and forensic toxicology, despite their inherent limitations.

## Liquid biopsy in blood malignancies

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Liquid biopsy contains disease-related biomarkers present in body fluids. Among them, diseased cells, circulatingcell-free deoxyribonucleic acid (cfDNA), microRNA (miRNA), and extracellular vesicles (EVs) are the key markers for various hematological diseases. Irregular concentrations of these components in liquid biopsies indicate abnormal dynamics of the target biomarkers, which may provide clinicians with important information about the pathological condition.

Recently, liquid biopsy has been used for early diagnosis, treatment guidance, prognostic decision-making, and tumor monitoring. In the context of hematological malignancies, liquid biopsy is a minimally invasive and real-time procedure that can potentially overcome the intrinsic limitations of tissue biopsies, which expose patients to procedural risks and cannot account for spatial intratumor heterogeneity. The use of liquid biopsy has rapidly expanded in the setting of lymphoproliferative disorders, in particular in diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL), where ctDNA analysis on the liquid biopsy recapitulates the mutational profile of the tissue biopsy and can identify mutations otherwise absent on the tissue biopsy.

This noninvasive method also enables monitoring of minimal residual disease (MRD), which may facilitate personalized treatment for patients with hematological malignancies.

Qualitative and quantitative detection of markers from liquid biopsies can provide an alternative when routine sampling involves painful and invasive procedures.

Once an abnormality is diagnosed, biomarkers can be used to track and evaluate the disease status and identify high-risk patients at risk of recurrence, which can effectively prevent disease progression and promote recovery.

In addition, real-time monitoring by liquid biopsies allows treatment plans to be made based on the patient's clinical outcomes.

Selecting the right technology to detect and manage biomarkers serves as a benchmark for individualized prevention and treatment.

## Janu-seq: a pan-cancer tumor agnostic platform to evaluate the prognostic role of plasma tumor fraction

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Recent advancements in sequencing technologies have provided substantial insights into the genomic architecture of human tumors. However, translating this knowledge into clinical practice remains a challenge due to the inherent genomic instability driving spatial and temporal heterogeneity within tumor cells. Conventional single-biopsy analyses often fail to capture this complexity effectively. Circulating-free tumor DNA (ctDNA) analysis emerges as a promising alternative, reflecting intra-tumor and inter-metastasis heterogeneity through non-invasive means. ctDNA, found in body fluids like blood, mirrors tumor genetic and epigenetic changes and offers advantages such as minimal invasiveness and longitudinal

sampling. Despite extensive studies, integrating ctDNA analysis into clinical practice remains limited, posing a challenge for effective disease monitoring and recurrence anticipation in oncology.

We have recently developed a novel platform for ctDNA analysis, termed Janus-Seq, aimed at longitudinal post-treatment monitoring in various solid tumors, particularly in ovarian cancer, mesothelioma, and head and neck cancer. Leveraging next-generation sequencing (NGS), Janus-Seq offers a two-step tumor-agnostic workflow. The first step employs shallow whole-genome sequencing (sWGS) to quantify somatic copy number alterations (SCNA) in plasma, yielding the tumor fraction (TF), an indirect measure of tumor burden. The second step employs targeted re-sequencing to identify pathogenic single nucleotide variants (SNVs) associated with therapy response or resistance. This comprehensive approach enables both quantitative and qualitative evaluation of ctDNA, providing real-time insights into tumor evolution and potential treatment strategies.

Data obtained so far confirmed the analytic validity of plasma TF as an accurate biomarker of minimal residual disease (MRD); demonstrated the clinical validity of plasma TF as an agnostic biomarker of MRD in different types of cancers and its clinical utility for individual patient surveillance and treatment is currently under investigation.

Overall, Janus-seq is a pan-cancer tumor agnostic platform that would expect to transform clinical practice by enhancing early relapse detection, treatment monitoring, and personalized therapy selection. These findings are expected to impact cancer patient management offering valuable insights into disease dynamics and optimize treatment strategies.

## *In vitro* molecular diagnostics for personalized medicine in Allergology

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Allergic diseases are among the top chronic diseases in Europe and developing countries, with a possible increase in prevalencenear to 50% in the latest generation. Intercepting, diagnosing and treating allergies, stopping their progression towards serious complications, is the role of the clinicalspecialist and Laboratory Medicine.

Clarifying the pathophysiology of the syndrome facilitates appropriate management but requires scientifically validated in vitro testsby new IVD-R regulation.Nowadays, the Allergy Diagnostic Laboratory is assuming a major role in helping to provide new approach based on the preliminary detection of IgE mediated sensitization for a precise differential diagnosis offering effective tools to the primary care.At the same time new biomarkers available and the molecular diagnostics guaranteeapersonalizedprofile, targeted and accurate management for the allergy specialist.

In the field of respiratory allergies, alongside tests such as ECP, FeNO and tryptase, the Molecular Allergology offers an important range of monomolecular IgE markers able to distinguish the primary sensitizations from cross-reactive ones.

Although the International Union of Immunological Societies (IUIS) Subcommittee on Allergen Nomenclature has classified well over 1800 allergenic molecules, biological and immunological studies have shown that many allergens behave similarly to their natural counterparts and a limited well selected number of allergen components are sufficient to diagnose most cases of respiratory allergies.According to the current advanced diagnostic protocols we described the most important algorithms for the respiratory allergies harmonizing the new molecular approach with the traditional one based on extractive allergen sources.Complete algorithms for suspected perennial inhalation symptoms caused by mites, animals and molds have been reported starting from the extractive allergen sources. Algorithms for suspected seasonal inhalation symptoms caused by pollen from grasses, weeds and trees complete the respiratory picture according to the Clinical Consensus of the most important Scientific Societies: EAACI, WAO, SIAIP, AAIITO and SIAAIC.

Therefore, illustrating the whole range of new resources that Laboratory Medicine can provide for initial diagnostic guidance for the allergy management, the first critical step for the physician is to determine whether the patient has a true allergy disorder. To direct selected patients into the appropriate pathwaythe second step is to recognize and manage simple allergy problems safely and effectively with primary care or identify those patients who really need an accurate specialist evaluationto the allergist.

In the next future the increase of the daily acquired informationwill populate the Big Dataand will represent a sector where it will be advantageous to apply the use of Artificial Intelligence, with desirable benefits in the correct interpretation and explanation of the multiple and complex sensitization profiles of the atopic patient.

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# Fraily, long-COVID and laboratory medicine

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Frailty is commonly defined as a state of increased vulnerability to stressors due to decline in physiological reserve and function across multiple organ systems. Coronavirus disease 2019 (COVID-19) and frailty have a complex relationship, as the virus tends to disproportionately affect older adults and individuals with underlying health derangements, both of which are common characteristics of frailty. On the other hand, the COVID-19 pandemic has had a significant impact on frailty, both directly and indirectly, contributing to increase the number of frailty people and also to deteriorate further the psychological and mental decline of already frail individuals. In particular, frail subjects who develop long-COVID may face unique challenges and complications due to the intersection of frailty-related vulnerabilities and the lingering effects of COVID-19. In this detrimental scenario, laboratory medicine may play a pivotal role in the comprehensive care of frail individuals with long-COVID, by facilitating diagnosis, assessing disease severity, monitoring organ function, evaluating coexisting conditions, assessing nutritional status, detecting biochemical abnormalities, monitoring treatment response, and supporting long-term follow-up and surveillance. A multidisciplinary approach that integrates laboratory testing with clinical assessment and other diagnostic modalities is essential for optimizing outcomes and enhancing the quality of care for these individuals. To this end, the identification of biomarkers of frailty in people with long-COVID is an area of ongoing research, and while specific biomarkers for this population may not yet be fully established, some of these are associated with frailty and COVID-19, and may thus provide useful insights into the intersection of these conditions. These specifically include biomarkers of viral persistence, hematological and hemostasis parameters, inflammatory biomarkers, cardiac and muscular biomarkers, biomarkers of oxidative stress, hormonal biomarkers, biomarkers of endothelial and immune dysfunction, along with biomarkers of neurological and cognitive impairment. Integrating biomarker assessments with clinical evaluation and functional assessments may help identify individuals at increased risk of frailty- and COVID-19-related complications, thus guiding targeted interventions to improve outcomes in this fragile and highly vulnerable population.

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# A new interference of serum protein electrophoresis looking as a rare plasma cell neoplasm

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This case concerns a serum protein electrophoresis of a 49-year-old man admitted to the Kidney and Pancreas Transplantation Unit of Padua University-Hospital on 05/01/24. The electrophoretic profile, performed on capillary electrophoresis (Capillarys 3 Tera, Sebia, France) showed an increase in the beta-2 globulin zone, which also appeared abnormal, with a concomitant decrease in the gamma globulin zone. This finding was suggestive of a monoclonal component in the beta-2 globulin area; therefore, immunotyping test was carried out (Capillarys 3 Tera, Sebia). This evidenced that the abnormal area disappeared only in the anti-IgG pattern, whereas the small gamma globulin zone was completely unaffected. To confirm this finding, immunofixation test on agarose gel (Hydrasys, Sebia) was performed. It revealed the presence of an abnormal band in the beta-2 globulin zone fixed only by anti-gamma heavy chains antiserum. These results, as well as subsequent determinations of serum immunoglobulin and free-light-chains, were compatible with “heavy chain disease”, a rare plasma cell dyscrasia. A following consulting with clinicians revealed that the patient, affected by chronic kidney disease, underwent to a kidney retransplantation after desensitization with Imlifidase on 04/01/24. Imlifidase is a cysteine protease that cleaves the heavy chains of all subclasses of human IgG leading to the elimination of Fc-dependent effector functions. It is used to deplete anti- HLA antibodies in highly sensitized transplant recipient in order to allow an HLA-noncompatible graft. Cleavage arises within a few hours from infusion, and reappearance of endogenous IgG occurs approximately 2 weeks after administration. As expected, serum protein electrophoresis performed 2 weeks later showed a polyclonal hypergammaglobulinemia with disappearance of the monoclonal fraction in the beta-2 globulin zone on immunofixation test. The presented case highlights a new type of pharmacological interference on the electrophoretic profile, not due to the nature of the drug administered (e.g., therapeutic monoclonal antibody) but related to the mechanism of action and underlines the importance of collaboration between laboratory specialists and clinicians for the correct interpretation of laboratory tests.

# OCCASIONAL DIAGNOSIS OF MULTIPLE SCLEROSIS IN A YOUNG PATIENT

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In February 2024, a 17-year-old woman, following a motor-vehicle accident, underwent brain Magnetic Resonance Imaging (MRI) which detected numerous periventricular lesions in the corpus callosum as well as juxtacorticals lesions typical of Multiple Sclerosis (MS). She was seen at the Multiple Sclerosis Center of the Neurosciences Department of the University of Naples, and clinical history and neurological examination were normal. The results of blood chemistry analyses carried out at the Department of Laboratory and Transfusion Medicine of the University of Naples were all within the normal range, as were the main Cerebrospinal Fluid Analysis (CSF) parameters, including absence of red blood cells, normal leukocytes, glucose and protein levels. However, the k index (ratio between CSF/serum kappa free light chains and CSF/serum albumin) was 15.9, much higher than the proposed cut-off level (6.1)[1], suggesting intrathecal immunoglobulin synthesis, while the serum /liquor isoelectrofocusing for Oligoclonal Bands (OB) detection, currently employed for MS diagnosis, was negative. A group of MS experts has recently developed a consensus statement[2] recommending the introduction of the k index in the forthcoming revision of MS diagnostic criteria as an additional tool to measure intrathecal immunoglobulin synthesis. The

high k index observed in this patient confirmed the suspected diagnosis of MS, but given her young age, disease-modifying therapy (DMT) was not recommended, and MRI follow-up is ongoing. This case is very unique for the occasional finding of MS-like brain lesions, due to MRI performed for other indications. This allowed the patient to be classified as having MS thanks to the concomitant findings at the k index, despite the complete absence of symptoms. Furthermore, this clinical case highlights the high diagnostic sensitivity of the k index for MS, which was markedly high even in the absence of OB. In this case, the early diagnostic classification will allow the young patient to be monitored with stringent clinical/laboratory follow-up to highlight the possible evolution of the pathology and, consequently, a timely administration of suitable therapy. Hegen et al. MSJ 2023, 29, 169-812. Hegen et al. MSJ 2023, 29, 182-95

## TSH TESTING IN AMNIOTIC FLUID FOR MONITORING OF DYSHORMONOGENETIC FETAL GOITER: A CASE REPORT

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Fetal goiter is a rare condition characterized by increased fetal thyroid gland volume as a sign of hypothyroidism and, if severe, can lead to cardiac failure, hydrops and intrauterine death. Fetal goiter can be treated by administration of levothyroxine in amniotic fluid (AF), umbilical vein or muscle. Current evidences show an efficacy of about 70% of intra-amniotic therapy. Fetal blood (FB) sampling is associated with 5% of complications and amniocentesis is considered a safer procedure (<1% complications). Although AF sampling poses fewer risks, a well-established, analytically validated, assay for AF TSH monitoring should be warranted. In this case report, we evaluate AF TSH measured on an automated chemiluminescent immunoassay (Alinity i Abbott) in a hypothyroid fetus with goiter. A 25- years-old hypothyroid woman treated with levothyroxine and with a history of multiple miscarriages was referred to the Fetal Therapy Unit at 20.4 gestation weeks for a suspected fetal goiter, confirmed by ultrasound. TSH assayed on FB resulted >100 mU/L and intra-amniotic injection of 100 μg of levothyroxine was performed. Treatment was repeated weekly from week 22 to 27. FB and AF sampling was performed at week 25, with TSH results of 24.2 and 5.6 mU/L, respectively. AF TSH levels at 27, 29 and 33 weeks were 2.9, 1.2 and 1.8 mU/L respectively. The decreasing values probably reflected the efficacy of therapy, with a subsequent rise few weeks after stopping therapy. In absence of hydrops, AF sampling was not repeated further to reduce prematurity risk. A liveborn weighing 3020g was delivered at 38 weeks, with blood TSH >100 mU/L and negative thyroid autoimmune screening treated with levothyroxine which normalized thyroid function in 4 days. Intrauterine treatment of fetal goiter is fundamental to prevent complications. The possibility of monitoring the dysfunction with minimal risk by measuring AF TSH is important. However, some limitations cannot be ignored: a) TSH commercial assays need to be analytically validated to avoid matrix effects when used on AF; b) physiological or pathological confounders influencing AF TSH levels should be investigated; c) clinical studies are needed to assess the actual power of the test in clinical decision- making.