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Nota dell'Editore: i riassunti sono stati riprodotti senza alcuna revisione dal materiale direttamente fornito dagli autori.

EP001

Managing the Immunohematology Laboratory with Artificial Intelligence

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Improving the management of the immunohematology laboratory through the use of artificial intelligence (AI) represents a significant step towards efficiency and precision in diagnostic and therapeutic practices. Immunohematology is crucial for ensuring the safety of transfusions and organ transplants.

Advantages of AI. One of the primary benefits is the ability to analyze large amounts of data quickly, enhancing the speed and accuracy of diagnoses. Advanced AI systems can identify patterns in antibody and antigen profiles, helping predict immune reactions and suggesting personalized treatments.

Additionally, AI can optimize the sample management process by automating labeling, preparation, and analysis phases. This not only reduces the risk of human errors but also decreases the time required to complete tests, enabling smoother workflow and faster responses to clinical emergencies.

Another critical benefit is AI's capacity to support clinical decision-making. AI systems can integrate clinical data, such as a patient's transfusion history and laboratory test results, to provide evidence-based recommendations. This assists healthcare professionals in making informed and timely decisions, improving patient care and optimizing healthcare resource utilization.

In the context of organ transplants, AI has the potential to revolutionize the management of antigen compatibility between donors and recipients. AI-driven predictive models can assess immunological compatibility more accurately than traditional methods, reducing the risk of rejection and enhancing long-term transplant outcomes.However, integrating AI into the immunohematology laboratory also presents challenges. Ensuring the quality and accuracy of data used to train AI models is essential, along with addressing ethical and legal issues related to data privacy and clinical responsibility in interpreting AI outputs.

In conclusion, using artificial intelligence to enhance immunohematology laboratory management promises to radically transform diagnostic and therapeutic practices. With the potential to increase operational efficiency, improve diagnostic accuracy, and support informed clinical decisions, AI stands as a valuable ally in advancing transfusion medicine and organ transplantation.

EP002

Ensuring Data Security in AI-driven Management of Immunohematology Laboratories

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Ensuring data security in the management of an immunohematology laboratory through the use of artificial intelligence (AI) is crucial given the sensitivity and importance of patient information in healthcare settings. AI technologies offer significant advancements in efficiency and accuracy, but they also introduce unique challenges related to data privacy and security.

Firstly, AI systems in immunohematology labs rely on vast amounts of patient data, including blood group information, antibody profiles, and medical histories. Protecting this data against unauthorized access, breaches, or misuse is paramount. Robust encryption protocols and secure data storage solutions are essential to safeguard patient confidentiality and comply with healthcare regulations like HIPAA (Health Insurance Portability and Accountability Act) in the United States or GDPR (General Data Protection Regulation) in Europe.

Furthermore, AI algorithms require continuous training and updating using real-world patient data to remain effective. This process necessitates careful management to prevent biases from compromising the integrity of diagnostic or treatment recommendations. Rigorous data validation and audit procedures are crucial to ensure that AI-driven insights are reliable and clinically relevant.

Collaboration between data scientists, healthcare providers, and cybersecurity experts is essential to develop AI systems that prioritize patient privacy and data security. Implementing robust access controls and regular security assessments can mitigate risks associated with AI deployment in immunohematology labs.

Moreover, healthcare institutions must establish clear policies and procedures for data handling and AI utilization. This includes defining roles and responsibilities for data stewardship, establishing guidelines for ethical AI use.

Despite these challenges, the integration of AI in immunohematology laboratories holds promise for enhancing diagnostic accuracy and advancing medical research.

In conclusion, while AI presents opportunities for transformative advancements in healthcare, safeguarding patient data through robust security measures and ethical practices remains foundational to responsible AI implementation in immunohematology and beyond.

EP003

The Necessity of Appropriateness in Laboratory Test Requests and the Role of Artificial Intelligence

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In the contemporary medical landscape, the precision and appropriateness of laboratory test requests are critical, especially for advanced tests that involve complex procedures and interpretations that can significantly impact patient management and outcomes. Inappropriately ordered tests can lead to unnecessary costs, patient discomfort, and misdiagnosis. Recent advancements in artificial intelligence (AI) and deep learning offer promising support and corrective measures in this field, aiding physicians and optimizing test utilization.Coagulation tests are often expensive and complex, requiring specialized interpretation. Misuse or overuse can result in false positives or negatives, leading to inappropriate treatment strategies.Microbiology tests, including cultures and molecular diagnostics for infectious diseases, play a crucial role in identifying pathogens and guiding antimicrobial therapy. Inaccurate or unnecessary testing can delay appropriate treatment and contribute to antimicrobial resistance.Serological tests and immunopathological assessments are essential for diagnosing autoimmune disorders, infectious diseases, and allergies. These tests require precise timing and context to avoid false interpretations. Overuse or inappropriate ordering can lead to unnecessary follow-ups and treatments.AI and deep learning algorithms can enhance the appropriateness of these requests by analyzing patient data and clinical history comprehensively. These technologies can screen for clinical indications and cross-reference with evidence-based guidelines, providing real-time decision support to physicians. Moreover, AI systems can continuously learn from new data, improving their accuracy and recommending the most appropriate tests tailored to individual patient profiles.Cardarelli Clinical Pathology lab has initiated a verification program aimed at assessing the accuracy of prescriptions. This program evaluates the performance of medical orders from various departments against established guidelines, taking into account patient clinical conditions and whether the prescription is routine or urgent. The primary objective is to provide a comprehensive assessment, ensuring that all prescriptions meet the required standards. This initiative is part of the lab's commitment to enhancing healthcare quality and patient safety.

EP004

Evoluzione dell'organizzazione del laboratorio ad alta automazione attraverso un middleware con algoritmi di "visual management"

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Scopo. Il laboratorio analisi ad alta automazione dell'Ospedale di Pistoia e il presidio di Pescia da febbraio 2024 hanno soluzioni analitiche e software di nuova generazione.Scopo: dettagliare l'utilizzo del middleware (MW) per la realizzazione degli obiettivi: riduzione TAT (Tourn Around Time) di validazione routine senza impatto su TAT urgenze; allineamento strumentale; gestione ancicipata non conformità; accelerazione e standardizzazione criteri rilascio risultati.
Metodi. Dotazione: sistema DxA 5000 connesso a 2 DxI9000, 1 DxI800, 1 AU5812, 1 AU 5822 (Beckman Coulter Brea CA USA), 1 Liaison XL (DiaSorin Saluggia IT); MW ATMOSphere (Dedalus Italia SPA, distribuito in esclusiva da Beckman Coulter srl).MW: creazione area di lavoro (dashboard, DS) per controllo del processo con indicatori personalizzati per aree analitiche, matrici biologiche, richiedenti, categorie pazienti, priorità, tempistiche, controllo di qualità strumentale, gestione non conformità; creazione DS di stato , definizione e monitoraggio efficacia regole autovalidazione (AV).Campionamento e statistiche con MW: marzo 2024; TAT (T), routine (R), urgenze (U) e per ISE, creatinina (CR), hsTroponinal (TN) e hCG; numero test (N) in minuti: mediano (ME), minimo (MN), medio (M) massimo (MX).
Risultati. DS controllo di stato per non conformità: permette di gestirle rapidamente, senza ricerca dei campioni da gestire a fine seduta. AV% giornaliera U e R=56,5% e 77,6%.TAT T: N=453813, ME=51, MN=18 M=54 MX=120; TAT R: N=381091, ME=55, MN=18 M=58 MX=120; TAT U: N=72722, ME=34, MN=18 M=35 MX=119.ISE T: N=9184, ME=47, MN=14 M=52 MX=120; ISE R: N= 6329, ME=57, MN=18 M=59 MX=120; ISE U: N=2855, ME=33, MN=14 M=35 MX=114.CR T: N=4833, ME=50, MN=16 M=53 MX=120; CR R: N=3899, ME=55, MN=18 M=58 MX=120; CR U: N=934, ME=33, MN=16 M=35 MX=113.TN T: N=681, ME=37, MN=20 M=38 MX=118; TN R: N=25, ME=53, MN=30 M=58 MX=118; TN U: N=656, ME=36, MN=20 M=38 MX=117.hCG T: N=134, ME=38, MN=19 M=41 MX=86; hCG R: N=37, ME=53, MN=25 M=55 MX=86; hCG U: N=97, ME=36, MN=19 M=36 MX=86.
Conclusioni. Le DS permettono attraverso il visual management la gestione coordinata e integrata degli analizzatori dalla postazione MW. L'analisi dei TAT evidenzia come media e mediana per R e U si avvicinino, dimostrando l'assenza di interferenze reciproche.

EP005

Development of an Excel-based form for reporting laboratory test results when the laboratory/hospital information system may be unavailable

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Background: The Laboratory Information System (LIS), usually connected to the Hospital Information System (HIS), has become an indispensable resource for all healthcare facilities worldwide, as it enables the receipt of medical orders, is bidirectionally connected to the analyzers and is the ideal platform for reporting test results. However, under certain circumstances, the HIS/LIS may be unavailable (e.g., software/hardware failure, cyber-attacks, backlog of orders/results), requiring alternative strategies for results reporting (1,2).
Methods: Following a disruptive cyber-attack, when both HIS/LIS become unavailable, we developed an Excel-based form in which urgent/stat laboratory test results can be entered. The form includes an initial section form entering demographic information (date, first name, last name, age at birth, sex, patient ID, clinical ward its fax and/or phone number), followed by a section for laboratory data. The first column contains the name of the test, the second column is empty and intended for the test results (i.e., the (numerical) value), the third column the unit of measurement and the last column the reference interval (RI). The RIs of each test are linked to a query with the patient's date of birth and sex so that they can be automatically updated when these data are entered in the first section of the form.
Results: Once all data is entered, a form for each patient and/or request with unique patient ID, date and time can be saved, transmitted by email (when available), printed (header and footer contain the same information as the official, LIS-generated lab report) and then conveyed by hand, pneumatic tube system or sent by fax. The saved form is also stored permanently in digital form, eliminating the need to keep paper forms.
Conclusions: The Excel-based form developed in our institution allows to temporarily replace the unavailability of HIS/LIS for efficient transmission of laboratory test results.
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EP006

CELL POPULATION DATA IN SEPSIS DIAGNOSIS: E. COLI AND S. AUREUS LIVE BACTERIA AND ENDOTOXINS AS MONOCYTE AND NEUTROPHIL ACTIVATING TRIGGERS

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Sepsis is one of the most challenging health-care problem, representing a major cause of morbidity and mortality worldwide. Early biomarkers are currently being investigated for rapid identification of patients in intensive care unit. Hematology analyzers generate Complete Blood Count (CBC) along with Cell Population Data (CPD) which are obtained through Volume-Conductivity-Scatter technology. CPDs provide morphological information of immune cells through impedance, radio frequencies and light scattering, which are potential time- and cost-saving biomarkers. This study aimed to investigate neutrophil (NE) and monocyte (MO) activation during infection by Volume (V), Conductivity (C) and Axial Ligh Loss (AL2) alterations. EDTA-K2 anticoagulated whole blood (n=24) were treated with live E.coli and S.aureus (10^6 and 10^8 CFU/ml), LPS (1 µg/ml), and LTA (200 µg/ml). CBC was performed at 0, 30, 60, and 180 min through DxH690T (Beckman Coulter). Our results showed that live bacteria, LPS and LTA induced an early and significant increase in the mean monocyte volume (MN_V_MO) ($p<0.0001$) with a time- and dose-dependent mechanism and also accompained by an increased anisocytosis, as demonstrated by the significant higher values of volume standard deviation (SD_V_MO) induced by Gram-negative and LPS at each time and by S. aureus 10^8 CFU/ml and LTA ($p<0.01$). A significant decrease of MN_C_MO was observed with E. coli 10^6, 10^8 CFU/ml, LPS, S. aureus 10^8CFU/ml and LTA treatment; a similar trend was observed for the MN_AL2_MO parameter with E. coli 10^6 and 10^8 CFU/ml treatments, whereas SD_AL2_MO was significantly increased by both live bacteria and LPS ($p<0.01$). Conversely, neutrophil mean volume (MN_V_NE) was significantly decreased by E. coli 10^6, 10^8 CFU/ml, LPS and S. aureus 10^8 CFU/ml ($p<0.05$). E. coli and LPS significantly increased MN_AL2_NE and decreased MN_C_NE values. All neutrophil SD of V, C and AL2 were significantly enhanced by both bacteria and PAMPs. CPDs, characterizing different leukocyte populations, have emerged as promising biomarkers able to discriminate sepsis conditions according to the pathogen, that could be used as part of an integrated approach for the early diagnosis and management of sepsis, without any additional time and costs.

EP007

An Explainable Method for Lung Cancer Detection and Localisation from Tissue Images through Convolutional Neural Networks

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Aim

Lung cancer (LC) is one of the leading causes of death worldwide, accounting for more than 20% of cancer deaths in Europe; a prevalent and life-threatening condition, necessitates early detection for effective intervention.

In the era of personalized medicine, lung cancer diagnosis and accurate classification strongly rely on cytological and histological

subtyping by microscopic evaluation with standard histochemical stains and ancillary immunohistochemical staining. With the recent

advancements in deep learning techniques, particularly in medical image analysis, which offer unparalleled accuracy and efficiency, we

propose a method aimed at designing and developing a method for the automated identification of cancerous cells in lung histological

tissue images. We aim to find a model capable of classifying histological images as positive or negative for LC.

Methods

The method adopted uses five main steps: composition of the dataset, selection of deep learning models, execution of the experiments,

generation of heatmap through Grad-CAMs, and analysis of the results. We explore various deep learning architectures: Standard_CNN,

AlexNet, VGG-16, VGG-19 and MobileNet, with the objective of identifying the most effective one based on both quantitative and qualitative assessments. In particular, we assess qualitative outcomes by incorporating the concept of explainability, enabling the visualization of areas within tissue images deemed relevant to the presence of lung cancer by the model.

Results

The experiments carried out to test and prove the functioning of this methodology were performed on a dataset of 15.000 lung

tissue images; 5.000 of which were labelled as adenocarcinoma, 5.000 as squamos cell carcinoma and 5.000 as benigne tissue. The

performances of the Standard_CNN and VGG-16 models proved to be the best to classify to correctly classify images with accuracy of

98.5% and 99.2%, and AUC values of 99.4% and 99.9%, respectively.

Conclusions

The results obtained are very promising; the identified potentials in terms of reliability and speed could serve as an excellent foundation

for future developments. The developed method serves as a supportive tool for pathologists, offering a second opinion on lung biopsy

diagnoses, significantly reducing analysis times, and alleviating the workload of medical professionals.

EP008

MR Net: A Method for Breast Cancer Detection and Localisation from Histological images through Explainable Convolutional Neural Networks

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Aim: Breast cancer (BC) is the most common cancer among women worldwide. This is the reason why, early and accurate breast cancer detection is crucial for proper treatment planning to save a life. This paper propose a method aimed to detect and localise brest cancer through deep learning, in particular convolutional neural networks. We aim to find a model capable of classifying histological images as positive or negative for BC.

Methods: We exploit several deep learning model: Standard CNN, Efficient-Net, ResNet50, VGG-16, VGG-19 and MobileNet. In addition to these networks, furthermore propose a novel deep learning model, named MR Net. The aim is to provide a more effective network, able to correctly detect and localise breast cancer, that could support the physician in making clinical decisions. It could also prove to be a successful model to speed up the diagnostic process and detect the possible presence of the disease at an early stage. Moreover, we propose a way to provide prediction explainability with the aim to understand where the model is looking to predict a certain label by drawing heatmaps, with the aim to reveal a proper detection of the presence of benign, atypical and malignant tumours. Thus, we assess both quantitative and qualitative outcomes of the proposed MR Net and the remaining models presenting also explainable results, enabling the visualization of areas within tissue images deemed relevant to the presence of breast cancer by the model.

Results: In the following case study, the BReAst Carcinoma Subtyping (BRACS) dataset was adopted, contains 5628 images of which 80% were allocated to training, 10% to testing and another 10% to validation. The performances of the MR Net model proved to be the best to classify to correctly classify images with accuracy of 69%.

Conclusions: Although the proposed model does not outperform state-of-the-art models in terms of BC detection, it does in terms of explainability, as the heat-maps generated using Grad-CAM reveal a proper detection of the presence of benign, atypical and malignant tumours. The results obtained are very promising, even when compared to the current state of the art, providing an excellent basis for possible future developments.

EP009

The potential role of monocytes cell complexity (MO-X) for diagnosis of sepsis in adult patients admitted to the intensive care unit

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Background: Sepsis is a time dependent process, in which the first 12 hours are fundamental to the patient's prognosis. The diagnosis of sepsis is often difficult and belated, substantially increasing the mortality in affected patients. Its early identification allows to choose the most appropriate therapies in the shortest time, improving patients' outcome and eventually their survival. The innate immune system cells, such as monocytes and neutrophils, are the first line of defence against invading pathogens. Upon infection, circulating monocytes undergo activation leading to morphological and functional changes. Thus, during the early stages of infection, a heterogeneous population of monocytes can be detected. The dynamic morphological changes of monocytes can be captured by the monocytes cell complexity (MO-X). The MO-X increases in the presence of greater amounts of granules, vacuoles and other cytoplasmic inclusions. Decreases in the presence of a lower cell complexity. Therefore, the aim of the study was to evaluate the role of monocytes cell complexity (MO-X) in the early diagnosis of sepsis.

Materials and Methods: Data from 95 patients consecutively admitted to the intensive care unit were retrospectively analyzed (55 non-septic and 40 septic patients). Septic patients were further divided between sepsis and septic shock according to the severity of the illness. Patients were subsequently classified according to renal function. The renal failure group included patients diagnosed with Chronic Kidney Disease (CKD) and Acute Kidney Injury (AKI) as according to the Kidney Disease Improving Global Outcomes (KDIGO) classification. Procalcitonin (PCT), C-reactive protein (CRP) and creatinine were measured using the automated clinical chemistry analyzer Roche Cobas 8000 (Roche Diagnostics©, Mannheim, Germany) and expressed in ng/mL, mg/L and mg/dL respectively. MO-X parameters were measured using a Sysmex XN 9000 hematology analyzers (Sysmex©, Kobe, Japan) and expressed in fluorescence intensity (FI). All parameters were measured following the manufacturer's recommendations.

Results: A significant difference between the non-septic and septic group was detected in the MO-X, CRP and PCT parameters (120.4 vs. 123.6, 15.0 vs. 150.0, 0.32 vs. 4.17; $p< 0.0001$). Similar results were observed among the septic group (sepsis vs septic shock) for MO-X and PCT parameters (122.1 vs 127.2; 0.59 vs 14.2; $p<0.05$) except to CRP values (142.0 vs 158.0 $p=0.4644$). For the diagnosis of sepsis, MO-X showed an Area Under Curve (AUC) > of 0,795, with a specificity of the 85.4% and a better positive predictive value than PCT and CRP (75.0 % vs 69.7 % and 74.2 %, $p<0.0001$). Unlike PCT (0.43 vs. 12.6 $p= 0.0025$), MO-X and CRP (122.3 vs 125.2 $p= 0.0951$, 107.2 vs 184.2 $p= 0.1485$) did not show significant difference within the septic group between patients with normal renal function and those with renal failure. While, a significant difference were observed among the non-septic group for CRP value (12.0 vs 80.0 $p=0.0484$) between patients with normal renal function and those with renal failure.

Conclusions: The results of this study suggest that the increase of the MO-X values could be useful for the diagnosis and management of sepsis in the ICU. Furthermore, MO-X values has proved efficient in discriminating the severity of sepsis at the time of admission and are not influenced by renal function. However, larger prospective studies are needed to confirm these results.

EP010

A machine learning approach for assessing patients' disease status by erythrocyte sedimentation rate (ESR)

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Background. Erythrocyte sedimentation rate (ESR) is a well-recognized indicator of the acute phase response which differs among different clinical conditions. Automated ESR determination uses a modified Westergren technique, measuring sedimentation rate after 20 minutes while also presenting the capability of recording a 20-minute kinetics. The aim of the study was to identify a machine learning (ML) algorithm able to differentiate different clinical conditions based on complete blood count (CBC) results, ESR and ESR kinetics.

Materials and Methods. Blood (K2-EDTA) leftover specimens were randomly selected at University-Hospital of Padua, Italy. Samples from rheumatological wards (n=32) (RP), oncological wards (n=103) (OP), patients with sepsis or acute inflammatory status (n=107) (SP) and outpatients from routine testing (n=104) (CTL) were included in the study. ESR was determined by reference manual Westergren method and by CUBE 30 Touch (Cube30) (Diesse Diagnostica Senese, Siena, Italy). Sedimentation was measured at different times every 2 minutes up to 20 minutes. Kinetics were calculated using the slopes at different time points (6-10m, 8-12m and 14-18m) after normalization of parameters by log-transformation. CBC was determined by XN10 and XN20 (Sysmex, Japan). Machine learning (ML) analyses were performed by R v4.2.2 using a 3:7 ratio for training, testing and fine-tuning of parameters.

Results. ESR results, measured by CUBE 30 Touch and Westergren methods, were closely correlated (Spearman's $r = 0.945$, $p < 0.001$). By ML, white blood count, ESR, age and 8-12m ESR slope were the most important variables. The study focused on SP prediction: the optimal sensitivity and specificity were obtained for slope values between 8 and 12 minutes, being 0.71 and 0.81, respectively. For CTL, sensitivity and specificity were 0.78 and 0.86, respectively. RP and OP presented suboptimal performances, being difficult to be efficiently predicted due to the low sample number and the variety of clinical conditions.

Conclusions. ML algorithms based on CBC and ESR kinetics can predict SP and CTL with good sensibility and specificity. Therefore, changes in the 20-minute kinetics may be associated to different pathologies, thus increasing the value of the simple ESR measurement.

EP011

Stability of leukocyte counts in body fluids: with or without EDTA?

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Background. The leukocyte count of body fluids is essential for the diagnosis of a variety of human diseases. Samples for body fluid analysis are received daily in our laboratory with the following instructions: Body fluid must be collected in an EDTA-containing tube when a leukocyte count with a simplified differential (i.e., polymorphonuclear and mononuclear cell counts) is ordered, while body fluid for chemistry tests (creatinine, triglycerides, amylase, etc.) must be collected in a tube without additives. These instructions are often ignored, and it is not uncommon to receive only a single tube without anticoagulant, for which the requesting physician orders both the leukocyte count and chemistry tests. Therefore, the aim of this study was to evaluate the stability of the leukocyte count in tubes with or without EDTA over time and thus define the sample stability on different days.

Materials and methods. Ten body fluids were analyzed, 4 pleural and 6 peritoneal, with different leukocyte counts. For each patient, the body fluid was collected in two consecutive test tubes, one with K2-EDTA, the other without additives. Both samples were analyzed using a Sysmex XN 9100 analyzer, immediately upon arrival at the laboratory, 2 hours later and then once a day for the following 7 days.

Results. The K2-EDTA samples remained relatively stable, as the leukocyte count decreased on average by 1.7% on the day after collection, by a further 5% on the third day and by a maximum of 10% on the fifth day. The samples without additives showed a decrease of 7% already 2 hours after arrival at the laboratory, followed by a decrease of 20% on the next day, 30% on the third day and a maximum decrease of 50% on the seventh day. The average difference between the two paired samples analyzed immediately upon arrival at the laboratory was 14.9%.

Conclusions. When a leukocyte count is ordered on body fluids, anticoagulated K2-EDTA tubes are the preferred samples.

EP012

Indirect evaluation of lung function by means of LF-NMR following chest physiotherapy or kaftrio administration in cystic-fibrosis patients

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Background. Cystic fibrosis (CF) is an autosomal recessive disorder most commonly caused by a deletion in position 508 (F508del) of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Dysfunctional CFTR induces the production of thick/viscous mucoid secretions in particular in the airways. This promotes mucus stasis with chronic inflammation/bacterial infection leading to progressive respiratory failure, the most common cause of death for CF patients. Thus, lung functionality assessment is pivotal. We previously employed Low Field Nuclear Magnetic Resonance (LF-NMR) to measure the transversal relaxation time (T2m) of the water hydrogens dipole present in the CF sputum. Our data showed that T2m indirectly correlated with circulating/local inflammation markers and directly correlated with the forced expiratory volume in the first second (FEV1). Thus, the assessment of sputum by T2m, provides a useful tool for the indirect monitoring of lung disease in CF patients.Aim. Here T2m significance was further investigated exploring: 1) the influence on T2m of sputum contamination by saliva, 2) the correlation with the effects of chest physiotherapy (CP) and 3) of the CFTR-modulator Kaftrio, recently approved by the FDA for the treatment of CF patients aged ≥12 years with the F508del in at least one allele.Methods. T2m was measured in the sputum of 16 CF-patients before/after CP and in 9/16 patients before/ after kaftrio administration. FEV1/C-reactive-protein-(CRP)/ erythrocyte-sedimentation-rate-(ESR) and sweat NaCl concentration were measured by standard techniques. Sputum contamination by saliva was determined mathematically. Results. Our data show that T2m can detect the lack of significant effects on lung function by CP (confirmed by FEV1). Moreover, T2m detects the kaftrio positive effects on lung function (evaluated by FEV1) and inversely correlates with the CRP/ESR/NaCl sweat concentration reduction. Finally, we provide evidence of the development of a novel mathematical approach to correct T2m value in sputum samples contaminated by saliva.Conclusion. Our data strengthen the rationale for T2m employment in CF lung disease monitoring in general and following therapeutic treatments.

EP013

Comparison between a new device for the semen quality analysis and the manual microscopic evaluation in a not specialistic clinical laboratory.

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Introduction: Semen analysis is a diagnostic qualitative and quantitative exam which investigate different parameters of human semen with a high relevance in fertility workup. The performance of seminal fluid examination, a manually technique, is characterized by poor reproducibility due to subjective interpretation preparations; this can cause misclassification of the semen quality. Aim of this study was to evaluate the performance of an automatic device in semen analysis by comparing its results with those obtained with the manual microscopy method.

Methods: 50 samples were analyzed with the manual and automated method. Semen parameters investigated, concentration, motility and seminal pH, were determined by means of standard manual semen analysis and by the automated LensHooke™ X1 PRO semen quality analyser following the WHO 6th Edition guidelines.

Results: First we compared the number of spermatozoa obtained from manual and instrumental count and their different classifications in normal, oligospermic, cryptospermic and azoospermic samples. The Bland-Altman plot comparing the manual and instrumental count showed a little positive bias, indicating a slightly higher value for the manual count. Second, we compared the morphology and the different samples classification in normal and abnormal groups. Third, spermatozoa motility obtained from the manual and instrumental count was compared and classified in normal total motility and asthenozoospermia. Statistical tests showed respectively for morphology and motility a moderate and a very good agreement.

Conclusions: Results of our work agree with those published by other authors which concluded that spermatozoa concentration and motility data derived from the analyzer are comparable with the manual microscopic evaluation. Our study demonstrates the LensHooke™ X1 PRO automated semen analyzer shows an acceptable agreement with the manual microscopic evaluation of seminal fluid coupled to the easiness of technique implementation, these attractive features suggest the opportunity for a wider adoption of this instrument technique in the clinical practice. The use of this simple device could help to standardize reports and clinical information in non specialistic laboratories.

EP014

Microbiota del liquido seminale e infertilità maschile: una revisione della letteratura

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Un microbiota sano e diversificato è fondamentale per la salute dell'individuo, comprendendo anche la salute del sistema riproduttivo sia maschile che femminile. In particolare, nel caso di quello maschile, il microbiota svolge un ruolo chiave nel supportare la funzione degli spermatozoi e nel favorire il successo della fecondazione. Recenti studi correlano la disbiosi del microbiota con la qualità degli spermatozoi. Queste alterazioni del microbiota possono influenzare la fertilità determinando stress ossidativo e la produzione di radicali liberi che danneggiano la membrana e il DNA degli spermatozoi, possono comprometterne la funzionalità, provocare reazioni infiammatorie sistemiche o croniche a livello del tratto genitale maschile, creando un ambiente ostile agli spermatozoi, determinare un' alterazione della motilità e della morfologia spermatica, influenzare la capacitazione spermatica, processo che prepara gli spermatozoi alla fecondazione; se alterato, il microbiota può compromettere questo processo, riducendo le probabilità di successo. Diverse ricerche hanno analizzato la composizione del microbiota del liquido seminale di uomini fertili e quello di uomini con anomalie nella motilità/concentrazione degli spermatozoi. Sono state evidenziate differenze significative nella varietà di specie rappresentate. Negli uomini che presentano alterazione nella motilità degli spermatozoi o una oligo/ azoospermia, si osserva spesso una minore diversità ed uno sbilanciamento nelle specie batteriche presenti, un aumento dei batteri patogeni e una riduzione dei microrganismi commensali benefici. Gli ultimi studi eseguiti attraverso NGS evidenziano, infatti, un ruolo critico del Lattobacillus iners e di membri del genere Pseudomonas nell'infertilità maschile.

La comprensione del ruolo del microbiota nella fertilità maschile apre nuove strade per lo sviluppo di terapie innovative contro l'infertilità. La manipolazione del microbiota mediante l'utilizzo di probiotici, prebiotici o antibiotici mirati, l'intervento sullo stile di vita e sul corretto uso di antibiotici non necessari potrebbe rappresentare un approccio terapeutico efficace per migliorare la qualità dello sperma e le probabilità di concepimento.

EP015

Are the dried blood spots (DBS) a reliable tool for analyzing the metabolic profiles of patients with Alzheimer's disease?

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Alzheimer's disease (AD) is the most common neurodegenerative disease associated with aging, whose prevalence is rising as the world's population ages. This has important socioeconomic implications and highlights the importance of early diagnosis.

The scientific community has been paying more and more attention in recent years to the search for disease biomarkers as new approaches for early and accurate diagnosis and novel therapeutic approaches for the identification or modification of the course of AD. Because metabolites play a significant role in the interruption of biochemical patterns caused by disease, metabolomics is becoming a more popular tool for the identification of biomarkers. Compared to conventional diagnostic approaches and conventional clinical biomarkers, metabolomics offers potential advantages in terms of sensitivity and specificity.

Dried blood spot (DBS) can be used as an alternative sample collection method to serum or plasma. The DBS technique has the following advantages over traditional blood sampling: less blood is required for analysis than in a venous sample, the method is easy to use, non–invasive, and doesn't require any specialized tools or personnel. Moreover, hemolysis and contamination are not very likely, and the samples are easy to store and transfer.

The aim of this study was to compare the ability of DBS versus serum samples in detecting metabolites that may be biomarkers for AD. Metabolomic analyses were performed by GC–MS on DBS and serum samples from n = 40 subjects, of which 20 affected by AD, enrolled at the Center for Research and Training in Aging Medicine (University of Molise, Campobasso). To confirm whether there were any differences in the metabolite concentrations between the serum and DBS samples, a comparison analysis was performed. The data were first normalized in respect to the internal standard after being aligned using the MetaboPredict to obtain areas. A paired T–test was used to compare the serum and DBS areas for each metabolite.

The results showed a substantial overlap between the metabolites present in DBS and those present in serum.

These preliminary data provided a promising result regarding the use of DBS for metabolomic analysis in AD patient.

EP016

CORREAZIONE TRA I LIVELLI PLASMATICI DI VIT D 25 OH E MALATTIE NEUROLOGICHE

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PREMESSA

In un'ampia e dettagliata revisione della letteratura, le proporzioni epidemiche raggiunte dalla condizione di deficit di vitamina D nel mondo, hanno evidenziato che la possibilità di avere livelli bassi di vitamina D nel sangue,possa influenzare negativamente lo sviluppo di patologie neurodegenerative, come la Sclerosi multipla e l'Encefalite autoimmune (1). Gli studi sulla relazione fra il sistema nervoso e la vitamina D hanno messo in evidenza la grande quantità di processi regolati da questa vitamina, tra i quali spicca la qualità dei processi cognitivi come la difficoltà di concentrazione, perdita della memoria, fatica nel trattenere ed elaborare informazioni nuove. OBIETTIVO DELLO STUDIO: L'obiettivo di questo studio e'quello di confermare la relazione esistente tra patologie neurologiche, quali Sclerosi Multipla e Encefaliti Autoimmuni, e carenza dei livelli plasmatici di Vit D 25 OH al fine di mettere in evidenza il ruolo di quest'ultima nella modulazione della risposta immunologica nei pazienti affetti da queste due patologie. A tale scopo sono stati effettuati dosaggi di Vit D 25 OH su 156 pazienti ricoverati presso l'UOC di Neurologia del Grande Ospedale Metropolitano di Reggio Calabria, da gennaio 2022 a gennaio 2024.

MATERIALI E METODI

In ogni paziente esaminato, sono stati presi in considerazione: dati demografici, quali età e sesso; dati laboratoristici, ossia data del prelievo, reparto ed esame chimico-fisico del liquor, e tramite metodica nefelometrica e'stata misurata l'íntegrita'di barriera. Il gold standard per fare diagnosi di SM e EA è l'Isoelettrofocusing (IEF) delle proteine del liquor cefalorachidiano (LCR) e del siero, che consente di dimostrare la presenza di bande oligoclonali (OCB). Tra i 156 pazienti reclutati, 71 presentano il pattern di tipo 2, 33 pazienti il pattern di tipo 3 e 42 pazienti il pattern di tipo 1. Il dosaggio della Vit D 25-OH è stato eseguito mediante tecnica di chemiluminescenza nel siero dei 156 pazienti.RISULTATI:I valori di riferimento della concentrazione di Vit D 25-OH seconda la metodica utilizzata oscillano tra 50- 250 nmol/L. Sulla base dei dati ottenuti abbiamo riscontrato che sia i pazienti con pattern di tipo 2 e di tipo 3 presentano un deficit di Vit D 25-OH. Facendo un ulteriore confronto fra le varie fasce di età interessate, si è osservato che i livelli di Vit D 25-OH sono piu' bassi tra i 20 e i 60 anni, con una media di 39,50 nmol/L, rispecchiando l'insorgenza precoce della sclerosi multipla. Nei pazienti con EA che presentano il pattern di Tipo 3, i livelli plasmatici d Vit D 25-OH risultano inferiori rispetto ai range di normalita' in pazienti tra i 30 e i 70 anni, con una media di 40,25 nmol/L. CONCLUSIONI: Come evidenzia lo studio condotto, più del 65% dei pazienti con diagnosi positiva di sclerosi multipla e nelle encefaliti autoimmuni presenta una concentrazione di vit D inferiore ai valori standard, (<50 nmol/L). Sono stati effettuati diversi trials clinici riguardanti la somministrazione supplementare di Vit D a diverse dosi, sia alte (20.400 IU) che a basse dosi (400 IU) a giorni alterni, e si è osservato quali effetti avessero sui marcatori clinici e di imaging dell'attività della malattia nei pazienti con SM (2). Gli autori hanno riconosciuto che la dimensione del campione di questo studio era sottodimensionata, ma non c'era alcuna differenza significativa tra i due gruppi, dopo 18 mesi.

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EP017

Laboratory Diagnosis of Intrathecal Synthesis of Immunoglobulins: the contribution of OCBs and K-index

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Background: The diagnosis of MS relies on a combination of imaging, clinical examinations, and biological analyses, including blood and cerebrospinal fluid (CSF) assessments. G-Oligoclonal bands (OCBs) are considered a "gold standard" for MS diagnosis due to their high sensitivity and specificity. Recent advancements have involved the introduction of kappa free light chain (k-FLC) assay into CSF and serum (S), along with the albumin quotient, leading to the development of a novel biomarker known as the "K-index" or "k-FLC index". The integration of the measure of the K-index with OCBs assessment emerges as a more precise method for MS diagnosis. This combined approach not only enhances diagnostic accuracy, but also offers a more efficient and cost-effective alternative.
Objective: Therefore, the aim of this study was to identify the possible advantages derived from the use of the K-index value by means of a study comparing the diagnostic performance of the K-index with that of the oligoclonal band analysis in a cohort of patients with suspected MS pathology.
Methods: For this study, we prospectively included 70 patients. We analyzed CSF and S samples by nephelometric measurements for the K-index assay and with immunofixation techniques to identify the possible presence of OCBs. Diagnostic performance was assessed by ROC curve analysis.
Discussion and conclusions: The accurate analysis of the K-index values in the cohort of recruited patients allowed us to define by means of ROC curves an accurate and clinically congruent measurement range allowing the identification of all MS patients with simultaneous identification of OCBs. By means of statistical analysis, we obtained: i) a K-index cut-off: 4,41 with a sensitivity and specificity of 93.10% and 95.12% respectively, ii) a slightly higher optimal cut-off value for multiple sclerosis in women (4,41) and in men (3,91), iii) an inverse correlation with albumin and K-index, iv) increased cellularity in MS patients and a cell count that increases in direct proportion with K-index and disease severity.

EP018

MULTIPLE SCLEROSIS DIAGNOSIS: THE CONTRIBUTION OF THE FREE LIGHT CHAIN TEST IN CEREBROSPINAL FLUID

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Introduction: Multiple sclerosis (MS) is a complex neurological disorder characterized by immune-mediated inflammation and demyelination of the central nervous system. Accurate and early diagnosis of MS is critical for effective management and treatment. Over recent years, the analysis of Free Light Chains (FLCs) in CSF has emerged as a promising diagnostic tool. This study investigates the role of FLC analysis in enhancing the accuracy of MS diagnosis. The research involves the examination of CSF and serum samples collected between 2019 and 2022. Key objectives include evaluating the predictive value of existing FLC indices, assessing specific patient profiles, and considering the utility of FLC analysis in paediatric cases.
Methods: 132 patients were included in this study. They were divided in five groups: 33 MS, 33 IND (inflammatory neurological disorders), 25 peripheral inflammatory neurological disorders (PIND), 28 non-inflammatory neurological disorders (NIND) and 13 symptomatic controls (SC) without central inflammatory neurological disease. KFLC, λFLC, IgG and albumin levels were quantified by turbidimetry on Optilite (The Binding Site®) in CSF and sera of patients. FLC index and IgG index were calculated and compared to OCBs.
Results: In terms of AUC, K index demonstrated superior diagnostic performance compared to the λ and IgG indices. Based on the data collected, it has identified an optimal cut-off value for the K Index at 6.56, which exceeds the current threshold value of 5 utilized at the Analysis Laboratory. Regarding λ Index, it has been determined an optimal cut-off value of 10.12, while for the IgG Index was 0.65. Additionally, FLC analysis reveals distinct patterns in patients with positive K indices and negative Oligoclonal Bands (OCB), shedding light on atypical MS presentations.
Conclusions: This study underscores the importance of FLC analysis as a complementary tool in the diagnosis of MS. Early and accurate diagnosis facilitated by FLC analysis can lead to timely interventions, thereby improving patient outcomes and enhancing the understanding of this complex neurological condition, especially for paediatric cohort given that, compared to adults, showed a generally inflammatory disease course, with more frequent relapses.

EP019

Plasmatic biomarkers of neurodegeneration in Alzheimer's patients: diagnostic performance in a real-world setting

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Introduction: neurodegenerative disorders like Alzheimer's Disease (AD) are a growing health concern. Biomarkers are becoming important for AD research and diagnosis. Commonly used cerebrospinal fluid (CSF) biomarkers and neuroimaging are invasive and have limitations. Plasmatic biomarkers could be optimal for detecting neurodegeneration, as non-invasive, cost-effective, reliable, and consistent tools. Our study aims to analyze plasmatic Aβ1-42, 1-40, and p-tau compared to CSF A+/T+ status. These non-invasive biomarker combination could pre-select patients for lumbar puncture.
Methods: we enrolled 69 patients, categorizing them as AD or non-AD using the ATN criteria, which classify Alzheimer's based on biomarkers for Amyloid plaques (A), Tau pathology (T), and Neurodegeneration (N). CSF and EDTA plasma samples were collected from each patient. Neurodegeneration biomarkers (Aβ1-42, 1-40, p181-tau) were measured using Lumipulse G600 (Fujirebio, Tokyo), an automated platform utilizing CLEIA technology. According to CSF profiles, 34 cases met the criteria for A +/T+ status, serving as reference for Receiver Operating Characteristic (ROC) analysis, which included plasmatic Aβ1-42, Aβ1-42/1-40 ratio, and p181-tau as diagnostic tests for A+ and T+ status.
Results: plasmatic p-tau was significantly increased in A+/T + patients ($p < 0.001$), while Aβ1-42/1-40 ratio decreased ($p = 0.022$). No significant difference was found for plasmatic Aβ1-42 and Aβ1-40. Plasmatic p-tau and Aβ1-42/1-40 ratio correlated with CSF measurements (Spearman $r = 0.612$; $p < 0.001$ and $r = 0.357$; $p = 0.003$, respectively). ROC analysis showed optimal diagnostic performance with plasmatic Aβ1-42/1-40 ratio < 0.081 and plasmatic p-tau > 1.285 pg/ml for A+ and T+ thresholds. Combining these, sensitivity and specificity for diagnosing CSF A+/T+ condition were 92.8% and 85.5%, respectively.
Conclusions: Preliminary data suggest that incorporating Aβ1-42/1-40 and p181-tau measurements, using the automated Lumipulse G600 platform, could effectively identify probable AD through non-invasive, reliable plasma biomarkers, though further studies are needed to confirm these findings for widespread clinical use.

EP020

The role of biomarkers in differential diagnosis of neurodegenerative dementias: a case report

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BACKGROUND
Here, we report the role of cerebrospinal fluid (CSF) biomarkers in the differential diagnosis of neurodegenerative dementias. Our patient, a 75-year-old Caucasian male (A.I.), was in a healthy state until May 2022, when cognitive and behavioral alterations with a delirious character appeared. In April 2023, worsening dysphagia for solid foods and liquids, odynophagia, and hoarseness evolved into aphagia. A.I. was taken to the Emergency Room of A.O.R.N "A. Cardarelli" in Naples, and after the exclusion of a gastrointestinal pathology, he was relocated to the U.O.C. of Neurology at A.O.U. "Federico II" in Naples. Neurologists performed a neuropsychological examination that highlighted deficits in visuoconstructive skills for complex material, short- and long-term spatial and verbal memory deficits. The overall clinical status undoubtedly indicated a condition of dementia. The two main diagnostic suspicions were Alzheimer's Disease (AD) and Frontotemporal Lobar Degeneration (FTLD). To discriminate between these diseases, we evaluated β-Amyloid1-42 (Aβ42) and the β-Amyloid1-42/β-Amyloid1-40 ratio (Aβ42/Aβ40), whose decrease is associated with amyloid plaques, and total tau protein (T-tau) and phospho-tau (p-tau181), which are associated with neurodegeneration.
MATERIALS AND METHODS
A CSF sample was obtained by spinal tap from patient A.I. We evaluated the four markers using a chemiluminescent enzyme immunoassay (CLEIA) instrument (Lumipulse, Fujirebio Holdings Inc., Tokyo, Japan).
RESULTS
We found increased levels of both T-tau (1571 pg/ml, reference range 0-410 pg/ml) and p-tau181 (266 pg/ml, reference range 0-59 pg/ml), which are compatible with both AD and some forms of FTLD. We also detected reduced levels of both Aβ42 (366 pg/ml, reference range >725 pg/ml) and the Aβ42/Aβ40 ratio (0.026, reference range >0.069). This finding suggests the presence of amyloid plaques and aids in the differential diagnosis between the two forms of dementia.
CONCLUSIONS
Our clinical case demonstrated how, in complex geriatric patients, the availability of reliable biomarkers can be particularly useful in defining the correct diagnosis, prognosis, and therapeutic path, which differs significantly between each form of dementia.

EP021

Evaluation of core Biomarkers of Alzheimer's disease in three biological matrices: cerebrospinal fluid, plasma, and saliva

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Introduction. Core biomarkers of Alzheimer's disease (AD), including amyloid peptide beta-42 (Aβ42), Aβ42/40 ratio, and phosphorylated tau (pTau), are valuable tools for diagnosing AD. In clinical practice, they are currently measured in the cerebrospinal fluid (CSF). However, the invasiveness of CSF collection limits widespread use. Consequently, intensive research aims at identifying alternative, noninvasive, and accessible biological matrices for measuring AD core biomarkers. In this study, we measured AD core biomarkers in CSF, saliva, and plasma using a fully automated platform.Methods. We enrolled all consecutive patients with cognitive decline. For each patient, we measured Aβ42, Aβ40, and pTau levels in CSF, saliva, and plasma by Lumipulse G1200 (Fujirebio). Results. We included forty-two patients, of whom 65% had AD. Biomarker levels significantly differed across the three biofluids, with saliva showing the lowest and CSF the highest levels of Aβ42, Aβ40, and pTau. A positive correlation between pTau and the Aβ42/40 ratio in CSF and plasma was detected, whereas no correlation was found between any biomarkers in CSF and saliva.Conclusions. Our findings suggest that plasma, but not saliva, could serve as a surrogate biofluid for measuring core AD biomarkers. However, further studies are mandatory to validate the clinical use of blood-based biomarkers in AD.

EP022

Comparison of chemiluminescent enzyme immunoassays and enzyme-linked immunosorbent assay for CSF and serum Neurofilament Light Chain measurement

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Introduction: Neurofilament light chain (NfL) is a key component of the neuronal cytoskeleton, critical to maintaining neuronal structural integrity. NfL has gained attention as a biomarker for neuronal injury and axonal damage. Its levels in cerebrospinal fluid (CSF) provide valuable insights into the extent of neurodegeneration, aiding in the diagnosis, monitoring, and prognosis of various neurological conditions, including Alzheimer's disease (AD). Enzyme-linked immunosorbent assay (ELISA) is the most widely used method for quantifying NfL in cerebrospinal fluid (CSF). Recently, fully automated immunoassays for measuring NfL in CSF and blood have enhanced reproducibility across laboratories, making NfL suitable for routine clinical practice. In this study, we compared the Uman Diagnostics NF-light ELISA with the fully automated Lumipulse platform for measuring NfL.Methods: We enrolled sixty patients with cognitive decline. CSF NfL levels were measured by NF-light ELISA kit (Uman Diagnostics, Sweden), and chemiluminescent enzyme immunoassay (CLEIA) on the Lumipulse G1200 platform (Fujirebio Diagnostics). Serum NfL levels were measured by CLEIA on the Lumipulse G1200.Results: We found a significant, robust correlation (Spearman rho = 0.94 [0.90 - 0.96]) betweenCLEIA and ELISA in CSF, and a significant moderate correlation between CSF and serum with bothanalytical methods (CSF CLEIA vs serum CLEIA 0.41 [0.16 - 0.61]; CSF ELISA vs serum CLEIA 0.40 [0.15-0.60]). NfL levels in CSF measured by Lumipulse G1200 were approximately 1.68 times higher than those measured by ELISA. Notably, CSF CLEIA measurements were approximately 136.12 times higher than the serum ones.Conclusions: Our findings show a robust correlation between ELISA Uman Diagnostic and Lumipulse G1200 for CSF NfL measurements, with improved accuracy with the CLEIA technology over ELISA. Despite the strong correlation, the differences between the two methods are non-negligible, suggesting that while they may be used interchangeably with caution, the differences must be accounted for, especially in a clinical setting where exact quantification is crucial. Thus, different decisional cut-off must be used for optimal disease detection.

EP023

Establishing the decisional cut-off of serum Neurofilaments light chain for Alzheimer's Disease

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Introduction: Neurofilament light chain (NfL) is a well-established biomarker of neuroaxonal injury in several neurodegenerative diseases, including Alzheimer's disease (AD). NfL is commonly measured in the cerebrospinal fluid. However, the recent introduction of new-generation immunoassay methods allows its reliable quantification in serum. In this study, we established the decisional cut-off of NfL for AD. Methods: We enrolled a total of 120 individuals, 60 AD and 60 healthy controls. NfL levels were measured by chemiluminescent enzyme immunoassay (CLEIA) on the fully automated Lumipulse G1200 platform (Fujirebio Diagnostics). Results: We found that NfL has good accuracy for detecting AD, with an area under the curve of 0.88. We chose an optimal serum NfL cut-off of 17 pg/mL, which was associated with a sensitivity, specificity, positive and negative predictive value of 0.78, 0.77, 0.92, and 0.68, respectively. Conclusions: Our findings show that NfL can detect AD with good accuracy. Additionally, we established the decisional cut-off for AD, representing a critical step for introducing a biomarker in clinical practice.

EP024

Serum Brain-Derived Neurotrophic Factor (BDNF): a new biomarker of Brain Aging

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Introduction: Brain-derived neurotrophic Factor (BDNF) is a member of a family of neurotrophins that controls the differentiation and growth of neural progenitor cells, neural plasticity associated with learning, memory and maintains normal brain function. Several studies have reported that BDNF likely plays an important role in neurodegenerative diseases, representing an early biomarker of dysfunctional cognitive deficit.Objective: This study aims to evaluate the serum BDNF concentrations in 30 patients with Mild Cognitive Impairment (MCI) due to Alzheimer's Disease (AD) and 30 patients with AD admitted to U.O.C. of Neurology at University Hospital "P. Giaccone" of Palermo, and 40 normal controls (NCs) with normal cognitive function to determine the possible association between BDNF levels and cognitive decline. Methods: Serum levels of BDNF, CSF levels of α-synuclein (α-Syn), and neurogranin (Ng) were measured using enzyme immunoassay (ELISA). CSF levels of amyloid-β42, total tau, and phosphorylated tau were measured using CLEIA technology (Lumipulse G platform). Results: Serum BDNF levels were significantly higher in the NC group (11645,5; ±2768,3) compared with MCI due to AD group (8131,54±3568,8) and AD group (9778,97±3414,5) ($p < 0.01$). Serum BDNF levels in the MCI due to AD were significantly lower than those in the AD and control groups (P-value <0.01). The correlation analysis revealed a significant negative correlation between BDNF and α-Syn levels ($p = 0.02$) and a significant positive correlation between BDNF levels and Ng concentrations ($p = 0.02$). Subsequently, a correlation analysis was carried out between CSF neurodegeneration biomarkers (Aβ-42, the Aß 42/40 ratio, tTau, pTau, α-Syn, Ng) and BDNF levels in the AD group, which, however, did not have any statistically significant data. Furthermore, no statistically significant correlation was observed between serum BDNF levels, and the age and gender of the subjects analyzed in the study.Conclusions: Our data analysis supports the hypothesis that serum BDNF can be a biomarker for neuronal damage and brain aging. An early diagnosis of cognitive decline using a noninvasive blood sample could lead to an early diagnosis before dementia progresses.
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EP025

Establishing Allowable Total Error for Osteocalcin Based on State-of-the-Art: Insights from a Bone Metabolism Biomarkers EQA Program

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Introduction: State-of-the-art for setting analytical quality specifications is one of the three models proposed by the 1st European Federation of Laboratory Medicine (EFLM) Strategic Conference held in 2014 in Milan. Since the External Quality Assessment (EQA) schemes can contribute to their definition, we analysed data from 22 Lombard Laboratories participating in the Bone metabolism Biomarkers EQA program for the osteocalcin marker, for establishing the Allowable Total Error (TEA).Methods: 176 results of two EQA cycle, 2022-2023, for a total of 8 exercises, were elaborated as follows: subdivision by exercise and by two peer groups based on the principle of method (electrochemiluminescence immunoassay and chemiluminescence immunoassay); calculation of the percentage deviation (%Bias) of each result from the robust average of the peer group, after outliers removal (16 outliers) according to Huber-Hampel approach. In addition, we calculated the %Bias of each result from the robust average of all results as a single group, with no subdivision in peer groups, after outliers removal (16 outliers). Finally, we calculated the 95th percentile of all the obtained %Bias considering two peer group and the 95th percentile of all the obtained %Bias considering all data as a single group. Results: It was found a 95th percentile of 11.6% considering the %Bias of results for different method peer groups and a 95th percentile of 15.4% considering the %Bias of all data as a single group. Both 95th percentile obtained were taken as a TEA based on state-of-the-art.Conclusions: The EQA schemes can be a productive source of state-of-the art analytical performance specification whose application is able to discover a no-good performance between laboratories. Furthermore, TEA identified aligns with EFLM Biological Variation Database for Osteocalcin, where the desirable limit for total allowable error is set at 15.7%.

EP026

D- and L-amino acid blood concentrations are affected in children with Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked disease caused by the absence of functional dystrophin in the muscle cells. Recent untargeted metabolomics studies identified amino acid metabolism alterations as biochemical pathways potentially involved in DMD pathogenesis. Here, in a well-characterized cohort of DMD children and pediatric controls, we investigated by High-Performance Liquid Chromatography (HPLC) the serum profile of a selected pool of amino acids in D- and L-configuration, including L-glutamate, L-glutamine, glycine, L-aspartate, D-aspartate, L-asparagine, L-serine and D-serine. These amino acids are known to modulate neurotransmission and play essential roles in energy and skeletal muscle metabolism.

HPLC determinations highlighted a general amino acid deregulation in DMD, compared to controls, including lower L-aspartate, L-asparagine, D-serine, L-glutamine, glycine levels, and D-/Total serine ratio. In control subjects, we observed a significant positive correlation between L-glutamine and age, which was lacking in affected children. Conversely, in DMD, we observed: i) a negative correlation of L-glutamate and L-aspartate with serum creatinine and creatine kinase levels; ii) a direct correlation of serum L-glutamine/L-glutamate ratio with the fat-free mass index (as determined by Dual Energy X-ray Absorptiometry) and with specific motor function scores (North Star Ambulatory Assessment); iii) no correlations between glucocorticoid treatment or cognitive function and the serum amino acid profile.

Our study highlights significant correlations between serum L-glutamate levels, L-glutamine/L-glutamate ratio, and the multidimensional measures of muscle wasting and motor impairment, suggesting that peripheral glutamine-glutamate metabolism can be a suitable biomarker of disease severity and progression in DMD patients.

EP027

New diagnostic weapons for Alzheimer's disease

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Cerebrospinal fluid (CSF) biomarkers have been shown to be highly informative, sensitive and specific in diagnosing Alzheimer's disease (AD). On the other hand, the regular use of CSF in the clinic is limited due to the invasive method of CSF collection. Therefore, plasma samples represent the most direct and convenient means to study the biochemical changes occurring in the central nervous system.Our study aimed to access the robustness of the LUMIPULSE G600II (Fujirebio®) in the detection of plasma biomarkers in the diagnosis of AD. A method comparison with the ultra-sensitive biomarker detection system (Simoa®, Quanterix) was also performed.Recruited patients underwent standard clinical and cognitive assessment; AD diagnosis was based on CSF biomarker evaluation. Plasma p-tau181, amyloid β proteins (Aβ42, Aβ40) and p-tau217 were measured by both LUMIPULSE and SIMOA. The study was developed in a first phase involving a smaller cohort of 133 participants: 55 AD subjects, 28 patients with other neurodegenerative diseases (NDD) and 50 cognitively normal (CN) participants. During this first phase, technical and clinical validation of plasma p-tau181, amyloid β proteins (Aβ42, Aβ40) was performed. In the second phase of the study, the cohort was expanded and 392 participants (162 AD, 70 NDD and 160 controls) were recruited, allowing the assessment of plasma p-tau217. The accuracy and precision of LUMIPULSE was assessed by testing plasma p-tau181, Aβ-42 and Aβ-40 in the first cohort against SIMOA (SR-X) and plasma p-tau217 in the expanded cohort against another SIMOA platform (HD-X, ALZpath). The ability of the plasma biomarkers assessed by both techniques to discriminate AD from NDD and controls was investigated using ROC analyses. The between-run CV was 3.58%, 2.64% and 0.89% in L1 and 1.89%, 3.19% and 2.15% in L2 for p-tau181, Aβ42 and Aβ40 respectively; moreover, 1.310 and 2.280 in positive and negative controls respectively for p-tau217.Conclusions: Plasma assay confirmed high biological validity with high discriminatory accuracy for the diagnosis of AD. p-tau181 and p-tau217 are promising biomarkers for the clinical diagnosis of AD, demonstrating high precision and diagnostic accuracy.

EP028

Progranulin measurement with a new automated method

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Background: Progranulin (PRGN) is a glycoprotein coded by the GRN gene, on the chromosome 17q21. Among the most frequent genetic causes of Fronto Temporal Dementia (FTO), the GRN mutations are responsible for 20% of familial cases. Until now, the plasma PRGN measurement was carried out using a traditional manual ELISA method (released for research use only RUO, Progranulin ELISA, Mediagnost, Germany) that provides, in our experience, useful and relevant clinical information.Aim of our study is to verify the analytical performance and the clinical usefulness of a new automated chemiluminescent immunoassay for progranulin measurement recently developed and applied on the Chorus EVO instrument (Diesse, Siena, Italy).
Methods: Residual plasma samples (K2EDTA) from patients suffering from different neurodegenerative disorders, sent to the laboratory for the biochemical monitoring of neurodegenerative diseases, have been used for progranulin measurement. Five plasma pools in those concentrations 2.5,11.5,21.3,35.2and 68,8 ng/mL have been adopted to evaluate the analytical performance during the study. Statistical analysis (Cohen's Kappa and ROC curve) is performed using MedCalc program.
Results and Conclusion: A total of 130 patients (60 males, 70 females; age 53-81 and 44-82 years respectively) have been recruited. In 61 out of 130 patients, the genetic screening for GNR or other mutations has been carried out according to standard procedures. The Kappa of Cohen test between Mediagnost ELISA and Chorus EVO is 0.65; the within series imprecision (CV%) was found to range from 3.8% (11.5 ng/mL) to 10.8% (2.5 ng/mL) and the between run CV % from 5.6% (68.8 ng/mL) to 10.7% (2.5 ng/mL), respectively. ROC curves have been performed to compare genetic testing (GRN mutation carriers) and progranulin results: genetic testing vs PRGN Mediagnost, was found to have an AUC of 0.817 (cut-off: ≤17.6 ng/mL, sensitivity = 66.7%, specificity = 92.5%); genetic testing vs PRGN Chorus EVO, AUC: 0.949 (cut-off ≤ 21 ng/mL sensitivity =100%, specificity = 82.7%). In conclusion, the reported results demonstrate satisfactory analytical and clinical performances of the new automated method, which is suitable for the adoption in clinical practice.

EP029

Evaluating the relationship between APOE #4 and biomarkers of neurodegeneration in Alzheimer's disease

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Background. Alzheimer's disease (AD) is the most common form of dementia worldwide. It is a multifactorial disease, and the allele ε4 of the apolipoprotein E (APOE) gene is the major genetic risk factor of sporadic AD. Literature evidence suggests that APOEε4 also influences the progression of AD. In this study, we evaluated the possible association between APOE genotype and biomarkers of neurodegeneration in AD. Methods. We performed a retrospective observational study at the University Hospital "P. Giaccone" in Palermo, Italy. For each patient, we measured amyloid beta-42 (Aβ42), Aβ40, tau protein phosphorylated at threonine 181 (pTau), total tau (tTau), neurogranin, alpha-synuclein, and NfL in cerebrospinal fluid (CSF). The relationship between APOE genotype and biomarkers' levels was evaluated by stratifying patients for AD vs. non-AD.Results. The study population compriserd 194 patients (123 AD and 71 non-AD). AD patients have significantly lower Aβ42 levels and Aβ42/40 ratio and higher pTau, tTau, and NfLs levels than non-AD patients. In AD patients, the APOEε4 allele is associated with a significantly lower Aβ42/40 ratio and higher levels of pTau, tTau, neurogranin, and alpha-synuclein. This association is not observed in non-AD patients. Conclusions: This study provides evidence of the significant impact of the APOE ε4 allele on neurodegenerative biomarkers in AD patients, highlighting its role in exacerbating amyloid and tau pathology as well as synaptic degeneration.

EP030

Nanopore sequencing for unravelling short tandem repeats, interruption patterns and epialleles in Myotonic Dystrophy type 1

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Background/Objectives: Myotonic Dystrophy type 1 (DM1) is a complex disease, characterized by the expansion of a CTG triplet in gene DMPK. The current diagnostic techniques based on southern blot and/or Triplet Repeat Primed PCR cannot furnish an accurate and quantitative repeat size estimate. Moreover, they are not able to detect neither the presence of interruption motifs with a stabilizing effect on clinical manifestations, nor epigenetic modifications such as DNA methylation. Methods: In this study, we employed nanopore sequencing to analyze STRs and interruptions in 10 patients with DM1. Native DNA from whole blood and from nasal brushing was processed for library preparation using the Ligation Sequencing kit V14 and loaded onto the GridION platform (Oxford Nanopore, UK). The “in silico” target enrichment adaptive sampling approach avoided PCR bias in amplifying GC-rich regions. Long PCR followed by long read sequencing was performed in parallel for confirmation. Resulting reads were filtered and mapped to the reference genome, and STRs were detected using EPI2ME, IGV and straglr. Results: The adaptive sampling method was able to characterize precisely CTG repeats in all samples in the DMPK gene. It also detected interruption motifs within the pathogenic tandem repeats, and DNA methylation patterns in both epialleles. The coverage depth range varies between 3 to 12 reads/locus. In DM1 patients we found CTG expansions in a range between (75-1412) confirming southern blot analysis in all cases. CTG repeat was often intermingled with CAA, TTG, CCG, CCC, CCT. Conclusion: The adaptive sampling approach resulted to be a powerful and unique technique for the diagnosis of expansion repeat disorders identifying simultaneously STR size, interruption patterns and DNA methylation of each epialleles. In case of large expansions, the PCR approach gave false negative results due to intrinsic difficulties in amplifying large GC rich regions typical of these motifs. These results will be integrated with imaging, neuropsychological and clinical data collected at Bellaria Hospital, exploiting machine learning algorithms. The outcome will be a model that will help understanding the genotype-phenotype correlation, from which both health-care system and patients’ quality of life can benefit.

EP031

Hematological indexes and morphological features of blood cells in COVID-19: a current review

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Background: Coronavirus disease 2019 (Covid-19) is a systemic infection characterized by acute respiratory distress syndrome, hyperinflammation and coagulation disorders. The hematopoietic system plays a critical role during the hyperinflammation that is particularly serious in severe patients.Several hematological abnormalities have been recorded using both: cytofluorometric imaging (scattergrams), and typical morphological abnormalities which are linked to the severity of disease. The major hallmarks of complete blood count (CBC) and leucocyte differential studies include: i) specific scatter-plot images ii) neutrophilia and lymphopenia and iii) various morphological anomalies observed in multi-lineage of peripheral cells.

Scope and Methods: We conducted a study by researching in PubMed platform the most recent articles published over the last three years (from February 2020 to November 2023), by using keywords such as: Covid-19, hematological parameters and morphological abnormalities. We analysed the hematological results reported from survivors (S) and not survivor’s patients (NS) who showed moderate and severe symptoms of Covid-19 infection respectively.

Results: Our analysis revealed an evident increase in white blood cells (WBCs) count (neutrophilia), lymphopenia and thrombocytopenia, which are typical of the disease. Additionally, the microscopic analysis of the blood cells in severe patients, revealed: i) numerous neutrophils with evident granularity, and sometimes showing toxic granulation and vacuolization, ii) atypical lymphocytes with abundant blue cytoplasm, iii) atypical monocytes with vacuoles, iv) platelet aggregation and v) basophilic stippling in red blood cells.Through the observation of scattergrams mainly recorded by using Sysmex XN-9000 and Mindray BC-6800 instruments, the common characteristics reported were: i) an increase of neutrophilic population and ii) typical “sandglass pattern”.

Conclusions: This review highlights the importance of hematochemical and cytomorphological analysis of blood in COVID-19 patients, contributing to support the clinicians in better identifying and understanding the typical signs of disease severity. These findings contribute to the ongoing effort to improve patient management and outcomes in COVID-19.

EP032

Valutazione della performance diagnostica del sistema Sofia 2 SARS COV2 per la rilevazione dell'antigene SARS-CoV-2 in POCT: un'analisi di 130 campioni

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I sistemi Point-of-Care Testing (POCT) si sono rivelati strumenti diagnostici in grado di assicurare una diagnosi rapida per la rilevazione dell'antigene nucleocapsidico N del virus SARS-CoV 2.
Scopo di questo studio è stato confrontare il sistema POCT Sofia 2 SARS Antigen+ (QuidelOrtho), in immunofluorescenza, con il sistema Lumipulse G600 (Fujirebio Italia Srl), in chemiluminescenza, di cui abbiamo già dimostrato una buona sensibilità (64%) e un'alta specificità (100%). Lo studio ha incluso 130 campioni di tampone nasofaringeo in terreno liquido UTM, raccolti presso il Dipartimento di Emergenza dell'AORN Sant'Anna e San Sebastiano di Caserta, nel periodo compreso tra Marzo 2023 e Novembre 2023, di cui il 50% con sintomatologia da SARS-CoV 2 e il 50% senza alcuna sintomatologia. Dei 130 campioni è emerso che 69/130 (53%) erano positivi al test Lumipulse G SARS-CoV-2 Ag (cut-off>10 pg/mL) e 61/130(46%) erano negativi allo stesso test (cut-off<1.0 pg/mL). Non sono stati inclusi nello studio campioni con risultati dubbi (cut-off tra 1.0 pg/mL e 10 pg/mL). Tra i campioni positivi al test Lumipulse è stata osservata una concordanza del 63%, (44/69) ed una discordanza del 36% (25/69) (Lumipulse positivo/Sofia 2 negativo). Tutti i 61 campioni negativi al test Lumipulse si sono confermati negativi al test Sofia. I 25 campioni discordanti, tutti da pazienti asintomatici, si collocano in una fascia di bassa positività (<10 pg/mL), come confermato anche dal test molecolare Standard M10 SARS-CoV-2 (SD Biosensor. inc) (Ct>28). La sensibilità del test Sofia 2, risulta, quindi, essere del 64% e la specificità del 100%. In conclusione questo studio ha dimostrato che il sistema POCT Sofia 2 SARS Antigen ha un'elevata sensibilità tra i campioni sintomatici. La discordanza tra i due test, verificatasi in campioni con bassa positività e assenza di sintomatologia, suggerisce che il test Sofia 2 potrebbe essere particolarmente utile nella diagnosi rapida di infezioni conclamate da SARS-CoV-2 mentre persiste la necessità del test Lumipulse per la diagnosi di SARS-CoV-2 in soggetti asintomatici.

EP033

Six-months kinetics of humoral and cellular immunity after BNT162b2 bivalent booster

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Background: Humoral and cellular immunity are crucial in protecting against contracting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and developing severe coronavirus disease 2019 (COVID-19). This study was planned to monitor immunity in recipients of bivalent BNT162b2 booster up to 6 months thereafter.Methods: The study population consisted of healthcare workers who already received a primary vaccination cycle and booster of monovalent BNT162b2 vaccine, and were administered the BNT162b2 bivalent booster. Blood samples were drawn before vaccination, 1, 3 and 6 months thereafter. Humoral and cellular immunity were assessed with Roche Elecsys total anti-SARS-CoV-2 antibodies and Roche Elecsys IGRA SARS-CoV-2.Results: The final study population consisted of 52 healthy healthcare workers (mean age 45±13 years; 27 women). Total anti-SARS-CoV-2 antibodies increased 1 month after vaccination from 11027 (IQR, 6850-18836) to 25000 (IQR, 17807-25000; p<0.001) kBAU/L, levels remained elevated compared to baseline at 3 months (22598 kBAU/L; IQR, 10121-25000 kBAU/L; p<0.001), but returned to baseline values at 6 months (12394 kBAU/L; IQR, 5641-21168 kBAU/L; p=0.318). IGRA SARS-CoV-2 did not increase significantly from 0.53 (IQR, 0.15-1.03) to 0.80 (IQR, 0.28-1.49; p=0.314) U/mL 1 month after vaccination, then continued to increase significantly from baseline to 1.34 (IQR, 0.54-2.90; p<0.001) U/mL after 3 months, and returned to baseline values after 6 months (0.62 U/mL; IQR, 0.26-1.47 U/mL; p=0.307). An inverse significant correlation was found between basal total anti-SARS-CoV-2 antibodies and delta increase at 1 (r=-0.93 and 95%CI, -0.96 to -0.88; p<0.001), 3 (r=-0.73 and 95%CI, -0.55 to -0.84; p<0.001) and 6 (r=-0.38 and 95%CI, -0.61 to -0.10; p=0.001) months, and also between basal IGRA SARS-CoV-2 and delta increase at 1 (r=-0.57 and 95%CI, -0.74 to -0.33; p<0.001), 3 (r=-0.63 and 95%CI, -0.78 to -0.41; p<0.001) and 6 (r=-0.55 and 95%CI, -0.73 to -0.31; p<0.001) months.Conclusions: The specific kinetics of immunity in recipients of BNT162b2 bivalent booster suggests that regular administration of booster doses may be required between 6-12 months to achieve enhanced protection against SARS-CoV-2.

EP034

POCT TECHNOLOGY INNOVATION FOR ASP OF RAGUSA BY U.O.C. LABORATORY ANALYSIS.

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Following the completion of a tender for the renewal of PoCT devices, as of August 2023, the ASP of Ragusa has expanded the technology fleet of devices in use by providing a total of 32 Nova Biomedical benchtop blood gas monitors and 13 Abbott handhelds located in different OUs of the 3 PP.OOs of Ragusa, Modica - Scicli and Vittoria - Comiso.
The first improvement for the ASP was the implementation of Middleware, specific to each type of device installed, which allows the Reference Analysis Laboratory to remotely monitor the operating status of the devices themselves, the outcome of scheduled CQIs and the execution of tests. Prior to the technological upgrade, PoCT devices had been deployed for years in the OUs in a highly personalized and self-referential manner lacking a connection network with the Reference Laboratory. This mode did not allow their traceability of activities performed remotely, as opposed to the relevant UNI EN ISO 22870:2017 and UNI EN ISO 15189:2022 standards.
The second and most relevant innovation is the connection between the PoCT devices and the LIS, which enables the transmission on the computerized medical record of the patient's biographical and analytical data related to the tests performed with PoCT. This ensures full traceability of the various stages of the testing process, such as: the request for the test by the prescribing physician, the identification of the operator performing the test, the unique identification of the sample after acceptance on the management software, the execution of the test and the transmission of the results on the electronic medical record and health record.
This represents a real achievement for the ASP of Ragusa, which until now did not guarantee the traceability of the entire PoCT analytical process with the production of results with simple instrumental printout to be attached to the folder.
This implementation of the system was possible thanks to the collaboration between the U.O.C. Clinical Pathology Unit, the U.O.C. Information Technology and Digital Transition Service and the companies supplying the PoCT devices such as Nova Biomedical and Abbott.

EP035

Point of Care tests between ward physicians and lab staff

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The rapid turnaround time of POCT is one of its primary advantages, giving access to clinical results in shorter time than main laboratory, allowing faster diagnosis and treatment and so improving outcomes. Currently, there are no national or regional regulations that precisely define who has to sign report produced by a POCT, especially concerning its use for diagnosis and therapy, while Campania Region guide lines state that the doctor and the nurse in charge within each department are responsible for the final result produced by the POCT entrusted to them.The necessity for point-of-care testing (POCT) reports to be signed by ward physicians rather than laboratory graduated staff, stems from several key considerations. Firstly, ward physicians are directly involved in patient care and possess the most immediate and comprehensive understanding of the patient's clinical context. In contrast, laboratory personnel, while experts in test accuracy and interpretation, may lack the detailed, real-time clinical insights that are critical for immediate patient management. Secondly, the rapid turnaround time of POCT is one of its primary advantages, aimed at accelerating diagnosis and treatment. If POCT results were to be reviewed by laboratory staff, this process could introduce delays that negate the benefits of swift testing. At Cardarelli Hospital POCT blood gas analysis instruments are programmed to run internal quality control (QC) at least once a day, excluding the single test not passing the QC; 21 out of 36 run External Quality evaluation in 2024 and all 36 will do in 2025. All the instruments run alignment tests four times a year for shared tests. Provided that all instruments run a full quality control program (FQCP), the Lab team suggested to identify the ward physician requesting the test as the person in charge to electronically sign the result: the Hospital interdisciplinary POCT committee approved the proposal. Signed result will flow into Laboratory Information System and patient's chart. Laboratory team will subsequently validate data in batch, to certify that the instrument properly run quality global control (FQCP). This implementation is expected to be applied by the end of 2024.

EP036

ATTUAZIONE DEL DECRETO REGIONALE N. 145 – ESPERIENZA DELL' ASL NAPOLI 2 NORD - P.O. POZZUOLI E PROCIDA

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In seguito all'entrata in vigore, con il Decreto Regionale n. 145, nell'Aprile 2021, delle "Linee di indirizzo dei Point of Care (POCT) nella Riorganizzazione dei Servizi di Medicina di Laboratorio in Regione Campania", l'Azienda Sanitaria Locale Napoli 2 Nord ha istituito il comitato esecutivo POCT che ha iniziato i lavori per l'implementazione della fornitura di emogasanalizzatori in conformità a tali indirizzi. La nuova fornitura è costituita dall'uso di 36 analizzatori dislocati su 5 Presidi Ospedalieri e Distretti, collegati al middleware di supervisione interfacciato bidirezionalmente con il Laboratory Information System (LIS). La procedura operativa implementata, orientata alla massima tracciabilità, prevede la richiesta dell'esame tramite applicativo, la stampa dell'etichetta identificativa da apporre sul dispositivo di prelievo, l'identificazione obbligatoria con password dell'operatore sanitario che esegue l'esame, la produzione dei risultati e l'archiviazione nella Cartella Clinica Elettronica. Con il supporto del fornitore e in parte della formazione a distanza sono stati addestrati e abilitati all'uso della strumentazione più di 450 operatori, per i soli Presidi Ospedalieri di Pozzuoli e Procida assegnando loro un account personale (ID e password), inserito in una specifica categoria di utenza con privilegi predefiniti. Nei primi 10 mesi di fornitura si può dire di aver centrato l'obiettivo della piena conformità al D.R. n. 145 per la fornitura POCT di sistemi per emogasanalisi, passando da strumentazione fuori dal controllo del Laboratorio ad una tecnologia non solo compliante a questo nuovo modello organizzativo ma che facilita il governo clinico dell'intero processo da parte del Laboratorio, negli aspetti cruciali della connettività, formazione, assicurazione della Qualità, allineamento, gestione dei dati, refertazione e validazione dei risultati. Questo aspetto ha contribuito a migliorare i percorsi di cura, garantendo la qualità analitica del Laboratorio anche in POCT, mediante il monitoraggio e la supervisione da parte del personale di Laboratorio al raggiungimento di obiettivi ancora più performanti.

EP037

Valutazione di performance del sistema Icon 5 rispetto al metodo di riferimento del laboratorio centrale

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INTRODUZIONE : L'esame emocromocitometrico è tra i primi e più richiesti esami di laboratorio, perché di valutare lo stato di salute generale del paziente ed evidenziare la presenza di condizioni patologiche, in particolare se comprensivo anche di nuovi parametri definiti di "conta estesa". Nelle zone di decentralizzazione della medicina di laboratorio, in cui la necessità è quella di ottenere informazioni diagnostiche (oppure informazioni cliniche) rapide e accurate circa lo stato di salute del paziente, questa necessità è ampiamente supportata dall'utilizzo dei PoCT, la cui governance afferisce al laboratorio centrale.
METODI:con il nostro studio abbiamo valutato le performance analitiche del POCT Icon 5 (Norma Diagnostika) rispetto a quelle dello strumento di riferimento DxH900 (Beckman Coulter) utilizzato nel laboratorio centrale. Abbiamo analizzato 40 campioni di verifica di allineamento, (sangue venoso in provetta EDTA), inviati al Laboratorio Analisi BLU dell'Ospedale Civile di Baggiovara. Di questi abbiamo valutato i parametri dell'esame emocromocitometrico riportati di seguito globuli bianchi (WBC), globuli rossi (RBC), piastrine (PLT), emoglobina (HGB) ed il dettaglio della formula leucocitaria a cinque popolazioni, ovvero linfociti (LY), monociti (MO), neutrofili (NEU), eosinofili (EOS) e basofili (BAS).
RISULTATI: L'agreement tra i due metodi per ogni parametro è stato valutato con test di Bland-Altman e l'analisi di regressione di Passing-Bablok rispettivamente di seguito riportati: WBC (bias: -0,29%, 95%CI= 0,18+0,99 e r2=0,995), RBC (Bias:0,04%,95%CI= 0,06+0,99 e r2=0,983), PLT (Bias:0,04%, 95%CI=-6,59+1,09 e r2=0,994), HGB: (Bias: 0,03%95%CI= -0,23+1,02 e r2=0,990). Per la formula leucocitaria: LY (Bias:0,05%,95%CI= 0.20+0.91e r2=0,980), MO (Bias:-0,04%,95%CI= 0,16+0,65 e r2=0,71), NEU (Bias:0,04%,95%CI= 0,36+0,96 e r2=0,993), EOS (Bias:0,03%,95%CI= 0,08+0,66 e r2=0,642) BAS (Bias:0,01%,95%CI= -0,01+1,42 e r2=0,79). Nessuna differenza significativa né in percentuale né in valore assoluto è stata identificata tra i due metodi, attraverso entrambi i test.
CONCLUSIONI: Considerata la grande innovazione della digitalizzazione dell'emocitometria, anche a supporto della diagnostica decentrata, si rende necessario un confronto diretto e continuo con il laboratorio centrale, per promuovere la valorizzazione della medicina di prossimità.

EP038

Comparative Analysis of Glucose and Glycated Hemoglobin (HbA1c) Using TASCOM Point-Of-Care Testing (POCT) System and Two Automatic Laboratory Instrumentations

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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 Point-Of-Care Testing (POCT) systems currently on the market accept the measurement of a wide range of analytes, often using the same analytical principles as conventional laboratory analysis instruments. In this study, we compared glucose and glycated hemoglobin (HbA1c) values measured through TASCOM Point-Of-Care Testing (POCT) system and two automatic Laboratory instrumentations (DXI analyzer Beckman Coulter and Ion-Exchange HPLC System Bio-Rad), aiming to assess the interchangeability of the analytical methods employed and the impact of any pre-analytical errors.
METHODS 104 consecutive samples with a glucose and HbA1c tests request from the Hospital, have been used for the study. To measured glucose levels were utilized two sample, one K3EDTA samples was utilized with TASCOM on whole blood and on DXI 800 were utilized samples after centrifuge. For HbA1c determination, whole blood samples were assessed using both analyzer POCT and Laboratory instrumentation. Comparison of methods was performed according to CLSI EP-09A2 protocol. Statistical analysis were performed with MedCalc® Statistical Software version 20.216 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023).
RESULTS Comparing whole blood K3EDTA results obtained with Tascom versus K3EDTA plasma tested on DXI 800, the Deming regression revealed a proportional error, whereas Bland Altman highlighted a minimal underestimation explained by the time factor. A good concordance was revealed for HbA1c and no constant and proportional error were present. No significant differences neither in percentage or absolute values were identified through the Bland Altman test. Considering values close to the cut off, no significance differences were found.
CONCLUSIONS Method comparison is an important step in the validation process of assays and instruments. It can be concluded that the results examined by the analyzer POCT and Laboratory instrumentation are comparable. The possibility to have a POCT for glucose and HbA1c determination could be crucial on some occasions. Today the POCT shortened the laboratory process and made results available faster than the central lab, its can be used as an effective tool for improving patient flow.

EP039

ASSESSMENT OF A NOVEL POINT-OF-CARE SYSTEM FOR CHEMISTRY PANEL TESTING

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Simplex TASTM 101 is the Point-Of-Care Testing systems currently on the market accept the measurement of a wide range of analytes, often using the same analytical principles as conventional laboratory analysis instruments.
In this study, we compared total cholesterol (TC), high-density lipoprotein cholesterol (HDL) values measured through POCT system and one automatic Laboratory instrumentations (DXI analyzer Beckman Coulter) for cardiovascular disease risk screening. K3EDTA whole blood specimens were analyzed on the by POC staff.
K3EDTA samples were primary employ for TC and HDL with Simplex TASTM 101 as whole blood, then the samples were centrifuge and measured on DXI 800 too.
Comparison of methods was performed according to CLSI EP-09A2 protocol. Constant and proportional errors were investigated with Deming Regression. Bias between method was evaluated with Bland Altman test.
Statistical analysis were performed with MedCalc® Statistical Software version 20.216 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023). Total cholesterol and High-density lipoprotein cholesterol correlated well with plasma samples on the DXI.
The regression slope was 0.977 to 1.177, along with a correlation coefficient (r) of ≥0.963 for all analytes. No significant differences neither in percentage or absolute values were identified through the Bland Altman test.
Considering values close to the cut off, no significance differences were found. Simplex TASTM 101 demonstrated a strong correlation with the comparative methods and excellent precision. The system's analytical performance and continuous quality management make it suitable for use in the ED or in resource limited settings (such as in the developing world, in doctors' offices, or directly at home) to provide rapid reliable test results, which could minimize the time to treatment and to help improve the patients' outcome.

EP040

Comparative Analysis of High-Sensitivity Cardiac Troponin I: Point-of-Care Testing (Pathfast) vs. Central Laboratory Analysis (Beckman Coulter)

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A high-sensitivity cardiac troponin (hs-cTn) must meet the criteria established by AACC and IFCC Committee for Clinical Application of Cardiac Biomarkers. Many authors have suggested that the development of Point-Of-Care-Testing (POCT) methods for cTnI and cTnT with high analytical sensitivity could represent a fondamental progress because these methods could further reduce the TAT of cTnI and cTnT measurement in patients with NSTEMI. The PATHFAST hs-cTnI assay is the first POCT assay with a high-sensitivity designation that received FDA approval for diagnosis of myocardial infarction. The aim of our work is to compare possible differences in hs-cTnI determination using different matrices, by comparing the results obtained with POCT Pathfast hs-cTnI and Access hs-TnI applied on DxI 800 (Beckman Coulter).
METHODS K3EDTA whole blood samples from 148 patients with acute chest pain suspected or hospitalized for ACS were collected. K3EDTA samples were primary employ for hs-cTnI with Pathfast as whole blood, then the samples were centrifuge and measured on DXI 800 too. Comparison of methods was performed according CLSI EP-09A2 protocol.Constant and proportional errors were investigated with Deming Regression. Pathfast CV for whole blood K3EDTA was respectively 6%, whereas CV of Beckman Coulter DxI 800 was calculated as 4.16% for plasma. Bias between method was evaluated with Bland Altman test.
RESULTS Deming regression between whole blood and plasma K3EDTA both investigated with POCT hs-cTnI and Access applied on DxI800 Good Pearson correlation coefficient of 0.99 (CI= 95%; [0.9697–1.0799]) and no constant and proportional error were present (y=-0.9684+1.0303x; slope CI=95%, [0.9697–1.0799]; intercept CI=95% [-1.2995 – 0.1203]). No significant differences neither in percentage or absolute values were identified through the Bland Altman test, thus showing a good concordance between the two methods.
CONCLUSIONS Method comparison is a crucial step in the validation process of assays and instruments. Our comparison demonstrated good agreement between the POCT hs-cTnI and Access hs-TnI assays. The results indicated excellent analytical performance for the Pathfast hs-cTnI. In conclusion, given that POCT has the potential to be the first choice for evaluating patients with suspected ACS, we recommend using the same method, matrix, and anticoagulant in the 0 – 1 hour algorithm.

EP041

THE TECHNOLOGICAL UPDATE ABOUT GLUCOMETERS IN ASST PAPA GIOVANNI XXIII HOSPITAL OF BERGAMO STEP BY STEP

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BACKGROUND-AIM. The upcoming technological update, replacing our actual Accu-Chek Inform II® glucometers with the new Cobas Pulse® ones (both Roche), is the opportunity to our Laboratory to improve the management of POC testing inside and outside our hospital, ensuring the quality of analytical and clinical results, improving Patient cares and safety by governing the process. METHODS. In 2022 we planned the activities by Gantt chart and SWOT Analysis, detailing the following: A.mandatory aspects; B.hospital state of the art; C.training; D.method evaluation; E.informatics (IT); F.supplying; G.Internal Quality Control (IQC); H.External Quality Assurance (EQA); I.operator and patient traceability; L.starting in a pilot department; M.Quality System (QS) documents; N.improving in areas outside the hospital; O.active monitoring. RESULTS. We: A:analyzed mandatory laws and guidelines; B.in 2022 collected 45 questionnaires from nurse coordinators (the most critical areas were patient/staff identification, IT deficiencies); C.trained 1800 operators on-site in 40 sessions (2 hours each, 10-12/2023) and planned a retraining on-line; D.got an optimum agreement between both analytical and clinical performances of both glucometers; E.got a POC report and improved wi-fi connection; F.organized the materials supply inside and outside the hospital; G.got an annual reservation for IQC materials, to align and compare all glucometers results; H.implemented EQA programs for each glucometer; I.got that the staff logs in by personal badge (rfid) and identifies each patient by the barcode on his bracelet; L.distributed Pulses to a pilot department to assess the entire process before the replacement throughout the hospital; M.got the QS documents; N.identified the external hospital locations in which we'll export this model; O.monitor actively the process. DISCUSSION. The POC management is a process that must satisfy ISO 9001, Regional Councils (N °XI/1863/7044) and DM71 requirements. The management of the POC process is strategic for the hospital because it allows the management of its external areas, meeting its Patients and their chronicity with low spending increase: it also becomes an opportunity for the laboratory to exercise its governance through the POC manager and its staff.

EP042

Methemoglobin decrease after methylene blue sample spike: evaluation on two different blood gas analyzers

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BACKGROUND-AIM

NaNO2 is becoming popular as it is contained in the so called “suicide kits” available on the dark web. Biochemically, once ingested, it favors the production and accumulation of methemoglobin (MetHb). The cases of suspected NaNO2 intoxication, must be managed with the prompt administration of antidotes, as methylene blue(MB) (1). Starting from a previous case of suicide by NaNO2 ingestion with unreported MetHb% by the blood gas analysis, and the report which reproduced in vitro the acute methemoglobinemia and the verification of the feedback of two gas analyzers (Rapid Point 500e Siemens Healthineers and GEM Premier 5000Werfen instruments)(2), the present work aims to provide a further step by evaluating the in vitro effect of MB administration(2mg/Kg)(3) in blood samples containing NaNO2

METHODS

The following experiments were conducted using a venous blood sample and measuring MetHb% by both the mentioned instruments: A) addition of MB; B) addition of NaNO2 and subsequent MB with MetHb% evaluation at each step; C) addition of NaNO2 and MetHb% evaluation after 30’, then addition of MB and immediate MetHb% evaluation (t=0’) and after further 30’(t=30’)

RESULTS

MB does not seem to interfere with the analysis (A) as MetHb values were provided by both instruments (0.2%). Results show a MetHb% decrement after the addition of MB to the NaNO2 spiked samples. B experiment, in fact, indicates that MetHb% changed from 30% to 19.9% (Siemens), and from >30 to 21.6% (Werfen). After 30' from NaNO2 spiking (C), when the peak in the MetHb% occurs, the adding of MB provided a determination of MetHb% at t=0’ decreasing from 47.2% to 32.2% with Siemens, while it remained >30 with Werfen. Also, the evaluation of the same sample at t=30' after the addition of MB, indicated MetHb values decrement to 8.9% and 9.4%, for Siemens and Werfen respectively

CONCLUSIONS

Results agree with the described MB pharmacokinetics providing the maximum effect at 30’ after administration. Also, although being an in vitro attempt to reproduce the effect of MB, the extended measuring range offered by Siemens respect to Werfen (0-100 vs 0-30) allows to better monitor the MetHb%. Of course, the described issue of~15% unreported MetHb% by Siemens in NaNO2 spiked blood samples must be considered.

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EP043

Immunoinflammation Signature in Neuronally Derived Extracellular Vesicles: Potential Diagnostic and Therapeutic Applications for Parkinson's Disease and Progressive Supranuclear Palsy

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Neuroinflammation plays a pivotal role in the pathogenesis of neurodegenerative diseases including Alzheimer's disease, type 1 diabetes mellitus, and acute brain injuries such as ischemic stroke. Significant efforts have been directed towards identifying biomarkers that could aid in early diagnosis of Parkinson's disease (PD) and Progressive supranuclear palsy (PSP). However, current advancements in biomarkers discovery fail to accurately predict disease progression or treatment response, complicating the differentiation between these neurodenerative dyscrasias.Extracellular vesicles (EVs) serve as crucial mediators of cell-to-cell communication and hold significant diagnostic potential due to their ability to concentrate protein biomarkers in bodily fluids. Because of their heterogeneity, the International Society for Extracellular Vesicles (ISEV) classifies EVs based on their size into small extracellular vesicles (<200 nm) and large/medium extracellular vesicles (>200). EVs carry a diverse array of biomolecules reflecting their parental cells, and, in particular, Small extracellular vesicles (sEVs) have garnered attention for their role in transferring cargo to host cells and inducing various cellular responses. Recent research highlights the enrichment of neuro-inflammatory proteins in tissue-associated sEVs derived from affected areas, showcasing their diagnostic relevance. Immunocapture of presumed neuronally derived circulating sEVs has emerged as a potential biomarker for various neurological diseases, serving as a proxy for brain pathology. In this context, liquid biopsy and circulating small extracellular vesicles (sEVs) may provide more information to overcome this challenge.Our preliminary data show for the first time a trend in immunomodulation key players expression in PD and PSP patients’ serum over time, suggesting its role as a promising biomarkers in neurodegeneration. Building upon this discovery and considering existing literature highlighting the involvement of inflammation in neurodegenetative diseases, we further investigated its expression both in total and neuro-derived sEVs from these patients. This brand-new approach presents a demanding avenue for enhancing standard diagnostic procedures, potentially offering more personalized insights for patients’ conditions.

EP044

Evaluation of C-Reactive Protein in Point of Care Testing to fight antibiotic overuse

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INTRODUCTION

C-Reactive Protein (CRP) measurement in Point of Care Testing (POCT) may be useful in patient management and therapeutic choices, particularly in basic and pediatric medicine.

MATERIALS AND METHODS

The study was performed at Federico II Clinical Departments and Laboratory, University Hospital in Naples; 196 patients were enrolled: 102 from the Pediatrics Department, in Day Hospital service; 70 hospitalized in the Infectious Diseases Department; 24 from other Departments. CRP-POCT method (Afinion, Abbott): solid phase immunochemistry; sample 2.5 microL; analysis time ~5'; measurement range 5-200 mg/L whole blood and 5-160 mg/L serum/plasma. CRP Laboratory method (Architect, Abbott): immunoturbidimetric; high sensitivity method; measurement range 0.1-160 mg/L, serum/plasma.

RESULTS AND DISCUSSION

Statistical analysis of 86 samples (110 not compared because POCT results showed levels < method sensitivity) was performed using linear regression and Pearson correlation coefficient showing excellent correlation (r=0.99) between the two methods. There was slight underestimation of POCT levels (y= 0.89x+0.03; y= 0.84x+0.94; y= 0.97x-0.34; for Infectious Diseases, Pediatrics and Laboratory, respectively). Reproducibility of the test performed (10 times) by two different operators was satisfactory: CRP 90.2 mg/L, CV 2 and 4% and CRP 5.8 mg/L, CV 7 and 9%. The results of a recent meta-analysis (trials on 9444 patients) confirmed the need for antibiotic therapy in the setting of lower respiratory infections, with CRP>100 mg/L, and no need for antibiotic therapy if CRP<20 mg/L[1]. Using these clinically relevant thresholds, the study highlighted that for levels <20 mg/L, only 2 out of 130 samples (1.5%) were slightly below threshold by POCT compared to laboratory reference method (19 mg/L vs 23, 1 mg/L and 19 mg/L vs 21.1 mg/L), while for a 100 mg/L threshold there was full agreement.

CONCLUSIONS

The CRP-POCT evaluated in this study was rapid, easy to use, operator-independent, well correlated with the results of the Laboratory–the latter always engaged in the 'governance' of POCT activities in front line. Although the test is not highly sensitive (>5mg/L), it could be used to screen patients undergoing antibiotic therapy.

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EP045

New technologies and innovation in POCT-based diagnostic

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Laboratory Medicine rides the wave of technological progress, looking at a multidisciplinary approach, leading to integrated diagnosis and the use of Point-of-Care-Testing (POCT) as important tool for diagnosis and treatment. Further, the recent pandemic of SARS-CoV2 forces us to re-think the use of POCT in settings outside of the hospital. Moreover, the pandemic has created unprecedented challenges for public health systems, with the need of innovative solutions. Inflammation is a set of complex responses strictly related to the pathophysiology of a broad disease range, including cardiovascular, renal diseases, and cancer. Also, COVID-19 and Long Covid (LC) are inflammatory diseases. An interdisciplinary approach is crucial to realize reliable POCT devices, endowed with required analytical sensitivity and specificity. Herein, are shown newly maiden POCTs, based on different technologies, with the aim to detect SARS-CoV2 and quantify inflammatory biomarkers. The new designed POCT for salivary detection of SARS-CoV2 is based on gold nanoparticles (AuNPs), able to bind spike, membrane, and envelope proteins. To facilitate the use of the device, we developed a comprehensive kit, along with a portable spectrophotometer customized for the purposes. This POCT is versatile, extending its applicability to other viruses. Also, to quantify inflammatory mediators, two technologies were selected: screen printed electrode-(SPE) and lateral flow assay-(LFA) biosensors. SPEs were coated with TEGO (Thermal Exfoliated Graphene Oxide) by 3D printing. The produced SPE detects IL-6 in saliva samples. Created LFAs are Multiplexed POCT, due to their ability in simultaneous detection of various analytes. Indeed, our LFAs can simultaneously detect IL-6 and TNFa or the panel VCAM-1, ICAM1, and E-Selectin in whole blood. The employed technology must be integrated with a connectivity process, supported by an accredited laboratory, that allows the quality assurance and the management of these services which are intimately linked to operator training and competency. In conclusion, the results are promising and represent a starting point for the application of innovative POCTs in the detection and clinical evaluation of inflammatory mediators

EP046

Project for the implementation of a Point of Care network at the University of Sassari Hospital Corporation

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INTRODUCTION: The University of Sassari Hospital Corporation (A.O.U.: Azienda Ospedaliera Universitaria di Sassari) was born from the aggregation of the Local Health Care Service (A.S.L.: Azienda Sanitaria Locale) and the care pavilions of the Hospital Corporation, logistically detached. The Operative Units with point of care (POC) are therefore distant from each other and connected via the computer network that manages the flow of data. Project 2024 proposes the creation of a network of POC in order to track the instruments present, define their accessibility, efficiency and effectiveness, with the aim of obtaining accurate results in the shortest possible time. OBJECTIVES: 1)To create a network for the management of POCTs by reference laboratory staff. 2)To strengthen collaboration and communication between the staff involved in the laboratory and in the Operating Units. 3)To activate a quality pathway for process management. 4)Define rules for the acquisition and management of equipment in order to meet the needs expressed by the clinicians according to treatment, levels of intensity of care, number of beds and relocation in the Operative Units 5)Ensure the efficiency of the entire process to guarantee continuity of service and timeliness of results. METHODOLOGY: 1)Mapping of tools. 2)Mapping of contact persons. 3)Integration of the middleware for POCT management with the hospital network for flow management. 4)Implementing a Multidisciplinary Committee composed of representatives of Health Management, Pharmacy, Nursing Coordinators, and laboratory referents. 5)Train healthcare personnel on the new protocols and technologies used in the POCT, with particular emphasis on the effective use of diagnostic instruments and communication between users. 6)Mapping of trained personnel. 7)Operator traceability pathway. EXPECTED RESULTS: 1)Creation of a stable working group 2)Reduction of Turn Around Times for results 3)Increase in clinicians' satisfaction with the overall care experience 4)Traceability of data flows. CONCLUSIONS: The implementation of a POCT network in the University of Sassari Hospital Corporation represents an important step forward in optimising patient care. With the use of innovative technologies and a more appropriate organisation, the aim is to guarantee greater accessibility, efficiency and quality of analytical data, improving the overall quality of diagnostics and care.

EP047

Esperienza di gestione dei POCT (Point Of Care Testing) secondo le indicazioni SIBIOC in una struttura ospedaliera specialistica cardiologica.

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Introduzione. Il documento Sibioc del maggio 2021 (BC 2021:45,3) riporta le indicazioni essenziali per la gestione dei POCT, adattabili alle specifiche realtà ospedaliere.Obiettivo. Descrivere il progetto di gestione dei POCT della UOC Medicina di Laboratorio dell'Ospedale del Cuore di Massa, centro cardiologico specialistico.Descrizione. Il progetto inizia nel 2022; nella prima fase, Biologi e Tecnici Sanitari di Laboratorio Biomedico (TSLB) hanno registrato gli strumenti presenti, definendo le istruzioni operative, sia per il personale di reparto che di laboratorio. Inizialmente la gestione ha riguardato glucometri, emogas e ACT (Activated Clotting Time). Sono stati elaborati moduli per tipologia di strumento, per registrare la verifica giornaliera della funzionalità dal laboratorio in remoto tramite software dedicati. Biologi e TSLB organizzano la formazione degli utilizzatori in collaborazione con la ditta fornitrice, sia in fase di installazione che a tempi successivi e/o su richiesta. Gli operatori con formazione certificata utilizzano gli strumenti in modo tracciabile. Il TSLB calendarizza e esegue i controlli di qualità da fare manualmente e verifica i risultati di quelli eseguiti in automatico, tracciando gli eventi e le azioni correttive; in caso di guasto tecnico attiva l'intervento della ditta. Il biologo verifica i risultati VEQ e pianifica le verifiche di comparabilità dei risultati fra strumenti uguali, secondo linee guida. Il laboratorio gestisce l'approvvigionamento dei consumabili centralizzato, ottimizzando la distribuzione del materiale, per evitare sprechi o carenze. Mensilmente è compilato il report di attività dei POCT, riportando i dati più significativi, condividendo con i responsabili dei reparti la discussione delle non conformità e delle azioni di miglioramento. I POCT sono stati interfacciati direttamente con la cartella clinica elettronica. Ad oggi, sono gestiti anche tromboelastogramma e aggregazione piastrinica, strumenti per misura di INR, proteina C reattiva e creatinina.Conclusioni. Il progetto secondo cui il laboratorio, attraverso la collaborazione con utilizzatori, ditte fornitrici e informatici, gestisce i POCT, prosegue ad oggi, adattando le indicazioni del documento Sibioc alle esigenze di un centro specialistico.

EP048

Valutazione delle misure degli elettroliti ottenute su strumento Stat Profile Prime ES Comp Nova Biomedical: differenza fra campioni di sangue intero e plasma eparinato e confronto con Cobas 6000 Roche.

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Introduzione. Stat Profile Prime ES Comp (PrimeES) Nova Biomedical misura Na+, K+, Cl-, iCa e iMg in campioni di sangue intero (WB), plasma eparinato (PE) o siero. L'utilizzo è semplice e pratico sia nel setting di laboratorio che di reparto, con tracciabilità dell'operatore e del paziente; i risultati numerici sono completati da indicazione visiva anche per i valori critici.Obiettivo. Valutare la comparabilità delle misure degli elettroliti ottenute da sangue intero e plasma eparinato con PrimeES; confrontare i risultati ottenuti con Cobas 6000 Roche.Materiali e metodi. Sono stati analizzati 150 campioni di sangue intero prelevato in provette con litio-eparina; entro 5 minuti (m) dalla misura su PrimeES i campioni sono stati centrifugati a 2400g per 10 m ed entro 60 m dalla centrifugazione sono stati nuovamente analizzati su PrimeES e su Cobas 6000 Roche per la misura di Na+, K+, Cl-. Risultati. La media delle misure su PrimeES (WB e PE) e Cobas (PE) è stata rispettivamente (media ±st.dev, range): Na+ mmol/L 141.6±3.36 (132.1-149.5), 140.1±3.31 (130.3-148.7), 138.8±3.40 (129.0-146.0), K + mmol/L 4.12±0.41 (3.24-5.56), 4.10±0.43 (3.15-5.54), 4.05±0.40 (3.2-5.3), Cl- mmol/L 106.8±3.37 (98-117.2), 108.1±3.48 (99.9-115.9), 103.0±3.69 (93.4-111.5) iCa mg/dL 4.69±0.23 (3.8-5.72), 4.40±0.21 (3.88-5.11).Le misure in WB e PE su PrimeES correlano per Na+,K+ e Cl-. È stata valutata anche la correlazione tra le due matrici per iCa, per il quale emerge dall'analisi di Bland-Altman un bias medio (WB vs PE) di 0.29 mg/dL, seppur non statisticamente significativo. Na+ e K+ da PE su PrimeES correlano con le rispettive misure su Cobas (r2=0.817, r2=0.951). Per il Cl-, nonostante la buona correlazione (r2=0.825), dall'analisi di Bland Altman emerge un bias significativo di 5.18 mmol/L. Conclusioni. Lo strumento PrimeES si è dimostrato di utilizzo pratico ed i risultati di Na+ e K+ sono correlabili con i dosaggi effettuati in laboratorio. E' stato osservato un bias nella misura del Cl- fra Prime ES e Cobas 6000. Si ipotizza una differenza di tecniche di misura tra i due strumenti confrontati. La diminuzione della concentrazione del iCa osservata nella misura su PE rispetto a WB potrebbe essere riferibile alla dilazione temporale nell'esecuzione del dosaggio dei campioni da PE.

EP049

Comparabilità delle misure di creatinina ottenute con POCT StatSensor Creatinine Meter (NovaBioMedical) e Cobas 6000 Roche

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Introduzione. Il POCT StatSensor Creatinine Meter (NovaBioMedical) è indicato per la misura della creatinina da sangue capillare, venoso e arterioso, da sangue o plasma prelevato con anticoagulante litio-eparina e richiede una minima quantità di campione (1,2 µL). Il sistema assicura la tracciabilità dell'operatore e del paziente tramite lettore di codice a barre e software dedicato (NovaNet). E' provvisto di 3 livelli di controlli interni di qualità e di 5 livelli di soluzioni per verificare la linearità di misura. Permette il calcolo della eGFR, con scelta dell'equazione fra CKD-EPI o MDRD. Obiettivo. Valutare la comparabilità delle misure di creatinina ottenute con POCT StatSensor Creatinine Meter e Cobas 6000 Roche. Materiali e metodi. Su POCT sono stati analizzati campioni di sangue intero, prelevato in litio-eparina; entro 5 minuti (m) dalla misura i campioni sono stati centrifugati a 2400 g per 10 m e entro 60 m dalla centrifugazione la creatinina su plasma litio eparina è stata misurata con Cobas 6000 Roche. Per verificare la ripetibilità in POCT, per 10 campioni la misura è stata ripetuta 3 volte consecutive. Risultati. Sono stati analizzati 100 campioni da 100 pazienti diversi (M/F 71/29, età 71.5±12.8, media±stdev, range 32-89 anni). Su POCT la creatinina era 1.5 ± 0.62 mg/dL (media ± st.dev, range 0.31-3.08); su Cobas 6000 invece 1.41 ± 0.75 mg/dL (media ± st.dev, range 0.45-4.28). Le 3 misure ripetute consecutive su POCT per 10 campioni hanno dato un CV% medio di 6.7% (range 0.9-16.8%).La misura in POCT correla con la misura su Cobas 6000 (r2=0.717, p<0.001). L'analisi di Bland-Altman mostra una buona concordanza la misura su Cobas Roche e su POCT StatSensor (media delle differenze= - 0.09 mg/dL ± 0.40; CI: -0.87/0.69). Si nota una minore correlazione quando i valori di creatinina sono >3.5 mg/dL.Conclusioni. Lo StatSensor Creatinine Meter mostra una buona correlazione con la misura effettuata su strumento di laboratorio. La visualizzazione immediata della eGFR consente un più rapido inquadramento del paziente. In caso di creatinina >3.5 mg/dL sembra più appropriato ripetere la misura con un secondo campione ed eventualmente confermare il risultato in laboratorio.

EP050

A prick is enough! Therapeutic drug monitoring of antiseizure medication through capillary microsampling devices

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Aims: Capillary fingerprick sampling requires a lower blood volume compared to venipuncture (reference-standard), resulting in a less painful and invasive procedure. This makes it attractive for therapeutic drug monitoring (TDM). Our aim was to assess the performance of fingerprick devices, VAMS-Mitra® and qDBS-Capitainer®, for antiseizure medications (ASMs) quantification in persons with epilepsy (PWE) for self-sampling. We also evaluated the reliability and real-life feasibility of VAMS devices performed at-home and shipped via mail to the lab. Methods: We focused on the most commonly used ASMs: carbamazepine (CBZ), lacosamide (LCM), lamotrigine (LTG) and levetiracetam (LEV). Quantification was conducted using a validated UHPLC-MS/MS method. The reliability of VAMS and qDBS devices was assessed comparing the ASMs concentrations obtained through self-sampling fingerprick in ambulatory settings with those from venipuncture (a). The same evaluation was conducted for VAMS samples collected in ambulatory settings versus those collected at-home (b). Reliability between the different results (a; b) was performed through Bland-Altman analysis and Passing-Bablok regression. Furthermore, to assess the at-home feasibility, we considered the percentage of VAMS devices that successfully reach the laboratory for analysis, along with an ad hoc survey submitted to PWE to assess qualitative data. Results: 76 PWE (69,7% females, mean±SD age 41,5±14,7), were enrolled. 13,2% were on CBZ, 18,4% LCM, 43,4% LTG, and 36,8% LEV (13,2% on polytherapy). Preliminary results by Bland-Altman analysis and Passing-Bablok regression, between (a) both devices sampled in ambulatory versus venous blood samples and (b) VAMS at-home versus VAMS ambulatory, revealed good agreement. More than 88% of PWE successfully sent the device to the laboratory, while the rest encountered difficulties with the shipment process. The ad hoc survey assesses the challenge of fingerprick procedure: 95% reported it as "easy" and painless. Conclusion: Our results promises an improvement in the accessibility of TDM in PWE. Moreover, the at-home VAMS study, despite the logistical challenges, demonstrates the feasibility and reliability of measuring ASMs levels, enhancing the use of these devices for telemedicine.

EP051

A novel Point of Care Testing (POCT) for Prothrombin time and INR evaluation: a comparison study versus a central laboratory analytical platform.

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Introduction: Hemostasis testing is mandatory whenever there is a possible alteration in coagulation mechanisms. Therefore, it's needed that medical departments can count on reliable platforms such as Point of Care Testing (POCT) to investigate on altered bleeding conditions and to decide the appropriate treatments as fast as possible. We compared the performance of a new POCT versus a fully automated laboratory platform for Prothrombin Time (PT) and INR evaluation.

Materials and Methods: 40 whole blood samples were collected from patients admitted to our hospital for monitoring of oral anticoagulant therapy and for routine evaluation. PT and INR data were compared according to CLSI EP-09 guideline, using POCT Wondfo® (Guangzhou Wondfo Biotech® Co.) and ACL TOP 350® (Instrumentation Laboratory®). Each whole blood specimen was first analyzed with Wondfo® and then tested with ACL TOP 350® after centrifugation.

Results: Deming regression highlights for PT (y = - 0.55 + 1.04 x, R = 0.96, CI 95% [0.92 – 0.98]) and INR (y = -0.11 + 1.09 x, R = 0.97, CI 95% [0.95 – 0.98]) a very good correlation with no constant and no proportional errors. Pearson correlation coefficient regarding PT and INR was respectively 0.96 (CI 95% [0.92 – 0.98]) and 0.97 (CI 95% [0.95 – 0.98]). Bland Altman shows no significant bias between these two analytical platforms.

Conclusions: The opportunity to count on POCTs for faster patient evaluation must always paired with the accuracy of performance. Since POCTs are not employed and used by laboratory personnel, it is our responsibility to guarantee that the results provided by POCT are reliable, enabling physicians to be more confident in managing their patient results. In our study, Wondfo® shows good performances for PT and INR determinations, thus considering it a possible option to quickly set the correct treatments for the patient with anticoagulant therapy and even in case of a possible oral anticoagulant overdose such as those patients presented to the emergency department with constant bleeding.

EP052

A Proposal of a Point-of-Care Testing (POCT) Network for Facilitated Management of Patients with Inherited Metabolic Diseases at High Risk of Metabolic Decompensation in the Marche Region

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Patients with inherited metabolic diseases are prone to rapid transitions from stable to unstable metabolic conditions. Timely identification of metabolic decompensation is crucial to prevent adverse health outcomes. Measurement of blood glycemia, electrolytes, ketones, ammonium, lactic acids, and anion gap is essential for assessing metabolic status. Point-of-Care Testing (POCT) tools enable timely assessment of these metabolites through minimally invasive sampling. However, peripheral regional care centers usually lack specialized expertise in managing metabolic diseases and a comprehensive POCT network, leading to diagnostic delays and a high risk of metabolic decompensation episodes.

To address these challenges, we have developed an algorithm utilizing POCT-derived blood glycemia, electrolytes, ketones, ammonium, lactic acids, and anion gap values for facilitated management of patients with inherited metabolic diseases at high risk of metabolic decompensation. This algorithm provides physicians with a "high risk" or "low risk" assessment for metabolic decompensation, supporting clinical decision-making. Results are secured in an online database shared among all care centers in the Marche Region. The software allows the recording of physicians' decisions and triggers actions, including emailing analysis results and patient histories to the regional referral first aid, sending informative brochures to parents on how the potential metabolic decompensation will be managed, and alert emails to the family doctor.

The first phase of the project will focus on standardizing instrumentation across regional care centers, installing PCs with the developed algorithm, and testing the network's functionality. Subsequently, a 3-year blinded evaluation study will assess the algorithm's agreement with medical decisions. The expected results include reducing the number of metabolic decompensation episodes and the costs associated with late interception of metabolic decompensation. This initiative aims to improve the quality of life for patients with inherited metabolic diseases and their families, as well as reduce healthcare costs in the Marche Region.

EP053

A validated and standardized method to analyze human tear proteins sampled by Schirmer test strips for clinical purposes

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Aims / Purpose: The identification of biomarkers in tears help in diagnosis and management of ocular diseases, and beyond. We aimed to develop and validate a method for extracting and quantifying proteins from human tears collected by using Schirmer test strips, which are typically discarded after clinical use. Methods: Tears from 18 healthy volunteers were collected either by aspiration with a micropipette and by paper strips for Schirmer test (5 minutes at closed eyes). Aspirated tears were centrifuged and stored at -80°C until processing. Tears from strips were eluted using a developed extraction buffer (ABC 100mM, Chaps X-100 0,25%), and both samples were analyzed using the Agilent 2100 Bioanalyzer. The conversion of the wetted portion (mm) into volume tears (mL) was accurately assessed. The ratio between mm and tear volume was determined to be 1.14 mm/µl (SD ±0.05). The method's accuracy (bias%), precision (CV%), matrix effect (ME) and sensitivity (LOD, LOQ) were determined. The results were further validated using UPLC triple mass spectrometer with a bottom-up proteomics approach.Results: The elution buffer enabled high recovery values for proteins such as albumin, lysozyme, zinc-alpha2-glycoprotein (ZAG), lipocalin A, transferrin, and lactoferrin, as compared to the results from the aspirated tears. The method demonstrated high accuracy (bias%<10%), precision (CV%<10%), and sensitivity, with a matrix effect of less than 5% for the studied proteins. The recovery rates were greater than 90% for lysozyme and albumin, and greater than 80% for ZAG, lipocalin, transferrin, and lactoferrin. Validation using mass spectrometry confirmed the reliability of protein identifications and quantifications.Conclusions: The developed protocol is simple, cost-effective, and achieves significant protein recovery, making it suitable for routine analyses in the clinical setting, potentially providing a decision guide for the clinician. The method's validation through mass spectrometry highlights its robustness and applicability for identifying and quantifying tear proteins.

EP054

Inflammatory markers and protein profile from very low volume samples. The case of human tears.

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Aim. Tears recently emerged as a novel fluid source for the analysis of disease biomarkers, taking the advantage of relatively easy, rapid and non-invasive collection method. Tear composition may reflect indeed the presence of pathological states in ocular surface and systemic diseases. However, the number of cells collected is too low for being properly analyzed by flow cytometry techniques. In the present study, we provide a method to fully analyze each tear sample to both characterize the main protein levels along with an immunophenotype profile for inflammatory signature.Methods. Tears were aspirated with a micropipette and sterile tips at the lower canthus of 22 healthy volunteers, and centrifuged for pellet and surnatant separation. Surnatants were processed for protein analysis with Agilent 2100 Bioanalyzer (BioA). Pellets were fixed with paraformaldeyde 4% , then incubated with HLA-DR and CD3 antibodies, markers of inflammation and T lymphocytes respectively. Total cell counting and expression of HLA-DR/CD3 were evaluated by the Countess FL automated cell counter (Thermofisher) equipped with light cubes for fluorescence detection, and data were compared with standard flow cytometry. Results. The total cell number in each tear sample correlated with the tear surnatant volume, which ranged 5-40 mL (mean total cell number: 1.25±0.1x104 /mL correlation analysis with tear volume:R2= 0.1, p value 0.04). CD3 expression was recorded for 33.5% ± 11.8 cells and HLA-DR for the 38% ± 9 (background ranging from 4 to 8%, respectively). Data were comparable to those obtained from standard flow cytometry analysis. Levels of total protein content, Lysozyme-C, Lactoferrin, Lipocalin-A, Albumin, and zincalpha2-glycoprotein (ZAG-2) were determined with the BioA micro-chip based equipment. Conclusions. Tears are rapid, easy to collect and contains biomarkers for healthy and pathological states, which can be determined even in very small volumes. A multiple analytical approach from protein profile, to immunophenotype and potentially nucleic acid analysis unveils the clinical potential of tears. Method standardization and technical improvements are necessary to strengthen tear application.

EP055

Performance Evaluation of Point-of-Care Analyzer for Differential White Blood Cell Count in Comparison with Centralized Laboratory Instrumentation for Hematology Performed at the Romagna Health Service

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Point-of-care testing (POCT) is becoming increasingly common in many healthcare systems. The health service of Romagna provides diagnostic support through a network of traditional laboratories. Recently, in order to improve healthcare organisation, various POCT analysers have been installed in fourteen points of first medical intervention outside the hospitals. Currently, the hematology POCT provide RBC, WBC and PLT counts. The future goal would be to implement these results with the leukocyte differential count. In our study, total leukocyte and differential counts (WBC-DIFF) (granulocytes, monocytes and lymphocytes) performed using Icon 3 device (Norma Instruments Zrt, Budapest, Hungary) and the routine laboratory method (Sysmex XN-9000, Sysmex, Kobe, Japan) were compared. Specifically, 37 normal and 126 pathological venous EDTA blood samples were compared between the two methods, including subjects with granulocytosis (4-292x10^9/L), monocytosis (1.5-13.4x10^9/L), lymphocytosis (7-240x10^9/L), acute leukaemia (blasts:0.2-61x10^9/L) and erythroblastosis (0.3-38x10^9/L). Deming linear regression analysis, Pearson's correlation coefficient (r) and Bland-Altman method were used.The correlation between two methods was highly positive (0.70<r>0.95) for all WBCs, except for monocytes which showed an excellent correlation (r>0.95) only for monocytosis. In the presence of blasts, the correlation of WBC-DIFF was low (r<0.5) except for leukocyte count (r>0.95). In cases of erythroblastosis the lymphocytes showed a low correlation r<0.5 because erythroblasts were counted as lymphocytes by POCT (an “insufficient lysis alarm” is highlighted in the result obtained). There was evidence of significant bias (95% of LoA) in each category examined.The total and WBC-DIFF performed by Icon 3 on venous samples appears to be a good tool for quantitative counts of neutrophils and lymphocytes, but its utility in terms of WBC-DIFF is not optimal in the presence of blasts or erythroblasts. In our experience, it is important to emphasise that leukaemic blasts cannot be visualised or reported in any way by POCT. Therefore, the POCT blood differential count should always be further investigated if symptoms and/or laboratory data are suggestive of haematological disorders.

EP056

The global quality assessment program of POCT in Cardarelli Hospital

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Point-of-care testing (POCT) represents a significant advancement in the field of medical diagnostics, enabling rapid and accurate results at the site of patient care. However, the widespread adoption of POCT necessitates stringent quality control (QC) measures to ensure the reliability and accuracy of test results.Quality control in POCT encompasses both internal and external mechanisms. Internal quality control (IQC) involves routine checks performed by the operators to ensure that the devices are functioning correctly and producing accurate results. External quality assessment (EQA) is another crucial component of POCT quality assurance. EQA programs provide an independent evaluation of the POCT performance by comparing results from different sites or operators to a standard reference or consensus value. Participation in EQA schemes helps to identify any systematic errors or biases in the testing process, allowing for corrective actions to be implemented.Aligning POCT data with central laboratory results is vital for maintaining consistency in patient records and ensuring accurate longitudinal monitoring. This alignment requires the harmonization of methodologies and calibration standards between POCT devices and central laboratory instruments. Regular correlation studies are necessary to assess the comparability of results from POCT and central lab tests. The recently installed POCTs in the hospital (NOVA Stat Profile Prime Plus), following the issuance of Campania Region guidelines, are undergoing a comprehensive global quality assessment program that includes self-check, quality control, external quality assessment, and instrument alignment. Over the course of the next 8 months, the various systems will be gradually implemented.Two different methods were used to assess the alignment of point-of-care testing (POCT) with central laboratory results. The first method involved comparing results from identical blood samples analyzed on the blood gas analyzer and in the central laboratory. The second method utilized external quality assessment samples, testing them on both the blood gas analyzers and the central laboratory instruments. This dual approach provided a comprehensive first evaluation of POCT accuracy and alignment with central laboratory results.

EP057

ACCU-CHEK INFORM II® VERSUS COBAS PULSE®: PRELIMINARY EVALUATION ABOUT ANALYTICAL AND CLINICAL HARMONIZATION OF ROCHE GLUCOMETERS

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BACKGROUND-AIM. The assessment of Point Of Care (POC) is essential to monitor the effectiveness of laboratory services and to highlight new requirements, especially in emergency situations: however, performance, ease of use, business connectivity and operator safety must be ensured. Due to the upcoming technological update, which sees the replacement of actual Accu-Chek Inform II® glucometers with the new Cobas Pulse® ones (both Roche), our Laboratory had to show their analytical and clinical harmonization, to satisfy both UNI EN ISO 9001:2015 and Regional Council N°XI/7044 requirements. METHODS. In April 2024 we tested on both glucometers N=110 glycemias from blood count samples (K3-EDTA tubes) of inpatients who had a serum glucose test required (collected in tubes with an inert, stable gel to separate the serum), performed on Siemens Atellica™ Solution. Then, we performed: A) Passing-Bablok and Bland-Altman correlations (software MedComp 1.0) to show the analytical agreement between the two glucometers; B) the Consensus Error Grid (Parkes Grid) to assess the clinical agreement of results. RESULTS. A) Passing-Bablok and Bland-Altman correlations showed a positive agreement of results, with differences beyond the limits of 0.9% compared to the pre-established theoretical value of 5%. Bland-Altman plot showed a bias of 0.827, while Passing-Bablok plot showed no proportional or constant systematic error. B) Parkes plot showed that all glucometer results fall in “A” Area (clinically appropriate measurements), while the comparison to Atellica glycemias presents results equally distributed between “A” and “B” (clinical effect low or absent) Areas. CONCLUSIONS. Our data showed an optimum agreement between both analytical and clinical performances of glucometers, so our Laboratory is confident in this technological update because its clinical impact will be guaranteed. So, the Laboratory ensures the quality of laboratory testing to Patient cares, because each POC is under its government. However, we are aware about the importance of monitoring all the POC activities over the time, to supervisor the entire process and to minimize the the opportunity for misinterpretation of test results that could adversely impact on patient outcome.

EP058

INTERNATIONAL NORMALIZED RATIO: A COMPARISON BETWEEN A POINT-OF-CARE TEST AND A REFERENCE CENTRAL LABORATORY METHOD USING DIFFERENT BIOLOGICAL MATRICES

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BACKGROUND-AIM: international normalized ratio (INR) is the most required test for patients treated with vitamin K antagonist. INR value is the gold standard for the anticoagulant therapy modulation in order to prevent thrombosis. A continuous INR monitoring is required in order to maintain an adequate dosage of the therapy. LumiraDx INR Test (London, UK) is a Point-of-care testing device which uses capillary blood, that ensures a quick result in few seconds, playing a possible crucial role in the monitoring of the chronic patients. The aim of our study is to compare the INR results obtained with LumiraDx INR Test and a well-known central laboratory method, considering different biological matrices.METHODS: 40 capillary blood and 40 citrate plasma samples from the same patients were collected and tested respectively with LumiraDx INR Test and ACL TOP 550. The comparison of methods was performed according to CLSI EP-09A2 protocol. Constant and proportional errors were investigated with Deming Regression. The Bias between methods were evaluated with Bland Altman test as percentage and relative value.RESULTS: a good concordance between LumiraDx INR and ACL TOP INR was reported (Pearson coefficient = 0.96, CI = 95%, [0.92 – 0.97]). Despite no proportional error was encountered, Deming regression highlighted a constant error (y = -0.3042 + 1.0677. Intercept CI = 95%, [-0.54 # -0.06], Slope CI = 95%, [0.95 – 1.17]). A very low underestimation and no significant bias were revealed by the Bland Altman test. CONCLUSIONS: the opportunity to have portable, user friendly and accurate technologies with a good reproducibility compared to central laboratory instruments, ensures the possibility to have reliable results useful for a quick managing of patients in emergency settings and for the regulation of anticoagulant therapy also directly to the patient's home. Comparing LumiraDx INR to ACL TOP INR, the presence of a negative constant error must consider very carefully when evaluating sufferers with results close to the cut off in order to avoid under or over dosing of the therapy. Whenever discordant results versus patient's clinical conditions are present, a confirmation sample is always mandatory.

EP059

Comparison of Troponin I Measurements: Point-of-Care Testing vs. Central Laboratory Analyzer Using Different Biological Matrices and Anticoagulants

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High-sensitive cardiac Troponin I (hs-cTnI) is widely used for diagnosis of acute coronary syndromes. Furthermore, in January 2023, the IFCC Committee on Clinical Applications of Cardiac Bio-Markers issued a document providing an in-depth analysis of the analytical characteristics and the clinical relevance of new methods for hs-TnI measurement. A Point of Care Testing able to guarantee these performances could be very useful in reducing the turnaround time and ruling out of patients suspected of Acute Coronary Syndrome (ACS), especially by using biological matrices that are not required for centrifuge. The aim of our work is to compare the results for hs-cTnI obtained using different biological matrices and anticoagulants, comparing Atellica®VTLi hscTnI POCT and Access hs-TnI DxI 800 performances.METHODS K3EDTA and Li-heparinized whole blood samples from 43 patients with acute chest pain suspected or hospitalized for ACS were collected. Li-heparinized samples were primary employed for hs-cTnI with Atellica®VTLi as whole blood, then centrifuged and tested on Atellica®VTLi and DxI 800®. K3EDTA samples were centrifuged and measured on DXI 800® too. The comparison of methods was performed according to CLSI EP-09A2 protocol. Constant and proportional errors were investigated with Deming Regression. The bias between methods were evaluated with Bland Altman test.
RESULTS Comparing whole blood lithium heparin results obtained with Atellica versus lithium heparin plasma and K3EDTA plasma tested on DXI 800, the Deming regression revealed a proportional error, whereas in both cases Bland Altman highlighted a minimal underestimation. A similar performance was revealed when considering plasma lithium heparin tested on Atellica versus lithium heparin and K3EDTA plasma obtained with DXI 800, confirming the same underestimation. Considering values close to the cut off, no significance differences were found. CONCLUSIONS The estimation of the bias between two different analyzers is pivotal in laboratory. Moreover, this is crucial when different biological matrices and anticoagulants are employed for the analysis. Our study demonstrates that no relevant differences among the two matrices are present comparing Atellica and DXI 800 performances, thus it seems possible considering to consider the interchangeability of matrices an opportunity.

EP060

ASSESSMENT OF NEW POCT FOR HIGH-SENSITIVITY TROPONIN I MEASUREMENT: CLINICAL IMPLICATIONS

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Introduction: The introduction of immunometric assays marked a milestone in identifying specific cardiac biomarkers, notably cardiac troponin I and T (cTnI and cTnT), crucial for diagnosing cardiovascular diseases like myocardial infarction (MI). The definition itself of MI has evolved following the measurement of cardiac troponin with highly sensitive methods: detection of increased and/or released cardiac troponin values with at least one value higher than the 99th percentile upper reference limit (URL). So, the cTnI has clinical relevance in acute coronary syndromes (ACS), and a faster and easier analysis is needed.AimThis study aimed to evaluate and compare the performance of the Point of Care Test (POCT), Atellica® VTLi Immunoassay Analyzer, and Alinity c-series for measuring high-sensitivity troponin I (hs-cTnI) levels in clinical samples, assessing their diagnostic agreement and analytical precision.
Material and methods: Blood samples were collected using lithium heparin tubes from 126 patients presenting with ACS symptoms at the University Hospital of Tor Vergata (Rome). Samples were analyzed using: the POCT Atellica® VTLi Immunoassay Analyzer and Alinity c-series, comparing plasma from lithium heparin and from EDTA tubes. Analytical methods included correlation analysis and assessment of reproducibility. Statistical analysis was applied.
Results: Both instruments demonstrated optimal agreement with Passing-Bablok analysis in diagnosing myocardial injury across all samples. The Atellica® VTLi Immunoassay Analyzer showed a high correlation with Alinity i STAT ($R2 = 0.98$, $p < 0.0001$) in plasma from lithium heparin tubes, while correlations between plasma from EDTA tubes were slightly lower ($R2 = 0.917$, $p < 0.001$), but still great.
Conclusion: The Atellica® VTLi Immunoassay Analyzer exhibits robust analytical performance for hs-cTnI measurement, validated against a standard device. Its accuracy and rapid turnaround time make it suitable for various clinical settings, including emergency departments or external locations such as ambulances or medical centers, enhancing early diagnosis and treatment initiation in ACS. This study underscores the device's potential to improve patient outcomes by reducing diagnostic delays and healthcare costs associated with ACS management.

EP061

Dosaggio del magnesio ionizzato e magnesio totale in ambito interventistico cardiochirurgico: studio di comparazione e valutazione dei risultati nel postoperatorio.

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Introduzione. L'infusione intraoperatoria di magnesio (Mg) in interventi di bypass aortocoronarico a cuore battente (Off-pump coronary artery bypass grafting -OPCABG) è usata per ridurre l'incidenza di aritmie ventricolari e sopraventricolari. La misura della concentrazione di Mg ionizzato (iMg), che rappresenta la quota biologicamente attiva, può essere utile per gestire la necessità di supplementazione.Obiettivo. Valutare la correlazione e l'eventuale errore proporzionale e sistematico fra i valori di iMg, ottenuti da POCT Prime ES (Novabiomedical) su sangue intero (SI) e plasma litio-eparina (PL) e quelli di Mg totale (tMg) dosato su Cobas6000 Roche su plasma litio-eparina (PL). L'obiettivo in ambito clinico è valutare la relazione fra tMg e iMg post intervento. Materiali e metodi. In 165 campioni è stato dosato iMg su SI e PL con Prime ES e tMg su PL con Cobas6000 Roche. In 20 pazienti sottoposti a OPCABG sono stati misurati tMg e iMg nella valutazione postintervento. Risultati. Tra iMg da PL e SI e tMg da PL esiste una buona correlazione ($r2= 0.937$, iMg plasma=0.87*tMg -0.44 e $r2= 0.853$, $n=165$, iMg siero=0.87*tMg -0.43 rispettivamente); per entrambi i confronti l'analisi di Bland Altman rileva un bias significativo di -0.73 mg/dL.Tra iMg misurato su SI e PL esiste una buona correlazione ($r2= 0.867$, $n=165$, iMg sangue=1*iMg plasma-0.03) e l'analisi di Bland Altman non evidenzia la presenza di una differenza sistematica o proporzionale, che invece è rilevata nel confronto tra tMg e iMg indipendentemente dalla matrice analizzata per il dosaggio.Si riscontra una differenza statisticamente significativa tra i valori di tMg e iMg misurati nel postoperatorio (test t, p-value <0.05). Conclusioni. Il dosaggio di iMg non è confrontabile con quello tMg: sarebbe quindi opportuno considerare i due analiti in maniera indipendente tra loro. Dato che le alterazioni di concentrazione di iMg hanno un ruolo fondamentale nella genesi di disturbi del ritmo cardiaco, si propone di ampliare il numero di pazienti coinvolti nello studio, per valutare l'implementazione del dosaggio di iMg in routine e individuare un livello target terapeutico/profilattico utile a prevenire eventi aritmici anche al fine di un corretto management dell'infusione intraoperatoria di magnesio.

EP062

I poct statstrip e cobas pulse: comparazione dei due nuovi glucometri network-capable

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Introduzione: Il Point Of Care Testing (POCT) è definito come "test diagnostico effettuato vicino o in prossimità del letto del paziente; è una branca della medicina di laboratorio che si sta affermando e migliorando sempre più rapidamente. I glucometri sono strumenti ben noti e utili per il monitoraggio della glicemia e la gestione terapeutica, in grado di fornire risultati immediati, ripetibili e affidabili. Riportiamo l'esperienza della “Fondazione Policlinico Universitario A. Gemelli IRCCS” di Roma, in cui abbiamo comparto nuovi glucometri POCT, collegati al CoreLab tramite rete.Materiali e metodi:Abbiamo preso in esame due sistemi POCT Cobas Pulse di Roche (COB) e StatStrip di Nova Biomedical (STAT), comparandone i risultati con Atellica CH 930 Analyzer di Siemens, utilizzato di routine nel nostro laboratorio. Nello studio, è stata arruolata una coorte di 150 pazienti ambulatoriali e ospedalizzati di età compresa tra i 18 e gli 86 anni (80 femmine, 70 maschi) afferenti alla nostra struttura tra Gennaio e Marzo 2024.Il dosaggio del glucosio è stato effettuato su sangue intero raccolto in plasma eparina per COB e STAT e dopo centrifugazione (4000 rpm per 5 min) su plasma per Atellica. La correlazione fra i dosaggi è stata effettuata mediante la regressione lineare Passing–Bablok e il coefficiente di correlazione di Pearson. La dispersione delle differenze, invece, è stata ottenuta mediante il grafico di Bland-Altman; la concordanza è stata calcolata con la percentuale di accordo e il test Kappa di Cohen.Risultati:Si è osservato che la regressione COB ha un’accurata sovrapposizione con il metodo di routine, (y = -0,094 + 1,050x), con un bias di -0,17 (-3,1%); la regressione STAT ha una buona sovrapposizione con il metodo di routine (y = 0,191 + 1,033 x), con un bias di -0,35 (-7,0%).Conclusione: Sia COB che STAT hanno mostrato una buona ripetibilità e precisione. Nonostante COB abbia dimostrato una migliore regressione con una maggiore correlazione di Pearson e un bias inferiore rispetto alla regressione STAT, entrambi gli strumenti risultano validi e di facile utilizzo. In conclusione, l'impiego dei due strumenti può essere valutato sia nell’ambito di assistenza sanitaria primaria che ospedaliero con un buon grado di accuratezza e una riduzione dei TAT.

EP063

Approccio multidisciplinare di Health Technology Assessment (HTA) nella gestione dell'emorragia post partum ROTEM guidata.

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Introduzione: L'Emorragia Post Partum (EPP) è una delle principali cause di mortalità materna a livello globale, con un’incidenza variabile dall’1 al 5% secondo diversi criteri di definizione. Per ridurre il rischio di mortalità e gravi complicanze, è essenziale un approccio multidisciplinare che coinvolga vari professionisti, tra cui clinici, chirurghi, intensivisti, trasfusionisti, biologi, farmacisti, ostetriche, tecnici di laboratorio, infermieri e bioingegneri. L'implementazione di protocolli operativi per la prevenzione, la diagnosi precoce e il trattamento tempestivo dell’EPP è fondamentale, secondo quanto riportato in diverse evidenze scientifiche, revisioni sistematiche e meta-analisi, nelle Linee guida nazionali ed internazionali e in recenti studi condotti sulla valutazione dell’efficacia clinica e sulla costo-efficacia dei test viscoelastici.
Obiettivo: L'obiettivo principale è adottare un approccio multidisciplinare per sviluppare, implementare e validare il protocollo operativo aziendale “Gestione dell’emorragia post-partum”, basato su un algoritmo diagnostico guidato dalla Trombo-Elastometria Rotazionale (ROTEM). Questo approccio mira a migliorare gli esiti di cura seguendo un percorso metodologico di Health Technology Assessment (HTA), basato su studi clinici di alta qualità e raccomandazioni forti.
Materiali e Metodi: Il processo diagnostico dell’EPP prevede la valutazione quantitativa della perdita ematica, il monitoraggio dei parametri vitali e l'uso di esami di laboratorio standard e test viscoelastici ROTEM. Questo test, eseguito in modalità Point of Care Test (PoCT), fornisce informazioni qualitative e quantitative sui fattori della coagulazione, permettendo un uso razionale e mirato di farmaci e emocomponenti secondo il programma di Patient Blood Management (PBM).Sono stati definiti criteri di selezione per la popolazione ostetrica da sottoporre al test ROTEM, integrati con profili analitici di laboratorio per lo screening, la diagnosi e il monitoraggio delle pazienti. È stata inoltre garantita la connettività informatica per l’interfacciamento al Sistema Informatico di Laboratorio (LIS) e al Sistema Informatico Ospedaliero (HIS) e avviato un programma di formazione continua della tecnologia in esame, con audit multidisciplinari periodici.Risultati e Discussione: L'implementazione dell’algoritmo diagnostico ROTEM-guidato nel 2023 ha mostrato una lieve riduzione delle complicanze emorragiche post-partum rispetto al protocollo precedente senza l’uso della tecnologia ROTEM. Questo miglioramento si è tradotto in un decremento nell'uso di prodotti del sangue, farmaci, isterectomie di emergenza, ricoveri in terapia intensiva e rischi di complicanze post-trasfusionali, ottimizzando gli esiti

post-partum. Tuttavia, sono necessari ulteriori studi osservazionali prospettici per confermare l'efficacia clinica e la costo-efficacia dei test viscoelastici.L'adozione del protocollo aziendale di gestione dell'emorragia post-partum, con un algoritmo diagnostico ROTEM-guidato, ha migliorato la tempestività e l'appropriatezza degli interventi terapeutici. Questo si allinea alle Linee Guida e al programma PBM, ottimizzando così gli esiti di cura. La prospettiva futura è di sviluppare percorsi mirati all'appropriatezza, efficacia e sostenibilità dell'intervento clinico-terapeutico, mantenendo la centralità del paziente e la sicurezza delle cure.

EP064

Mieloma Multiplo : dal laboratorio alla clinica.

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INTRODUZIONE Presentiamo il caso di una paziente donna di anni 69 , che giunge in Ematologia, con sospetto Mieloma Multiplo , per febbre ricorrente, insufficienza renale e grave compromissione delle vertebre cervicali. MATERIALI E METODI L'esame emocromocitometrico mette in evidenza i seguenti dati: WBC 6.11 *10³/ uL , con relativa formula leucocitaria, N 68 % L 20 % M 7 % E 0 % B 0 % HFLC 5 % HGB 8.1g/dL PLT 24 .000 uL. Analizzando gli scattergram di distribuzione cellulare, sul quadrante WDF, si osserva la presenza di linfociti ad alta fluorescenza (HFLC), probabilmente patologici, rilevabili lungo l'asse delle Y. Il sistema di assi cartesiani X Y rileva e colloca le cellule in base alla complessità maturativa ed alla fluorescenza grazie a specifici fluorocromi che legandosi le cellule, ne definiscono la natura collocandole in specifici clusters di distribuzione cellulare. Allo striscio periferico si evidenzia una quota di plasmacellule pari al 5%. Successivamente l'esame citofluorimetrico rileva la presenza di plasmacellule patologiche CD 38+, CD 138+,CD 56+, Lambda 56+, CD 45+, CD 27-, CD 19-, pari al 70% della popolazione in toto.

CONCLUSIONI La leucemia plasmacellulare è una rara forma di mieloma, ossia un tumore maligno che si sviluppa a partire dalle plasmacellule. La particolarità di questa variante è che non rimane localizzata nel midollo osseo, come accade invece per il più noto mieloma multiplo; in caso di leucemia plasmacellulare, infatti, le cellule tumorali si diffondono in maniera estesa. Rispetto al mieloma multiplo, la leucemia plasmacellulare è una condizione neoplastica molto più aggressiva, caratterizzata dalla formazione di un maggior numero di masse tumorali. In genere i pazienti che ne sono affetti vengono colpiti da anemia severa ed ipercalcemia. La diagnostica ematologica è un settore complesso e fortemente dinamico della Medicina di Laboratorio, caratterizzato dalla coesistenza di una rapida e continua evoluzione tecnologica con la tradizionale ed imprescindibile analisi morfologica sullo striscio di sangue venoso periferico o midollare in microscopia ottica. Sebbene siano stati fatti enormi passi in avanti nello sviluppo tecnologico, l'esame morfologico dello striscio di sangue periferico costituisce tuttora un punto cardine della diagnostica ematologica; questo caso clinico ne è la prova. La diagnosi precoce su sangue venoso periferico di Leucemia Plasmacellulare offre al clinico la possibilità di intervenire con tempistiche e metodiche piu' efficaci ed innovative per garantire al paziente le migliori e piu' tempestive cure.

EP065

Diagnosi emometrica di laboratorio in un caso di Leucemia Mieloide Acuta

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INTRODUZIONE. Presentiamo il caso di una paziente donna (aa 87) , giunta in Pronto Soccorso per forte astenia e lieve stato febbrile. L' esame emocromocitometrico mette in evidenza un quadro ematologico suggestivo e degno di approfondimento . WBC : 13.220/µL N : 18 L : 25 M : 57 HGB : 11.1 g/L PLT : 122.000 µL MCV: 102.3 fL MATERIALI E METODI. L'osservazione e l'analisi dei dati numerici e degli scattergram di distribuzione cellulare inducono a pensare ad un possibile disordine mieloproliferativo acuto. Difatti, sullo scattergram WDF (White differentation) si osservano dei clusters di distribuzione cellulare anomali, sia nell'area dei monociti che appare molto intensa ed estesa lungo l'asse della fluorescenza, che in quella dei neutrofili (presenza cellule mieloidi immature). Viene allestito uno striscio di sangue periferico : N 54 L 22 M 6 E / B 1 MC 2 MMC 5 PMC 3 BL 7 Il quadro che si va delineando diviene sempre più concreto e pertanto si consiglia al reparto di appartenenza di richiedere una consulenza ematologica. Vengono eseguiti gli approfondimenti del caso. Esame citofluorimetrico: aspirato midollare ipercellulare (406.000 cells/ml). L'analisi citometrica evidenzia la presenza di una popolazione blastica mieloide CD 33 ++ CD 56 ++ CD 117++ CD 38 + CD 13+/- CD 11C+ / - DR+ CD15- CD 14- CD 19- CD 64- CD 34- pari al 67% della cellularità globale. Quota linfoide residua normale pari al 2%. CONCLUSIONI. Il percorso logico seguito nel porre il sospetto diagnostico da prospettare al clinico vede alla base del medesimo l'esame di laboratorio , considerato "procedura diagnostica" fondamentale alla prognosi e all' intervento sanitario. L'esame di laboratorio, l'emocromo in questo caso, assume un significato più ampio nell'ottica della Medicina di Laboratorio in quanto ha un ruolo centrale non solo nel diagnosticare, ma anche nel monitorare e gestire la malattia.

EP066

Evaluation of reference values of glucose-6-phosphate dehydrogenase (G6PD) enzyme activity in the pediatric population

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Introduction: glucose-6-phosphate dehydrogenase deficiency (G6PD), caused by X- linked genetic variants, can be associated with severe hemolytic anemia, thus leading to serious jaundice and irreversible brain damage. Early recognition and monitoring is essential to ensure the health of affected children.

Methods: our aim is to determine reference values of G6PD enzyme activity in peripheral blood in newborns. The study includes two-year data from 426 infants (274 males, 64%). The study population is divided by age groups: under 5 days, under 10 days and within one year of life. Using the AU5800 platform (Beckman Coulter, USA), G6PD enzyme activity is measured in IU/Hb, monitoring NADPH formation spectrophotometrically at 340 nm. Values indicating deficiency in the adult population for our laboratory are <2.71 IU/Hb in males and <2.95 in homozygous females, while values up to 10.22 identify heterozygosity.

Results: forty-four children have been considered deficient (median value, CI 95%; min-max: 1.36, 0.99 - 1.71; 0.33 - 2.71) based on adult reference values and excluded from the subsequent analysis. In newborns within 5 days of life (n=55), median levels are 17.10 (CI 95%: 15.74 - 19.42; min-max: 3.55 - 29.02) and 15.66 (CI 95%: 13.96 - 17.48; min-max: 5.66 - 28.78) for males and females, respectively. Among subjects under 10 days of life, males (n=124) have a median value of 17.16 (CI 95%: 16.24 - 18.37; min-max: 3.55 - 29.02) and females (n=61) have a value of 16.62 (CI 95%: 15.0 - 17.42; min-max: 5.66 - 28.78). Extending analysis to children within one year old, males (n=244) have a median of 16.27 (CI 95%: 16.34 - 17.44; min-max: 3.0 - 29.54) and females (n=149) have a median of 15.33 (CI 95%: 14.27 - 16.19; min-max: 4.87 - 29.12).

Conclusion: blood G6PD enzyme activity values in infants are higher than in adults. Preliminary data show slight differences between sexes, both at five days and one year, with lower levels in females. A threshold of 4.5 IU/Hb could be suggested as a potential attention limit for considering G6PD deficiency in children. Since the request for the assessment of G6PD activity may be of an emergent nature, it is important that laboratory provides reliable decision limits based on its case-mix reference population.

EP067

Cytokines in Chronic Lymphocytic Leukemia: The Role of BAG3 in Tumor Microenvironment and Clinical Implications

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The Bcl2-associated athanogene-3 (BAG3) protein is increasingly recognized as a critical regulator of cellular survival pathways and has emerged as a potential therapeutic target in various malignancies, including B-cell Chronic Lymphocytic Leukemia (B-CLL). This study explores the role of BAG3 within stromal fibroblasts and its consequential interactions with B-CLL cells, highlighting its influence on tumor proliferation and survival. The silencing of BAG3 expression in stromal fibroblasts was observed to diminish cell viability and perturb the survival of co-cultured B-CLL cells, implicating the cytokine network as a critical mediator of leukemic cell viability within the tumor microenvironment. A detailed analysis demonstrated a robust correlation between BAG3 expression and the levels of the chemokine CXCL12 and the anti-inflammatory cytokine IL-10, both in co-culture systems and patient-derived specimens. These cytokines are pivotal in the leukemic niche, influencing tumor cell homing, growth, and resistance to apoptosis. These findings underscore the importance of understanding the complex interplay of cytokines within the tumor microenvironment. The elucidation of BAG3's role in this context not only provides valuable insights into the pathophysiology of CLL but also underscores the potential of targeting the BAG3-cytokine axis as a potential therapeutic strategy. Clinicians and researchers alike must consider the dynamic cytokine landscape when evaluating prognostic factors and therapeutic targets in CLL, as these soluble mediators hold significant promise as biomarkers for disease progression and treatment response.

EP068

Chronic lymphocytic leukaemia and Autoimmune Haemolytic Anaemia : case report

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Background: Chronic lymphocytic leukaemia (CLL) is one of the most common types of leukaemia. The median age at diagnosis is between 67 and 72 years. CLL has an extremely heterogeneous clinical course, ranging from years of stable disease to rapidly progressive disease.A widely known phenomenon in patients with CLL is the presence of profound immune disorders, which determines an increased risk of developing secondary neoplasms, opportunistic infections, and autoimmune complications, including autoimmune haemolytic anaemia(AIHA) .We report a clinical case in which haemolytic anaemia has made it possible to recognize and diagnosticate leukaemia in a timely manner.

Case report :Patient of 74 years who arrived in the Emergency Department for asthenia, dyspnoea from exertion, edema of the lower limbs. For about 10 days feverish episodes and night sweats. History: Hypothyroidism under treatment. Polymyalgia. Type I diabetes mellitus. No history of bleeding and/or nosebleeds. On physical examination: alert, T/S oriented, cooperative, pallor, cutaneous mucus, no objective acuteness, no macroscopic bleeding.

Results: Laboratory tests: RBC 1.600000mm³, Hb 5.7g/dL mcv 107.5fl, WBC 26.300 µl, lymphocytes 63.7 %, neutrophils 34.5% , reticulocytes 43.40%, Plt 273 mm³ Total bilirubin 4.50 mg/dL, Indirect bilirubin 4.10 mg/dL, LDH 511 U/L Smear: lymphocytes with irregular nuclei, Gumbrecth's shadows. Lymphocyte typing required, and bone marrow aspirate. On bone marrow aspirate: 60% small-medium lymphocyte infiltrate .Direct Coombs Test Positive (score +++-).Immunophenotype :CD5+, CD19+, CD20-, CD23+, CD22+, CD43+, CD81+, CD200+, with sIg high chain expression restricted to kappa : Autoimmune haemolytic anaemia associated with CLL.

Conclusion: AIHA is the most common autoimmune complication in CLL, and its treatment is often correlated to disease status. In the reported clinical case, AIHA was the first manifestation that allowed a timely diagnosis of CLL, thanks to the collaboration of pathologists and clinicians.

EP069

Myeloperoxidase deficiency: early evaluation in oncoematologic patient

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Background: Myeloperoxidase (MPO) has an important antimicrobial function, the enzyme is stored in azurophilic granules of polymorphonuclear neutrophils and macrophages and released into extracellular fluid during inflammatory processes . Traditionally, MPO acts as a bactericide by catalysing the conversion of hydrogen peroxide and chloride ion to form hypochlorous acid. The measurement of myeloperoxidase can also show a relative deficiency, caused by a hereditary alteration. This situation is often associated with disseminated candidiasis.
Case report: 59 years old female patient ,a diagnosis of acute myelomonocytic leukaemia, arrived at the emergency room for the presence of severe chest tightness, fever, lucid but uncooperative. Blood chemistry and instrumental test required.
Results: The peripheral blood samples of patient were analyzed with ADVIA 2120, system that uses the myeloperoxidase (MPO) staining characteristics of cells and, once the MPO deficiency was detected. The samples were evaluated by flow cytometry (FCM) and afterward for confirmation on Beckman Coulter DX 900.FCM observed that neutrophils stained with CD13, CD33, CD11b, CD16 monoclonal antibodies but not the MPO. From the reading of the parameters provided by Coulter we note a Monocyte distribution width (MDW) of 30, an early indicator of sepsis, confirmed by the positive blood culture for Candida parapsilosis.
Conclusion: Candids are yeasts that normally colonize healthy individuals. In most cases the infections are asymptomatic or paucisymptomatic, early recognition of opportunistic infections is important, especially in fragile, immunocompromised patient in whom candida could cause sepsis. The identification of candida infection with traditional microbiological tests requires a few days and can be complex in immunosuppressed patients. Therefore, the exact interpretation of MPO deficiency and early identification of sepsis through MDW contributed to a correct clinical -diagnostic work-up of patient. The comparison between instruments with different technology offers a valuable aid to manual counting with peripheral smear.

EP070

L'immunosottrazione come alternativa al trattamento con agenti riducenti nelle gammopatie monoclonali di tipo IgM: analisi di un gruppo selezionato di pazienti.

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Background: Le gammopatie monoclonali sono un gruppo eterogeneo di patologie ematologiche e la gammopatia monoclonale di significato indeterminato (MGUS) è la condizione più comune di premalignità. Le MGUS con isotipo IgG e IgA possono precedere l'insorgenza di Mieloma Multiplo (MM), mentre le MGUS IgM evolvono in Macroglobulimenia di Waldenström (MW) e, in rari casi, in Mieloma Multiplo. La MW è caratterizzata dalla presenza nel siero di una proteina monoclonale (proteina-M) con isotipo IgM legante catene leggere kappa o lambda, che può inficiare l'interpretazione della immunofissazione sierica (s-IFE). Una corretta identificazione della proteina-M è importante sia per la diagnosi che per il trattamento. Metodi: Il test per identificare e quantificare la proteina-M è l'elettroforesi delle siero proteine (SPEP), mentre la caratterizzazione immunologica viene effettuata o con immunofissazione su gel di agarosio (s-IFE), ritenuto il gold standard, o con metodica di immunosottrazione in elettroforesi capillare (ISE). La IgM, per le sue caratteristiche chimico-fisiche, può polimerizzare e generare artefatti sul gel di agarosio, rendendo necessario l'utilizzo di agenti riducenti come il Ditiotreitolo (DTT). In questo studio è stato analizzato un gruppo selezionato di 4 pazienti (A,B, C e D) che presentavano bande monoclonali di tipo IgM e IgG con la stessa motilità elettroforetica sul gel di agarosio. I campioni sono stati sottoposti a SPEP, s-IFE, ISE e ulteriore s-IFE previo trattamento con DTT. Risultati: L'ISE e s-IFE dopo il trattamento con DTT del paziente A ha evidenziato una IgMk monoclonale. Le ISE e le s-IFE dopo il trattamento con DTT dei pazienti B e C hanno mostrato una gammopatia biclonale: IgMk e IgGλ, IgMλ e IgGk rispettivamente. L'ISE del paziente D ha evidenziato la sottrazione di 4 proteine monoclonali: IgMk, IgMλ e 2 IgGk confermate anche dalla s-IFE dopo il trattamento con agente riducente. Conclusioni: Il laboratorio ha un ruolo centrale nella diagnosi, prognosi e terapia di queste patologie e il vantaggio di una metodica semplice e automatica come l'ISE potrebbe consentire ai laboratori ad elevata produttività di evitare l'esecuzione di s-IFE con DTT che non solo porterebbero ad un allungamento del T.A.T, ma anche ad un aumento dei costi.

EP071

Hb Köln: identificazione e caratterizzazione di una variante instabile dell'emoglobina a partire dalla misura dell'emoglobina glicata

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INTRODUZIONE: La variante emoglobinica di Köln è una emoglobinopatia caratterizzata da anemia, reticolocitosi e splenomegalia. Tale variante, a causa della sostituzione del residuo amminoacidico della valina in posizione 98 con la metionina nella catena beta-globinica, risulta instabile, presenta una ridotta sopravvivenza eritrocitaria e un aumento dell'affinità per l'ossigeno con minore cessione di quest'ultimo ai tessuti. Nei pazienti portatori di tale variante si osservano crisi emolitiche ricorrenti.

CASO CLINICO: Presentiamo il caso clinico di un uomo di 70 anni che, a causa di un'insufficienza respiratoria, si reca in ospedale. Inizialmente si riscontra un valore di emoglobina basso che richiede la prima di una serie di trasfusioni: il paziente infatti è soggetto a frequenti crisi emolitiche. In seguito viene sottoposto ad alcune analisi che evidenziano un quadro glicemico alterato. Giunge poi al nostro laboratorio dove, nell'ambito di un controllo generico, viene rilevata la presenza di una variante emoglobinica.

RISULTATI: Con l'analisi dell'emoglobina glicosilata (Hb A1c), eseguita su campione di sangue in provetta con EDTA mediante elettroforesi capillare tramite il Minicap Flex-Piercing (Sebia, Francia), il valore risulta non determinabile per la presenza di un profilo anomalo verosimilmente riferibile a una variante. Nello studio dell'assetto patologico emoglobinico, eseguito ancora mediante elettroforesi capillare, vengono evidenziate le seguenti frazioni: HbA 93,4%, HbA2 3,0% e picchi minori in zona HbD 0,9% e in zona HbE 2,7%. Quindi viene effettuato lo studio molecolare dei geni globinici attraverso il sequenziamento NGS mediante libreria costituita da ampliconi dei geni delle emoglobine HBA1, HBA2 e HBB. L'analisi molecolare ha evidenziato la variante HBB:c.295G>A p.(Val98Met); Hb Köln allo stato eterozigote.

CONCLUSIONI: La misura dell'Hb A1c in presenza di varianti instabili di solito mostra valori bassi per la ridotta sopravvivenza eritrocitaria di tali varianti, ciò può rappresentare un indicatore utile nella gestione di questi pazienti. In generale, vogliamo osservare che l'individuazione di un difetto emoglobinico a partire dal monitoraggio di HbA1c può facilitare la prevenzione delle emoglobinopatie e la formulazione di diagnosi corrette.

EP072

A rare case of JAK2 V617F-positive primary myelofibrosis with de-nucleated cells in the peripheral blood.

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The loss of the nucleus is the final step of erythrocyte maturation, which occurs in the hematopoietic bone marrow at the stage of orthochromatic erythroblast. Only occasionally - in cases of regenerative anemia, hemoglobinopathies, myeloid neoplasms, myelodysplastic syndromes, and others - suggestive images of enucleation (nucleus "half in-half outside") of acidophilic erythroblasts can also be observed in the peripheral blood (PB). We describe a light microscopy observation of PB in which a massive amount of empty cytoplasm and naked nuclei were present because of the incongruous enucleation of basophilic and polychromatophilic erythroblasts and other cell types. The non-erythroid cells involved were megakaryocytes, megakaryoblasts, immature granulocytes, and other unidentifiable. We are referring to an 83-year-old male with JAK2 V617F-positive primary myelofibrosis. CBC-DIFF and PB review showed dysplastic neutrophilia (23.0x10^9/L) with immature granulocytes (2.4x10^9/L), RBCs anisopoichilocytosis, blast cells, micro megakaryocytes, and megakaryoblasts. Bone marrow aspiration gave "punctio sicca." Besides the numerous naked nuclei in the PB, the results of this pathological de-nucleation are in large cytoplasm, often with homogeneous basophilia or with vacuoles and granulations that make us speculate or guess the cell of origin. In some cases, this empty cytoplasm retains the negative image of the leaked nucleus or maintains some coarse chromatin residues and Cabot-like rings. More rarely, the de-nucleated cytoplasm of originally phagocytic cells has maintained a large vacuole containing cellular residues of the engulfed cell. Numerous cytoplasmic fragments (clasmatosis) of varying sizes, generally large, completed this unusual pathological picture. The presence in the PB of megakaryocyte nuclei is a frequent observation described in the PB of myeloproliferative neoplasms and, therefore, could be considered congruent with the diagnosis of primary myelofibrosis. On the contrary, there are no matches in the literature for the incongruous nuclear expelling from the basophilic and polychromatophilic erythroblasts or the enucleation of various leukocyte elements.

EP073

Donatori abituali: popolazione sana fino a prova contraria, utilità della elettroforesi proteica nel profilo standard di donazione.

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• Background: la ASL N.1 Avezzano-Sulmona-L'Aquila presso la UOC Medicina di Laboratorio - PO San Salvatore, effettua analisi per una popolazione di circa 100.000 abitanti afferenti al "comprensorio L'Aquila". L'elettroforesi proteica è una tecnica usata nei laboratori clinici per la ricerca delle anomalie del profilo proteico, in particolare per la rilevazione di componenti monoclonali. Il settore di proteinologia e tecniche separative della UOC Medicina di Laboratorio esegue circa 40.000 elettroforesi proteiche l'anno.

• Metodi: Le elettroforesi eseguite con strumento SEBIA Capillarys 2 FLEX-PIERCING che utilizza il principio dell'elettroforesi capillare in fase libera separando le proteine secondo la loro mobilità elettroforetica in un tampone alcalino a pH specifico Lo studio è stato condotto analizzando le elettroforesi dei donatori di sangue afferenti alla UOC S.I.T. aziendale nel periodo gennaio 2014 - giugno 2024. In alcuni donatori con evidenza di componente monoclonale è stata effettuata la tipizzazione tramite immunofissazione con strumento SEBIA HYDRASYS 2 o immuntyping SEBIA Capyllaris.

• Risultati: Nel periodo preso in considerazione sono state eseguite una media annuale di 1850 elettroforesi nei donatori abituale. Tra queste sono risultate positive per la presenza di componente monoclonale circa lo 0,54% tra i quali il 79% uomini ed il 21% donne, con una età media di 52 anni.

• Conclusioni: i donatori di sangue, considerati appartenenti alla popolazione sana, al pari della popolazione generale, possono presentare alterazioni nel profilo elettroforetico. L'età evidenziata dallo studio, in accordo con le linee guida SIBioC, ci consente di confermare l'elettroforesi come metodica di screening nella popolazione generale di età >50 anni e pertanto si suggerisce di inserire il profilo elettroforetico nella valutazione ematochimica del donatore abituale.

EP074

Is automated digital microscopy capable of discriminating non-random morphological changes? The case of rod cell crystalline inclusions in lymphocytes of chronic lymphocytic leukemia.

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Automated digital cell imaging is becoming increasingly common in laboratories and tends to replace manual light microscopy. These analyzers show different capabilities in peripheral blood cell pre-selection: good for leukocyte five-part differential or moderate/poor for abnormal cells. Limitations in pre-classifying these latter cells (e.g., blast cells, atypical lymphocytes, plasma cells, and others) suggest increased attention and integrated evaluation with other available data (microscopic review, instrumental CBC, and flags). We describe a Chronic Lymphocytic Leukemia (CLL) case showing rod crystalline inclusions in 15.2 percent of lymphocytes whose presence has been "ignored" by the Sysmex analyzers DI-60. We are referring to a 93-year-old female with CLL, diagnosed several years before. CBC-DIFF showed moderate anemia (Hemoglobin 117g/L) and WBC 32.3x10^9/L with lymphocytes 24.8x10^9/L. Crystalline inclusions (one or two per cell) are shaped like hyaline rectangles arranged at the inner cell edge or partially overlapping the nucleus. Other granular inclusions (not with crystal appearance) were also present in about one percent of lymphocytes. Previous reports stated that cytoplasmic inclusions are composed of IgMλ—often isotypic with the surface immunoglobulins—in most described cases (Rodriguez et al., 2017). The inclusions have not yet been typified in our case, but the clinical and morphological data seem to agree with that hypothesis. The presence of the rectangular bodies does not seem to have affected the DI-60 digital analyzer's "interpretive capabilities," which pre-classified cells as "lymphocytes" only and did not place any of them in other boxes (for plasma cells, activated lymphocytes, unidentified, so on). Crystalline inclusions can be observed in about 5-10% of CLL cases (Kalfkiaer et al., 1982) but have also been described in multiple myeloma and lymphocytic lymphomas. Failure to pre-classify these cells in a box different from "lymphocytes" (e.g., atypical lymphocytes or unidentified) could not be relevant in known cases of CLL. Conversely, losing this sign (non-pathognomonic but narrowly correlated with CLL) in undiagnosed cases with a low-expressed anomaly and/or hasty or uncareful re-classification could cause a diagnostic delay.

EP075

MONOCYTE AND NEUTROPHIL ALTERATIONS IN AN IN VITRO SEPSIS MODEL: INSIGHTS FROM DIGITAL MORPHOLOGY ANALYSIS

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Sepsis is a medical emergency caused by a dysregulated host response to infections. Monocyte and neutrophils are prominent effectors of the innate immune responses and undergo early and significant morphological changes after their activation. Escherichia Coli and Staphylococcus Aureus are the most commonly gram-negative and gram-positive bacteria found in sepsis, with Lipopolysaccharide (LPS) and Lipoteichoic acid (LTA) as their major bacterial endotoxins, respectively. Histones are intranuclear proteins acting as Damage-Associated-Molecular-Pattern proteins (DAMPs) when extracellularly extruded at high levels in sepsis conditions. This study aimed to analyze the morphological changes of monocytes and neutrophils after in vitro stimulation with sepsis triggers. EDTA-K2 anticoagulated whole blood samples (n=24) from healthy donors were incubated with live E. coli and S. aureus (10^6 and 10^8CFU/ml), LPS (1 µg/ml), LTA (200 µg/ml) and a mixture of histones (200 µg/ml) for 3 h at 37 °C. Manually prepared smears were May-Grunwald-Giemsa-stained and processed through the automated slide preparation system (Sysmex SP-50) for digital cell morphology analyses with the CellaVision DM software (DI-60).Blood treatment with live bacteria was associated with increased cell size, activation of phagocytic mechanisms, vacuolization, nuclear and membrane structural alterations, including chromatin decondensation in monocytes. Neutrophils were also activated, as highlighted by the presence of intracellular bacteria, vacuoles, nuclear abnormalities. Similar features of monocyte and neutrophil activation in LPS, LTA, and histone stimulated blood samples have been observed, excepted for the presence of phagosomes.Our findings demonstrated that sepsis triggers, including live bacteria, associated endotoxins, and histones, were able to early promote monocyte and neutrophil activation in vitro, leading to morphological changes which can be identified and monitored by digital morphology, providing additional tools for the early recognition of sepsis patients.

EP076

MONOCYTE DISTRIBUTION WIDTH IN SEPSIS: DIFFERENCES AMONG LIVE BACTERIA, PATHOGEN- AND DAMAGE-ASSOCIATED MOLECULAR PATTERN PROTEINS

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Monocyte Distribution Width (MDW) is an FDA-approved early sepsis biomarker that quantifies monocyte heterogeneity and mirrors their morpho-functional changes. Sepsis is a critical medical emergency characterized by a dysregulated host response to infection. Escherichia Coli and Staphylococcus Aureus are the most commonly gram-negative and gram-positive bacteria found in sepsis, respectively. Lipopolysaccharide (LPS) and Lipoteichoic acid (LTA) are the major bacterial endotoxins and represent the most studied Pathogen-Associated Molecular Pattern proteins (PAMPs). Histones are cationic intranuclear proteins acting as Damage-Associated-Molecular-Pattern proteins (DAMPs) when released extracellularly under pathological conditions, including sepsis, where they have been found at high levels. This study aimed to investigate the ability of live bacteria, PAMPs and DAMPs to induce MDW alterations in an ex vivo whole blood model od sepsis. EDTA-K2 anticoagulated whole blood samples (n=24) were treated with live E. coli and S. aureus (10^6 and 10^8CFU/ml), LPS (1µg/ml), LTA (200µg/ml) and a mixture of histones (200µg/ml). MDW was evaluated at 0, 30, 60, and 180 min through DxH690T Hematology Analyzer (Beckman Coulter). MDW values were early, significantly and dose- and time-dependently increased by live bacteria. At 180 min of treatment with E. coli 10^6 and 10^8 CFU/ml, MDW increased to 21.7 (p=0.0059) and 26.1 (p=0.0008), while with S. aureus to 18.1 and 20.9 (p<0.0001), compared to the respective controls. LPS and LTA effect confirmed this species-specific difference, inducing significantly different MDW changes overlapping those obtained with E. coli and S. aureus 10^8 CFU/ml, reaching 28.4 and 21.9, at 180 min respectively. Histone treatment induced MDW variations (22.2 at 180 min) similar to those obtained with LTA, at each time point, and significantly lower than LPS (p=0.05-0.001). Our findings confirmed the clinical usefulness of MDW in early sepsis diagnosis, suggesting time- and dose-dependent MDW increase and possible species-specific MDW variations, as proved by both live bacteria and associated PAMPs. Sepsis-associated DAMPs promoted significant MDW changes highlighting that histones are critically involved in morpho-functional alterations of monocytes.

EP077

Un rapido ed innovativo biomarker di sepsi per la medicina d'urgenza: MDW (Monocyte Distribution Width)

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INTRODUZIONE

La sepsi è una rara complicazione di un'infezione, le cui conseguenze possono essere molto gravi e potenzialmente mortali.

Lo scopo dello studio è la correlazione tra l'insorgenza della sepsi con l'incremento del parametro MDW (Monocyte Distribution Width) dei pazienti adulti afferenti al pronto soccorso dell'Ospedale Evangelico Betania.

MATERIALI E METODI

Lo studio è stato condotto nell'unità di Patologia clinica dell'Ospedale Evangelico Betania.

A tale scopo sono stati analizzati 120 campioni clinici provenienti da pazienti afferenti al Pronto Soccorso del suddetto ospedale,

tutti con sospetto clinico di sepsi e selezionati secondo i criteri q-SOFA score (punteggio >=2).

A tali campioni è stato effettuato il dosaggio sierico di CRP (proteina c reattiva), e PCT (procalcitonina) (COBAS PRO-Roche), e l'MDW con un contaglobuli automatico (DxH900 Beckman Coulter).

Per l'MDW è stato scelto un cut-off di >= 25.

RISULTATI

Dei 120 pazienti esaminati, 43 di questi (35,8%) hanno confermato diagnosi di sepsi attraverso esami clinici

con l'incremento dell'MDW con un cut-off >= 25, aumento pcr e procalcitonina ed esame colturale positivo,

mentre i restanti 77 avevano altre patologie (64,16%). L'incremento della PCT e della PCR è associato ad un aumento, in parallelo dell'MDW.

CONCLUSIONI

La diagnosi precoce è fondamentale per ridurre la mortalità della sepsi.

Sebbene l'emocoltura resti il "gold standard" poiché suggerisce una diagnosi eziologica e soprattutto una terapia antibiotica adeguata.

I biomarcatori sono uno strumento rapido ed accurato, e soprattutto l'MDW, sulla base dei nostri dati preliminari, può migliorare il processo decisionale nella gestione precoce della sepsi.

EP078

Identification of rare haemoglobin variants by capillary electrophoresis system: two years experience in a large Italian laboratory

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Clinical Laboratory of AUSL Romagna provides diagnostic care to a 6500 km2 territory with 1,200,000 inhabitants. Quantification of HbA1c, HbA2, HbF, and Hb variants is centralized in the hub laboratory in Pievesestina, which is accessed by a population with heterogeneous ethnic origins. In recent years, this activity has led to the identification of a large number of rare hemoglobin variants. Quantification of all haemoglobin fractions was performed using capillary electrophoresis technology (Sebia Capillarys-3 Tera): HbA1c was measured with the CAPI 3 HbA1c kit; HbA2/HbF/Hb variants were measured with the CAPI 3 Hemoglobin kit. The study covered a two-year period (May 2022 - May 2024) during which Sebia provided a molecular identification service for the suspected variants identified. In addition, a short, detailed observational study was conducted on the ethnicity of patients referred for haemoglobinopathy testing. About 400,000 HbA1c tests and 20,000 HbA2/HbF/Hb variant tests were performed in the two years considered. Data analysis relative to subjects undergoing the HbA2/HbF/Hb variant assay showed that about 65% of the patients were of Italian origin, while 35% were of non-Italian origin, of which 24.2% were of non-European origin and 7.7% were of European origin. During the study period, 24 cases of rare Hb variants (10 alpha, 14 beta) were detected and molecularly characterized. The detection and numerosity of rare variants may be more frequent today because of increased migration (12 cases are not of Italian origin) or because of method's sensitivity (7 cases are detectable only by the HbA1c method); in addition, the availability of a molecular study increases staff expertise. Despite the rarity of these Hb defects, it is important to report their presence because they may have clinical consequences if associated with other hemoglobinopathies or present altered pathophysiologic states to be monitored. Limited information on many of these defects has not prevented the ability to document as many as 11 cases with phenotypes significantly different from normal haemoglobin, such as altered protein stability (e.g Hb Lansing, Hb Takoma); altered affinity for oxygen (e.g. Hb Desirade, Hb Roanne) or increased resistance to oxidative stress (e.g. Hb Providence).

EP079

SARS-CoV-2 spike protein impairs red blood cells parameters measured with Sysmex XN and DI-60

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Background: Evidence has emerged that severe acute respiratory syndrome coronavirus disease 2 (SARS-CoV-2) may influence biology and morphology of blood cells, including erythrocytes. We have hence planned these experiments to explore the possible role played by the spike protein of different SARS-CoV-2 variants in influencing erythrocyte biology.Methods: Whole blood samples were collected from seven ostensibly healthy volunteers (mean age 50±7 years; 5 females), into blood tubes anticoagulated with buffered sodium citrate to prevent EDTA-induced erythrocyte and platelet aggregation. Serial samples were spiked with saline (control sample) or SARS-CoV-2 recombinant trimeric spike protein of Ancestral, Alpha, Delta and Omicron variants, at final concentrations of 2 and 20 ng/mL for each spike protein variant. After 5 min incubation with recombinant spike proteins or saline, a 0.8 mL aliquot was taken and used for measuring red blood cell (RBC) parameters on a Sysmex XN hematological analyzer, connected to a DI-60 digital morphology analyzer. Test results were expressed as mean ± standard deviation (SD) of raw values or as percent variation from the corresponding values obtained in the paired control specimen (saline-spiked) of each participant. Results: The values of RBC count, hemoglobin concentration and mean corpuscular hemoglobin (MHC) did not vary across all samples challenged with both concentrations of the four different SARS-CoV-2 recombinant spike proteins. Significant increase of mean corpuscular volume (MCV) was noted in samples treated with SARS-CoV-2 Alpha and Delta recombinant spike proteins, at both 2 and 20 ng/mL final concentrations. RDW values significantly increased in samples challenged with 20 ng/mL of all SARS-CoV-2 recombinant spike proteins, reaching the highest values in those treated with Omicron recombinant spike protein. Blood smear revision evidenced hemagglutination and rouleaux in all samples where SARS-CoV-2 recombinant spike proteins were added, especially in those with Alpha and Delta variants.Conclusions: RBC morphology is impaired when erythrocytes are challenged with SARS-CoV-2 spike proteins, especially those of Alpha and Delta variants.

EP080

Big data approach for MPV reference limits estimation

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Background

The hematological analyzers calculate the mean platelet volume (MPV) according to volume distribution of the platelets during blood cell counting. Markedly enhanced or abnormal thrombocytopoiesis, or the effect of activating factors on blood platelets, may lead to changes in the relationship between MPV and platelet count. Moreover, MPV correlates with platelet activity, and is thus considered a marker of hyperactivation. MPV values have large interindividual variability in healthy subjects due to multiple factors, so that reference limits play a crucial role for the accurate interpretation of test results. Although direct calculating methods are considered the gold standard for quantifying MPV, we aim to calculate the reference limits with an indirect approach, which is especially useful when routine data from a large database are available (e.g., from the LIS) and direct sampling of non-diseased individuals is unfeasible.

Methods

We performed an extraction of cell blood count data from outpatients referred to our center (Borgo Roma, AOUI Verona, Italy) from the LIS between January 1 and June 30 2023. We used the following inclusion criteria: hemoglobin 120-180 g/L; platelets 150-400 x10^9/L; white blood cells <=10 x10^9/L. Data analysis was performed using R 4.4.0 with RStudio 2024.04.1, and the package “reflimR” 1.0.6, which applies an indirect method for deriving reference limits from mixed populations containing an unknown proportion of diseased individuals (typically <25%).

Results

An original dataset of 12263 cell blood counts was retrieved from the local LIS, reduced to 9291 after applying the inclusion criteria. The mean MPV was 10.34 fL (SD 0.86). The reflimR package calculated lower and upper reference limits of 8.6 fL (95% 8.55-8.67) and 12.0 fL (95% 11.92-12.05) using 8646 data from the original dataset.

Conclusion

The reflimR package is extremely fast, mainly because it does not use complex procedures to optimize a theoretical distribution model. The method used in this study provides a simple and robust three-step approach for estimating reference intervals from routine laboratories based on the use of a large amount of laboratory data without the need to recruit patients.

EP081

DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): USEFULNESS OF CELL POPULATION DATA OF LYMPHOCYTES

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Background:Chronic lymphocytic leukemia (CLL) is a disorder characterized by monoclonal B cell proliferation. Diagnosis involves complete blood count (CBC) showing increased lymphocytes, predominantly mature small lymphocytes in peripheral blood smears and additionally flow cytometry to confirm the disease. In CBC, Cell Population Data (CPD) obtained by Hematological Analyzer reflect the morphological and functional characteristics of lymphocytes.Aims:In this study we evaluated the applications of Cell Population Data of lymphocytes on the Hematology Analyzer in the diagnosis of CLL. Methods:CBC analysis were performed by DxH900 Hematology Analyzer (Beckman Coulter) through VCS technology that measures Volume (V), Conductivity (C) and Laser Scatter (S). The system also provides CPD expressing Mean (M) and Deviation Standard (SD) of neutrophils, lymphocytes (LY), monocytes and eosinophils. Our study included 30 patients with absolute lymphocyte count >5.0 x109/L and proven CLL admitted to the Clinical Pathology of SS Trinità Hospital to perform a CBC in the last month. We analysed about lymphocytes the absolute count, all CPD parameters (LY-M V; LY-SD V; LY-M C; LY-SD C), morphological alarm of suspect CLL (Variant Lymphs), 5PD1 plot (Scatter vs.Volume) and 5PD2 plot (Opacity vs. Volume).Results:We compared all parameters analysed to the normal values: LY-M V 83; LY-SD V 13.7; LY-M C 118; LY-SD C 8.8. The mean values obtained from 30 cases of CLL were as follows: LY-M V 86.1; LY-SD V 18.8, LY-M C 114, LY-SD C 11.3. In 90% cases we also found the morphological alarm “Variant Lymphs”. In all patients 5PD1 and 5PD2 plots showed abnormal patterns with wide variation in LY and second population of low volume LY.Summary/Conclusion:Our study showed values increased of Lymphocyte Volume SD in all cases of CLL and increased of Lymphocyte Conductivity SD in 70% cases compared to normal specimen. Therefore we believe that CPD could be a valid support for diagnosis of CLL promptly available with every CBC performed with no additional costs. To confirm our preliminary observations, further studies are necessary.

EP082

Myelodysplastic syndromes: our experience

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[1]

Background: Myelodysplastic syndromes (MDS) are a very heterogeneous group of myeloid diseases characterized by peripheral blood cytopenia, bone marrow hyperplasia, dysplasia with an increased risk of transformation into acute myeloid leukaemia (AML). The apparent dissociation between peripheral cytopenia and bone marrow hyperplasia is due to a bone marrow maturation defect, which leads to an accumulation of immature precursors with consequent failure to release mature elements into the circulation. MDS occur more frequently in older males (median age of onset 75yy.) and in individuals with prior exposure to cytotoxic therapy.

Materials and methods: Out of 5000 patients examined between January and December 2023, 70 MDS were identified and diagnosed based on the parameters of the blood count and blood chemistry. Patients had symptoms related to anaemia.Myelodysplastic syndrome has been suspected in patients aged 65 to 85 years who showed anaemia, leukopenia, or thrombocytopenia. The diagnosis was suggested by the finding of morphological abnormalities found in the peripheral blood smear and by the study of the bone marrow and confirmed by demonstrating specific cytogenetic abnormalities.

Results: On Blood count, the haematological picture shows mono-bi- or tri-linear cytopenia: normocytic or mildly macrocytic anaemia (haemoglobin range 7-10 mg/dL) with normal reticulocytes · neutropenia (< 1800/μL) · thrombocytopenia (< 100,000/μL). Examination of the peripheral blood smear revealed morphological abnormalities of neutrophil granulocytes, red blood cells, and/or platelets. Myeloaspirate showed bone marrow hypercellularity, with morphological abnormalities affecting one or more maturation lines: dyserythropoiesis, dysgranulopoiesis, dysmegakaryopoiesis.

Conclusions: MDS can be considered an emerging disease with an increasing burden on the healthcare system due to the aging of the general population with multiple pathologies, improvements in diagnostics and treatment choices. Symptomatic patients were transfused, and secondary iron overload (serum ferritin > 1,000 ng/mL) was found in many cases. The infections, resulting from neutropenia, in our study were mostly bacterial, relapsing, and slowly resolving.

EP083

Red Blood Cell Parameters in the Total Blood Count of Patients with Chronic Kidney Disease

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The complete blood count is a laboratory exam useful to acquire information about patients and for monitoring pre-existing diseases.

In our study we evaluated all parameters for RBC and PLT in chronic kidney disease (CKD) patients (157: 56 females and 110 males) comparing them with a control group composed of blood donors (985: 240 females and 745 males). We calculated mean and standard deviation for the first blood sample of every patient and we divided all the people in 4 groups: nephropathic female patients, female blood donors, nephropathic male patients and male blood donors. Then we compared RBC and PTL parameters in nephropathic female patients vs female donors and in nephropathic male patients vs male blood donors.

CKD patients suffer from anemia as a result of erythropoietin, vitamin B12, folate and iron deficiency. Patients with anemia of CKD are treated with erythropoietin. In particular, erythropoietin administration induces the hyperstimulation of bone marrow leading to an early release of reticulocytes.

As expected, there is a significant statistical difference between nephropathic patients and healthy blood donors for all the parameters evaluated, except for MicroR, that is a parameter that evaluates the presence of microcytosis, a condition where RBC have a smaller diameter than the normal RBC.We obtained lower values for RBC, HBG, HCT, MCH, MCHC and higher values for MCV, RDV, MacroR in nephropathic patients. Instead, MacroR is a parameter that evaluates the presence of macrocytosis, a condition where RBC have a bigger diameter than the normal RBC.Moreover, we noticed lower values of PLT, PCT, PDW in nephropathic patients most likely due to the fact that CKD causes platelet dysfunction.

In conclusion, with the cytometric blood count we can determine the type of anemia and monitor the health condition of nephropathic patients.

EP084

THE ROLE OF NEW RESEARCH PARAMETERS OF THE BC 6800 PLUS ANALYZER IN THE DIFFERENTIAL DIAGNOSIS OF LYMPHOID NEOPLASMS

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BACKGROUND-AIM. A precise and standardized classification system in hematology is essential. The latest edition of the WHO classification emphasizes the importance of the complete blood count (CBC) and the assessment of cell morphology in peripheral blood and bone marrow smears for diagnosing lymphoid neoplasms. This study aimed to evaluate the diagnostic role of Research Use Only (RUO) parameters from the BC-6800 Plus Mindray analyzer in differentiating B-cell chronic lymphocytic leukaemia (B-CLL), acute lymphoblastic leukaemia (ALL), and lymphoma, to develop new diagnostic algorithms to improve the sensitivity and specificity of CBCs in diagnosing lymphoid neoplasms.
METHODS. In this retrospective study, a complete blood count (CBC) was performed in 90 patients (M: F 66:34%, median age 67 years) admitted at the emergency department of Novara's Hospital with a pathological blood count (ALL, n=14; B-CLL, n=47; lymphoma, n=29).The association of basic (WBC, Hb, RDW, NE#, LY#, MO#, PLT) and research cell parameters (NLR or NE/LY ratio, NMR or NE/MO ratio, LMR or LY/MO ratio, NeuX, NeuY, NeuZ, LymX, LymY, LymZ, MonX, MonY, MonZ) was evaluated by univariable and multivariable logistic regression.
RESULTS. In multivariable analysis, Hb (p=0.02), NeuY (p=0.04), MonY (p=0.01), were found to be independent predictors of B-CLL compared to ALL. This multivariable model correctly classified 93.4% of cases with an AUC of 0.91 (95%CI 0.81-1.0). Independent predictors of B-CLL comparing to lymphoma patients were MO# (p=0.003), LymY (p<0.0001) and MonY (p=0.004). This multivariable model correctly classified 77.6% of cases with an AUC of 0.86 (95%CI 0.78-0.95). For the comparison between ALL and lymphoma patients, NeuZ (p=0.01) and NeuY (p=0.04), were identified as independent predictors. This model correctly classified 93% of cases with an AUC of 0.98 (95%CI 0.95-1.0).
CONCLUSIONS. The utilization of morphological research parameters may provide valuable help, without additional costs, in the early diagnosis of ALL, B-CLL and lymphoma.

EP085

Identification of acquired haemophilia A: 6 years experience of the "Area Vasta Romagna" laboratory

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Acquired haemophilia A (AHA) is a rare disease with an incidence of approximately 1-4 cases per million inhabitants/year. The diagnostics of this pathology for Romagna area (approximately 1.2 million inhabitants) is centralized at the "Area Vasta Romagna" laboratory located in Pievesestina. Moreover, in agreement with the Center for Congenital Bleeding Disorders of Cesena hospital, a 24-hour diagnostic service is guaranteed for coagulation/ bleeding emergency. This study collected a large case series observed from 2017 to 2023 in the "Area Vasta Romagna". AHA was considered observing an unexplained prolonged aPTT with normal PT whether or not in presence of any bleeding symptoms. An isolated low FVIII activity and the presence of a specific FVIII inhibitor confirmed the diagnosis. Twenty-four cases with AHA was observed in the six years considered by the study: 21 patients (11 males and 10 females) developed inhibitors when they were 70 to 93 year-old, while 3 female patients in the age comprised between 30 to 40 year-old in association with pregnancy. An average of 4.4 days (0-22 days) occurred between the first isolated elongated aPTT and the diagnosis of AHA, obtained by the calculation of the FVIII activity and inhibitor. The average value of aPTT at diagnosis was 2.18 ratio (1.58-3.48). At diagnosis, half of cases (12 subjects) had 5.1 to 34.2% plasma FVIII activity, 7 cases had 1.0 to 5.0% and 5 patients had <1.0%. The levels of inhibitor titer are very heterogeneous ranging from 0.8 to 281 UBh. Idiopathic AHA occurred in 33.28%, while the most common associated conditions were: MGUS, cancer, rheumatic diseases, pregnancy and autoimmune diseases. At time of diagnosis 5 patients were on concomitant anticoagulant therapy (VKA or DOAC). Half of AHA patients were identified thanks to the availability of the 24-hour diagnostic service. The incidence and case history of AHA in Romagna proved to be in line with what reported in literature. It is important to highlight that in almost 30% of cases the diagnosis was made within 24 hours from the incidental finding of prolonged APTT. This would be impossible if diagnostic service were not centralized and readily available. Efforts are needed to improve speed of AHA diagnosis in the remaining cases.

EP086

Differential diagnosis of lymphocytosis in routine laboratory practice: the contribution of Sysmex-XN9100

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Background: This study investigates the possibility of distinguishing clonal lymphocytosis from non-neoplastic lymphocytosis by using some of the data provided by the haematology analyzer XN9100 (RDW-SD, PLT, LYMPH#, IG#, HFLC %, LY-X).

Materials and methods: Data from 161 samples with lymphocyte counts between 4.00 and 20.00x10^9/L were initially analysed, 90 of which were diagnosed with non-malignant lymphocytosis (NON LPD) and 71 with malignant lymphocytosis CLL, lymphoma and ALL (LPD) from the analysis of the ROC curves. For two parameters of equal significance (e.g. LINFO% and LINFO# in absolute value or RDW-SD and RDW-CV), that with greater discriminatory power was selected according to p-value and are under the curve (AUC). The selected parameters were assessed using multiparametric logistic regression, with only non-redundant data included, as they were even more useful (than the single parameter) in distinguishing the two groups.

Results: The logistic regression equation resulting from the statistical analysis with MEDCALC and ANALYSE-IT is: logit (GROUP) = 57.3 - 0.07799 RDW-SD + 0.01244 PLT - 0.6164 LYMPH# - 39.18 IG# + 1.403 HFLC % -0.644 LY-X. If this formula is applied to the results of each sample, a "score" is obtained which, when the ROC curves are evaluated, gives an AUC value of 0.976. With a score value >1.5, only 1 sample with malignant lymphocytosis was identified as "non-malignant" (specificity 98.6 %). Of the 90 non-malignant samples, 73 were correctly classified and 17 were classified as suspected malignant. The "performance" of the score in terms of specificity was further verified by inserting the data of new samples to be assigned to the corresponding group into a spreadsheet. 107 of the 116 samples analyzed with lymphocytes between 4.00 and 20.00x10^9/L were correctly identified, 6 viral infections were false positives and only three were false negatives.

Conclusions: This study shows that Sysmex-XN9100 may help in the differential diagnosis of lymphocytosis.

EP087

The determination of Monocyte Distribution Width (MDW) in blood samples with hyperbilirubinemia

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Sepsis is a major cause of mortality in intensive care units (mortality rate 25-30%) and has an incidence rate of 535 cases per 100,000 person-years.The early identification of sepsis has become a primary healthcare goal for surveillance and clinical management.In the sepsis cascade monocyte differentiation in the circulation starts relatively early. The new generation of haematology analysers DxH 900 (Beckman Coulter,USA) measure automatically, in each CBC-DIFF (WBC differential analysis), monocyte activity and morphological alteration as MDW (Monocyte Distribution Width). MDW is the standard deviation of a monocyte cell mean volume. Excess bilirubin in the blood sample could interfere with CBC-DIFF. Interfered samples can be analysed by microscopic count or by repeating the CBC-DIFF by diluting the blood sample with saline solution. The aim of our study is to evaluate if dilutions influence the determination of MDW.MDW was determined in 18 CBC specimens collected in K2EDTA tubes (9 males and 9 females, age 68.2±16.0) with request for CBC-DIFF. In all samples, the total bilirubin value was in range of normality (0.28-0.83 mg/dL). Each sample was diluted with saline solution (1:3) and CBC-DIFF were performed on the whole and diluted samples on two different instruments (DxH 900 Hematology Analyzer_ Beckman Coulter, USA). No difference (p=0,138) was observed among undiluted and diluted samples (Student's t-test). Furthermore, the mean coefficient of variation (CV) of MDW resulted 5,4±2,1 (CVmin 2,2%; CVmax 9,6%).Finally we compared the diluted CBC-DIFF of 5 samples (3 males and 2 females, age 58.8±22.5) with a high value of total bilirubin (3.97-14.16mg/dL) with CBC-DIFF obtained on whole samples with CellaVision™ DM96 automated microscope (CellaVision AB, Sweden).From the comparison between the microscopic count and CBC-DIFF in terms of percentage of monocytes on total WBC, no difference was observed (p=0.810; Student's t test).In conclusion, MDW is easy and quick to measure and inexpensive. MDW can also be determined in samples with hyperbilirubinemia. Currently, MDW has been validated as a screening for suspected sepsis in the emergency department, but further studies need to verify its diagnostic efficacy in other clinical settings.

EP088

A comparison between the recommended multicolor assay and a simplified method for PNH screening, applied to UK NEQAS PNH samples

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder resulting from a somatic mutation of PIG-A gene that causes the inability to synthesize glycosylphosphatidylinositol anchor, which tethers over 20 proteins to cell membrane. Highly sensitive Multiparametric Flow Cytometry (MFC) represents the gold standard method for PNH diagnosis. UK NEQAS PNH scheme is a valid support to monitor MFC performance.
The aim of the study was a comparison between the standard multicolor assay and a simplified method for PNH screening, retrospectively applied to UKNEQAS PNH samples.
44 UKNEQAS PNH samples, collected between 2021 and 2024, were analysed following the standard assay described in the 2018 ICCS/ESCCA PNH Consensus Guidelines. Highly sensitive MFC analysis was able to quantify a PNH clone down to a lower limit of quantification (LLOQ) of 0.01% for Neutrophils (NE) and 0.5% for Monocytes (MO). Samples were reanalysed blindly, applying the simplified CD15/FLAER approach for leucocytes. The detection and quantification of NE and MO PNH clones by both methods were compared to consensus results.
In the 44 re-evaluated UKNEQAS PNH exercises, 32% were negative for leucocyte PNH clones, whereas 68% were positive, according to consensus results. We found a 100% agreement between the two methods, when applied to NE population both in negative and positive cases, with a median of variation of 0.2% in the clone quantification in positive samples. Concerning MO, with the simplified method we identified 79% of cases as truly negative, one as false positive with a 1% PNH MO clone, and in other two cases rare PNH events for MO were detected, despite the pre-defined LLOQ of 0.5% for MO. In 30 positive samples we identified 83% as truly positive, but in 5 cases, with a median consensus result of 2.5%, we were unable to detect the MO PNH clone with the simplified method. The overall concordance of the two methods within the MO compartment was about 60%.
We showed a complete agreement between the standard MFC method and the simplified approach, when applied to NE analysis in UKNEQAS PNH samples, confirming the validity of the simplified PNH screening method for NE. However, the simplified method did not show a satisfactory performance when applied MO analysis.

EP089

Focus on new-onset severe thrombocytopenia in Emergency Setting

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Aim: Baggiovara CoreLab in Modena's district serves 700000 inhabitants, performing 12 million tests/year with 30000 patients from Emergency Department (ED). The aim of this study is to report specific algorithms for ED patients with new-onset severe thrombocytopenia.
Methods: For ED management, CoreLab's clinicians Full Time Equivalent (FTE) 24 hours/day 7days/ week are represented by 3 hematopathologists and 9 non-hematopathologists, with 26 technicians. Hematopathologists cannot be on duty 24 hours/day 7days/ week but are available for remote second opinion.The available equipment in automation line comprises 4 hematology analyzers DxH900-2S (Beckman Coulter Inc., BCI) performing CBC-DIFF and smear, 4 coagulation analyzers ACL Top Plus (Werfen), 2 clinical chemistry analyzers AU5800 (BCI) and 2 DxC700 (BCI). Equipment off-line: 1 digital images reader DM 9600 (Cellavision AB) pre-classifying leukocytes on smear, 1 HEMOSIL® ACUSTAR for ADAMTS13 activity Assay (Werfen), 1 Polymerase Chain Reaction Rapid test Illumigene Malaria LAMP (Meridian).
Results: Immune thrombocytopenia has usually normal red blood cells and leukocytes morphology.Pseudothrombocytopenia is highlighted by DxH flags, microscopic visualization of platelets clumping and platelet count normalization in citrate tube. In acute promyelocitic leukemia (almost 10% of acute leukemias), thrombotic thrombocytopenic purpura and malaria the combined use of automated CBC-DIFF, blood smear revision, digital images, chemistry, coagulation tests, ADAMTS13 and rapid PCR (malaria) supports and completes severe thrombocytopenia evaluation, allowing differential diagnosis.
Conclusions: The three main causes of new-onset severe thrombocytopenia are related to hematological and infective emergencies.High productivity laboratories must define specific diagnostic pathways, accordingly to clinicians, adopting technological equipment adequate for second level analyses.This organizational model standardizes the laboratory diagnostic and guarantees to the clinicians a rapid reporting time, optimizing patient care setting definition. Thus, high quality service for the patients is established 24 hours/7days even if a hematopathologist is not present, albeit available for remote second opinion.

EP090

Lymphoproliferative disease vs reactive plasma cell proliferation in a case of HIV infection

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Introduction: A 35-year-old male patient presented to the Emergency Department due to extensive lateral submandibular, cervical and axillary lymphadenopathies with buccal bleeding and high fever for three days, associated with the appearance of petechiae. Immediately an aggressive lymphoproliferative disease was suspected.
Methods: Peripheral whole blood cell count was performed on Sysmex XN-9100TM hematology analyzer. The peripheral blood smear was performed using the automatic smear-making/staining. Flow cytometry analysis was carried out with BD FACSLyricTMII and InfinicytTM software. Serum protein electrophoresis and serum immunofixation were performed respectively on Sebia capillarys 2 flex piercing and Hydrasys. Optilite analyser (The Binding Site) was used to quantify K and λ serum free light chains. HIV RNA was detected by PCR Real-Time.
Results: Whole blood cell count showed normal WBC (8.9x109/L), anemia (Hb 10.3 g/dL) and thrombocytopenia (PLT 1x109/L). The differential leukocyte count showed an inverted formula (lymphocytes 4.37 x 109/L), and an anomalous WBC scattergram with flags of suspected presence of atypical lymphocytes. The microscopic revision of the blood smear confirmed the inverted formula and the presence of lymphoplasmacytoid lymphocytes (13% of total cells). Peripheral blood flow-cytometry analysis indicated a marked decreased of the CD4/CD8 ratio (0.3), CD4+ T cell lymphopenia (0.17 x109/L) and increased activated CD3+HLA-DR + T cells (28.5%); 20.0% of circulating plasma cells with normal immunophenotype (CD45+/CD38+/CD138+/ CD19+/CD56-/CD27+/CD81+/CD117-) were also detected. Qualitative analysis of serum protein electrophoresis showed a quantitative increase in the gamma globulin fraction, suggestive of polyclonal gammaglobulinemia. This finding was confirmed by serum immunofixation and measurement of free K and λ light chains. An advanced HIV infection (viral load: >10000 copies/ml) was subsequently diagnosed.
Conclusions: This case confirmed the importance of a multidisciplinary collaboration to address a rapid diagnosis and a prompt treatment and illustrates how HIV can provoke a profound plasma cell response, leading to benign atypical hematologic findings.

EP091

Flag strumentale pRBC dell'analizzatore XN-Sysmex: ruolo di screening del Laboratorio Generale nelle parassitosi da Plasmodium spp.

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Introduzione: La malaria rimane globalmente la più comune delle parassitosi umane trasmesse da vettore. In Italia è una malattia da importazione e la specie prevalente è P.falciparum di origine sub-sahariana, seguita da P.vivax diffusa in Asia. Un uomo pakistano di 27 anni effettua un accesso al Pronto Soccorso dell'Ospedale Careggi-Firenze, manifestando dolori addominali e vomito. Viene ricoverato in reparto di Degenza Malattie Infettive con sospetta sepsi ad eziologia sconosciuta.
Metodi: Presso il Laboratorio Generale si esegue l'emocromo con formula mediante piattaforma ematologica automatizzata Sysmex XN-9100TM. L'analizzatore identifica e quantifica le cellule ematologiche in base a Forward-Scatter (FSC) Side-Scatter (SSC) e Fluorescence Intensity (SFL) che misurano rispettivamente la dimensioni cellulari, granularità/complessità e contenuto DNA/RNA. Lo striscio di sangue periferico viene eseguito tramite preparazione e colorazione automatica. Un operatore esperto esegue la lettura impiegando l'analizzatore di immagini DI60.
Risultati: La conta totale dei leucociti mostra una generale leucopenia (4,67x109/L), anemia microcitica (Hb 11,8 g/dL, MCV 67,3 fL) e piastrinopenia (79 x109/L). Lo scatter associato al canale WDF evidenzia una popolazione anomala caratterizzata da basso segnale SFL e un alto SSC tale da generare il flag strumentale pRBC (pRBC=300), e una popolazione di cellule ad elevata intensità di fluorescenza di possibile origine linfocitaria reattiva. L'analisi morfologica rileva la presenza di emazie parassitate da trofozoiti e forme mature di Plasmodium spp. L'infezione è stata confermata con test LAMP. La lettura dello striscio sottile da parte del Microbiologo ha confermato la presenza di P. vivax con parassitemia pari a 0.2%.
Conclusioni: Il canale pRBC si è dimostrato un potente strumento di screening per indirizzare la diagnosi di infezione malarica e risulta pertanto un importante supporto nella pratica clinica per la diagnosi precoce di tali parassitosi ematiche.

EP092

An artificial intelligence system based on MicroNIR spectroscopy and Chemometrics for the quality assessment of plasma-derived medicines.

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The coupling of MicroNIR and chemometrics are now well recognized as promising analytical tool for totally digitalized platform development. The click-on spectrophotometer, the MicroNIR, controlled through a low-power wireless (Bluetooth) interface, ensures rapid analysis of complex matrices without any sample pre-treatment and chemometrics allows to obtain rapid and accurate results. A totally digitalized platform MicroNIR/Chemometrics is here proposed for the control of plasma-derived products. Blood products are obtained from voluntary donors of blood or plasma, through industrial manufacturing processes, and therefore a careful investigation is required prior of their distribution on the market. Currently, efficacy, quality and safety for recipients is ensured by performing complex and laborious tests, which require high expertise to handled samples and avoid any contaminations. Therefore, the novel analytical strategy based on a prompt and unbiased approach was considered for the study of Human Albumin Solutions (HAS). Spectra were collected placing the MicroNIR directly on the sealed flasks and then were processed by using a chemometric software. First, the Principal Component Analysis (PCA) was performed to point out correlations within samples according to protein content, manufacturing process and raw materials features. Supervised techniques, especially the Partial Least Squares regression (PLSr) and the Partial Least Square-Discriminant Analysis (PLS-DA) were then used to provide prediction models for the quality assessment of the biological products. Performances of the MicroNIR/Chemometric approach were evaluated by analysis of unknown HAS samples and results confirmed the effectiveness of the promising tool. An innovative artificial intelligence system, able to investigate HAS samples through a single click and associate results to the examined batch by the QR codes, may be developed connecting the miniaturized and click-on device to a smartphone, where the validated models were imported.The digitalized platform would allow a real-time evaluation of pharmaceuticals quality, defending health of patients.

EP093

Evaluation of the new diagnostic infection score ICIS (Intensive Care Infection Score) in patients hospitalized in Intensive Care Unit

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Introduction: Sepsis is one of the main causes of mortality in Intensive Care Unit (ICU), therefore it is essential to diagnose it early. However, there are difficulties in differentiating sepsis (systemic inflammation with infection) from the non-infectious systemic inflammatory response syndrome (SIRS). Therefore, the detection or exclusion of systemic infection quickly and reliably before the microbiological outcome, is still an open, unresolved question. The combination of multiple parameters of the CBC (Complete Blood Count) can be one of the most promising approaches, such as the new Sysmex ICIS (Intensive Care Infection Score) tool. The aim of the study is to perform an evaluation of the ICIS tool, in terms of effectiveness and diagnostic accuracy for the identification of infection in critically ill patients in ICU.
Materials and methods: Patients admitted to the ICU of the San Donato Hospital in Arezzo between august 2023 and april 2024 were enrolled, and the ICIS was performed using the samples collected with K2EDTA anticoagulant. The ICIS tool is calculated from the following 5 parameters of the CBC performed with XN instrumentation (Sysmex, Japan): average fluorescence intensity of segmented neutrophils (sNFI), hemoglobin difference between newly formed and mature erythrocytes (dCHC), absolute counts of segmented neutrophils (sN#), antibody-secreting lymphocytes (ASL) and accurate counts of immature granulocytes (aIG#). For statistical processing, ROC (Receiver Operating Characteristic) curve analysis was used.
Results: 196 patients (75 females + 121 males) were included in the study, of which 65 with infection and 131 without infection at the time of admission. The ICIS ROC curve at cut-off value >5 shows an area under the curve of 0.73 (compared to procalcitonin (PCT) of 0.66 at cut-off > 2.99) with sensitivity 62.5 %, specificity 74.6, NPV 80.0% and PPV 55.0%, which for PCT are 51.0%, 76.5%, 75.4% and 52.4% respectively.
Conclusion: Based on the preliminary results of this study, ICIS is a candidate as a further support for the classification of the infected patient in an important setting such as ICU. This study also confirms the role and importance of the CBC in contributing to the early diagnosis of the infectious process.

EP094

A twenty years observational and retrospective analyses of cryoglobulins at IRCCS San Gerardo

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Cryoglobulins are abnormal immunoglobulins that precipitate at < 37C° dissolving on warming. They are composed mainly of mono or polyclonal IgG, IgM, or IgA antibodies. They clump in small blood vessels, leading to vasculitis with symptoms such as purpura, arthralgia and asthenia. The presence is associated with autoimmune, viral and lymphoproliferative diseases [1]. Our aim is a 20 years (2004-2024), observational and retrospective study of cryoglobulins, at IRCCS San Gerardo, collecting about 10803 results with non-conform of about 10% (1.103). Moreover, among the positive outcome, we isolated the population with a cryocrite higher than 3%, which represent the sub-population subjected to the characterization, if requested by the clinicians, according to our procedure. Out of the 9700 findings, 5100 (47%) were from outpatient cases. Cryoglobulins were confirmed in 1860 samples (36%) compared to 3240 negative ones (64%). Around 11% (207) of the samples presented a cryocrite higher than 3%. The remaining 4600 (53%) results were from inpatients, with 2500 (54%) negative and 2100 (46%) positive. Among the positive results, 435 (21%) had a cryocrite >3%.Hematology, Nephrology, and Infectious Disease showed the highest percentage of patients affected by cryoglobulinemia (1082, 51%). The distribution was: Inf. Disease 720, (67%), Hematology 119 (11%) and Nefrology 243 (22%) of positive inpatients. Moreover, we analyzed the percentage of the results with a cryocrite >3% in these units, obtaining: 20% in Nefrology, 17% in Hematology and 6% in Inf. Disease. Interestingly, although the Inf. disease unit barely requests cryocrite characterization, 100% of all the samples from the Nefrology are classified as type II cryoglobulin, according to the Brouet classification. Moreoever, in Hematology we observed a distribution of 55% of type II cryoglobulin and 45% of type I. In conclusion our data suggest that in our hospital cryoglobulins are considered a secondary clinical evidence and rarely are requested for characterization by the clinician. Nevertheless, collaboration between clinic and laboratory is crucial for managing cryoglobulins properly, as distinguishing between type I and II cryoglobulins facilitating a more accurate diagnostic approach.

[1] Diagnosi di crioproteinemia: preziosa collaborazione tra laboratroio e clinica per a corretta gestione di una patologia rara. Natali et al. 2022

EP095

Comparative analysis of the performance of automated digital cell morphology analyzers for leukocyte differentiation in hematologic malignancies: Mindray MC-80 versus West Medical Hema Vision

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BACKGROUND-AIM. The hematology laboratory has enhanced its diagnostic capabilities by using advanced artificial intelligence tools to analyze digital images of peripheral blood cells. The Mindray MC-80 (MC80) has performed excellently in various independent studies.This study aims to compare the leukocyte differential performance of the MC80 with that of HemaVision (HV) and the gold standard, manual microscopy.
METHODS. 75 patients (M: F 53:47%; median (min-max) age 63 ys (1-90)), with hematological malignancies (ALL= 4, B-CLL=20, AML=20, CML=5, lymphoma= 20, infection=6) were analyzed. Their smears were compared using the MC-80, HV, and manual microscopy. According to REF, the agreement between microscopy (reference method, REF), HV, and, MC80, was expressed as the median (IQR) of a given cell population/feature, with REF-HV and REF-MC80 differences expressed as bias and 95% limits of agreement.
RESULTS. Concordance was calculated for all complete blood count parameters, but only the following are reported: Neu% [REF: 23.5% (6.5-36.7); REF-HV: 0.09 (-0.35 to 0.54); REF-MC80: 0.21 (-1.16 to 1.57)]; Ly% [REF: 45% (12.5-77.8); REF-HV: -2.56 (-6.72 to 1.60); REF-MC80: 23.03 (16.99 to 29.08)]; Mo% [REF: 2.00% (0.50-4.9); REF-HV: -2.15 (-3.57 to -0.73); REF-MC80: -1.47 (-2.42 to -0.51)]; Eo% [REF: 1.0% (0.0-2.0); REF-HV: -0.44 (-0.77 to -0.11); REF-MC80: 0.08 (-0.25 to 0.40)]; Baso% [REF: 0.0% (0.0-0.5); REF-HV: -0.76 (-1.73 to 0.21); REF-MC80: -2.22 (-3.17 to -1.28)]; band cells [REF: 0.5% (0.0-1.5); REF-HV: -0.01 (-0.19 to 0.17); REF-MC80: -1.87 (-2.52 to -1.23)]; myelocytes [REF: 0.00% (0.00-0.5); REF-HV: 0.18 (-0.16 to 0.51); REF-MC80: -4.10 (-5.81 to -2.40)]; metamyelocytes [REF: 0.00% (0.00-0.4); REF-HV: 0.33 (0.04 to 0.63); REF-MC80: -0.56 (-0.98 to -0.14)]; blasts, all samples [REF: 0.0% (0.0-34.6); REF-HV: 10.07 (5.17 to 14.97), REF-MC80: -2.05 (-7.06 to 2.96)]; blasts, in acute leukemia [REF: 61.2% (31.5-91.5, 2.0-98.0); REF-HV: 32.55 (21.55 to 43.56), REF-MC80: 17.70 (10.18 to 25.23)]; smudge cells in CLL [REF: 64.8% (42.9-100.3); REF-HV: 0.67 (-2.03 to 3.36), REF-MC80: -48.43 (-70.86 to -26.00)].
CONCLUSIONS. The study shows that MC80 has a higher sensitivity in identifying blasts than HV. However, HV shows better agreement with microscopy than MC80.

EP096

Impact of preanalytical variability on plasma microRNA expression profile

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Background: miRNAs represent potential non-invasive circulating biomarkers for oncological diseases as the myelodysplastic syndrome (MDS). Although the effect of biological variability on miRNA expression profile is well documented, limited data are available on the role of preanalytical variability, both relevant in the prospective of an imminent clinical application of these molecules. Indeed, very few data on the combined effects of time and temperature storage on the recommended Platelet-poor plasma (PPP) for miRNAs are available. Aim: The aim of this study was to: 1) investigate the impact of these preanalytical variables in PPP samples on miRNA expression profile; 2) identify potential circulating miRNAs as diagnostic biomarkers of MDS, using a comparative approach for preanalytical variability in the differential expression analysis.
Methods: EDTA K2 plasma from 12 patients with MDS and 12 healthy donors (HD) were collected, centrifuged to obtain PPP within 3 hours (h). PPP aliquots were stored before miRNA extraction as follows: A= 4°C for 30 minutes; B= 4°C for 24 h; C= RT for 24 h; D= -20°C for 10 days. miRNA libraries were prepared using QIAseq miRNA Library Kit (Qiagen), sequenced in the Next-generation sequencing platform NextSeq550 (Illumina) and analysed using CLC workbench (Qiagen).
Results: We showed that plasma miRNA expression profile (MEP) is significantly modified by the preanalytical conditions: MEP was affected the most by the C condition (RT for 24 h) followed by B; A and D showed not significant differences. 58 miRNAs were significantly modulated in MDS compared to HD: only 16 miRNAs were constantly differentially expressed in A, B, C and D; in particular, 3 miRNAs upregulated (miR-34a-5p, miR-409-3p, miR-411-5p) and 2 downregulated (miR-16-5p, miR-486-5p) in MDS were strongly significative although influenced by preanalytical conditions.
Conclusions: Our results highlight that the miRNA extraction immediately after PPP separation or PPP long-term storage at -20°C represent the best preanalytical conditions for a comprehensive and unbiased miRNA expression profile analysis. The standardization and declaration of preanalytical conditions should be always recommended in both miRNA research studies and in their clinical translation.

EP097

Trombofagocitosi: un raro caso di pseudopiastrinopenia

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La pseudopiastrinopenia (PTCP) è dovuta principalmente ad aggregazione e talora a satellitismo piastrinici in vitro e causa conte piastriniche falsamente ridotte. L'identificazione tempestiva di questo artefatto è essenziale per evitare un processo decisionale clinico e terapeutico inappropriato.Una signora di 85 anni è giunta al PS per episodio di cefalea associata ad afasia. Nega febbre, vomito e sincope. L'emocromo evidenzia una piastrinopenia con conta piastrinica automatizzata (CPA) di 82 x109/L. Le altre indagini risultano normali. L'anamnesi evidenzia ricorrenti emicranie con aura, artrosi, sindrome dell'intestino irritabile, stenosi carotidea, ipercolesterolemia, non sanguinamento e lividi. E'riferita sospensione di cardioaspirina a causa di modesta piastrinopenia, evidenziata precedentemente e non indagata.La valutazione dello striscio di sangue rivela un'estesa fagocitosi delle piastrine da parte dei granulociti neutrofili, talora dei monociti, senza aggregati piastrinici, e un satellitismo piastrinico limitato.Nel sospetto di PTCP, si è provveduto a un nuovo prelievo sia in EDTA che in CPT e a CPA al tempo 0', 30', 60' e 120', in ottico e in impedenziometrico. La CPA, immediatamente dopo il prelievo, è stata di 160 ± 5 x 109/L nei due anticoagulanti. Nelle seguenti misurazioni, la CPA in CPT è rimasta stabile, mentre in EDTA si è ridotta con il passare del tempo.La valutazione degli strisci non ha indicato alcuna anomalia sui campioni con CPT, mentre nei campioni in EDTA si sono rilevate le stesse caratteristiche morfologiche precedenti, con trombofagocitosi dei neutrofili. Questo fenomeno è causa rarissima di PTCP e risultava più accentuato sul vetrino al tempo 60', concordando con una conta piastrinica ridotta del 32,5% rispetto al tempo 0'. Si è concluso per pseudopiastrinopenia EDTA-dipendente.La morfologia resta indispensabile per verificare la stima CPA, eventuali interferenze e per un'analisi morfologica delle piastrine. In caso di PTCP, la valutazione va eseguita nel suo complesso, osservando tutte le linee cellulari, non solo quella megacariocitaria.

EP098

ACTIVATED PARTIAL THROMBOPLASTIN TIME (aPTT): HOW SENSITIVITY VARIES DEPENDING ON THE REAGENTS USED.

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Background: The APTT test is a very common clotting test.
It can be prolonged in a variety of conditions, including factorial deficiency (factors VIII, IX, XI, XII, prekallikrein-PK), in the presence of specific and /or nonspecific inhibitors (including Lupus Anticoagulant -LA), and for liver disease. Several reagents are available for the aPTT test, which differ, among other things, in the presence of phospholipids of plant and non-plant origin. This affects the ability to detect factorial deficits and the presence of LA. Here, we present an assessment of the change in sensitivity by comparing 2 different reagents: aPTT-SP and SynthAFax on a patient population already known at the Hemostasis Center.
Aims: The study was designed and implemented at the Haemostasis Centre of AORN S. Moscati (AV, Italy). The aim is to highlight, if possible, the most suitable reagent for the practice of clinical screening.
Methods: The data were obtained from tests carried out in the laboratory of the Hemostasis Center. All tests were performed with the automated coagulometer TOP-500 ACL (Werfen, Madrid, Spain). The following reagents were used for APTT: HemosIL APTT-SP (Werfen, non-vegetable phospholipids and colloidal silica), HemosIL SynthAFax (Werfen, plant phospholipids and ellagic acid). Results: 217 patients, already known to the Haemostasis Centre, were tested for aPTT. 93 were LA positive and 124 had factor deficiencies (13.7% FVIII, 31.3% FIX, 23.4% FXI, 25.6% FXII, and 6% PK). 100% of patients had an aPTT-SP ratio > 1.20 (i.r. aPTT ratio 0.85 - 1.20); but only 57% of the population had an aPTT- synthAFax ratio >1.20.
Conclusion(s): This study showed that a reagent consisting of plant phospholipids and ellagic acid is not able to detect the presence of non-specific inhibitors. This is a reality already known to the world of laboratory medicine. However, very often in common practice, both plant-based and non-plant-based phospholipid-based reagents are used. This does not allow to detect any aPTT prolongation due to the presence of non-specific inhibitors. To make matters worse, the bad habit of not specifying the nature of the reagent used for the aPTT test in the report makes matters worse.

EP099

PROLONGED ACTIVATED PARTIAL THROMBOPLASTIN TIME OF UNKNOWN ETIOLOGY: A SYSTEMATIC EVALUATION OF CAUSES.

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Background: A prolonged activated partial thromboplastin time (APTT) is one of the most frequent reasons why outpatients are referred for hemostasis consultation. Nevertheless, very few data are available on the relative contribution of individual causes of this common clinical scenario. Here, we present a systematic evaluation of all causes of APTT prolongation in a consecutive population of outpatients referred for specialized hemostasis consultation during a 25-year period (from 1997 to 2022).

Aims: The study was designed as a retrospective cohort study performed in the outpatient hemostasis clinic of Hemostasis Centre of AORN S. Moscati (AV, Italy). The aim of providing data on the relative contribution of different conditions for APTT prolongations.

Methods: Datas were obtained from the tests done in the laboratory of the Hemostasis Centre. All assays were performed in hemostasis laboratory with in automated coagulometers (Siemens XP Healthcare, Munich, Germany; ACL TOP-500, Werfen, Madrid, Spain). For the APTT, the following reagents were used during the study period: Actin FSL (Siemens Healthcare) from 1997 to 2010, and HemosIL APTT-SP (Werfen) from 2010 to 2022.

Results: Among 732 consecutive patients, the most frequent causes were antiphospholipid antibodies in 51.50%, coagulation factor deficiencies in 33.90%, and vitamin K deficiency/liver disease in 5.90%. Consumption coagulopathy in 3.60%; interference from drugs in 2.83%, inhibitor deficiency in 1.55%; Hemophilia A in 0.71%. A definite cause was not identified in 0.01% of patients.

Conclusion(s): A specific diagnosis for the prolonged APTT was defined in 99.9% of patients. In conclusions, our study provides contemporary data on the relative distribution of the causes for APTT prolongation in one of the most common clinical scenarios of consultative hemostasis, confirming the antiphospholipid antibodies as the main cause of this laboratory alteration, and highlighting the significance of a prolonged APTT in absence of a specific disease of hemostasis.

EP100

INCREASE OF DEEP VEIN THROMBOSIS IN ATYPICAL LOCALIZATION : THE EXPERIENCE OF THE HEMOSTASIS AND THROMBOSIS CENTER FCSA n °123

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Background :Venous thromboembolism (VTE), which can debut in the clinical form of deep vein thrombosis (DVT) or pulmonary embolism (PE), represents the third most frequent acute cardiovascular syndrome. Approximately 4% of deep vein thromboses (DVT) have an "atypical" localization . They involve venous segments other than the veins of the lower limbs, such as the splanchnic, renal, gonadal and cerebral districts.

Objectives : To monitor the increase of DVT in atypical localization in patients of the Hemostasis and thrombosis Center FCSA n°123 in the period from 2018 to 2024. To monitor the therapy efficacy and clinical evolution up of these patientsMethod : In 2018- 2021 period no DVT in atypical localization were registered at Hemostasis and Thrombosis Center FCSA n°123. In 2022 two patients have DVT (two females : one splanchnic and one gonadal district) . In 2023 four patients (three males and one female : two splanchnic , one cerebral,one upper limb. In the first five months of 2024 two patients (one male and one female : one splanchnic, one upper limb). In order to monitor the observed cases of DVT in atypical localization , the following indicators were considered: Number of cases and sex of patient; Anatomical localization of DVT; Therapy and follow up.

Results :. Increased number of cases (from 0 to 8) was registered in the observation period in equal number between males and females. In most of them the interested district was splanchnic (portal and mesenteric veins). All cases were treated with AVK, with complete clinical resolution in 4 cases.

Conclusions : DVT in an atypical districts is rare, but clinical debut may be severe and urgent. The therapy is complex. A limited number of clinical studies with large case series is available, mostly based on expert consensus rather than on consolidated guide lines. It is frequently associated with pre-existing haematological and oncological pathologies, with the consequent need to customize the therapeutic scheme, DOACs are a promising approach but not yet on the label. Further clinical studies are strongly needed.

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EP101

Andamento delle richieste del test D-dimero tra i periodi pre-covid e post-covid

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La misurazione del D-Dimero (DD) rappresenta un cardine nella diagnosi/esclusione e nella prognosi del tromboembolismo venoso (TEV) e della coagulazione intravascolare disseminata (CID), quando incorporata in algoritmi clinici validati e utilizzando soglie diagnostiche adeguate all'età. Durante il recente periodo pandemico ha inoltre rappresentato uno tra i parametri più rilevanti per seguire l'evoluzione delle possibili coagulopatie presenti nei pazienti affetti da COVID-19 con un aumento notevole delle richieste. Scopo del nostro lavoro è stato quello di verificare se, terminato il periodo pandemico, il numero di richieste di DD sia ritornato ai livelli precedenti il 2020. Nell'analisi sono stati considerati i dati relativi alle accettazioni effettuate nei primi 6 mesi degli anni 2019, 2021 e 2024. I dosaggi di DD sono stati eseguiti presso il laboratorio di Carate Brianza (MB) con strumentazione STA R MAX2 Stago con kit STA Liatest D-Di. Il totale complessivo delle accettazioni considerate è stato di 1.101.383 delle quali 7.543 includevano il DD. Analizzando le percentuali nei tre anni si evidenzia che la numerosità di richieste contenenti il DD è passata dallo 0.310% nel 2019 all'1.042% nel 2021 e allo 0.480% nel 2024. La crescita delle richieste di DD nel confronto dei periodi 2019-2021 è stata del 232% mentre tra il 2019 e il 2024 del 55%. L'analisi dei dati considerati sull'ampio numero di accettazioni ha evidenziato come la richiesta del DD sia rimasta, ancora oggi, più elevata rispetto al periodo pre-pandemia. Rimane da valutare se la misurazione del DD sia stata effettuata rispettando le indicazioni delle linee guida, o se invece la richiesta sia rimasta inappropriata, come anche suggerito in diversi studi scientifici.

EP102

Acquired haemophilia A caused by Factor VIII autoantibodies

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Background: Acquired hemophilia A is a rare hemorrhagic disorder caused by the development of autoantibodies directed against coagulation Factor-VIII (hemophilic factor) resulting in its inhibition. Data describe acquired hemophilia type A as the most prevalent form with a mortality rate of between 8% and 22% of cases. Here we discuss the diagnosis and treatment of a patient with hemorrhagic manifestations in which has been discovered the presence of autoantibodies directed against the hemophilic factor of the coagulation cascade.

Case presentation: an 80-year-old female patient has been clinically and molecularly investigated for the presence of severe anemia and increased aPTT ratio (2.2) in the absence of anticoagulant therapy. The patient showed massive hematomas in the upper limbs and torso, she denies previous traumas and furthermore does not report known coagulopathies or hemorrhagic manifestations previously. The patient referred the use of aspirin at the moment. Physiological parameter of note was a systolic hypertension (150/80 mmHg) with a heart rate of 90 BPM.

Conclusions: Due to patient symptoms, diagnostic hematological tests have been performed to evaluate the coagulation cascade, including mixture test, factors assay, LAC autoantibody, and the analysis of the possible presence of autoantibodies. After performing the mixture test the lengthening of coagulation times falls beyond normal limits after 2 hours of incubation at 37°C. The analysis of intrinsic pathway of coagulation showed a low residual Factor-VIII activity under 1%, and LAC research displayed negative result. Due to these preliminary results, we performed an assay of autoantibodies directed against the hemophilic factor, that showed the presence of an inhibitor at rate of 2.24 BU/ml, which demonstrated the development of acquired hemophilia by the patient. Clinically the patient has been treated with a bypassing agent (Novoseven) and subsequent immunosuppressive therapy.

EP103

LABORATORY ADAMTS13 TESTING IN PATIENTS WITH SUSPICION OF THROMBOTIC THROMBOCYTOPENIC PURPURA, "ANNUNZIATA" A.H. (CS, ITALY), YEARS 2020-2023

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is a rare disorder characterized by thrombocytopenia and microangiopathic hemolytic anemia, and very high-mortality rate (90%) in untreated patients. Severe ADAMTS13 deficiency (activity <10%) leads to accumulation of large von Willebrand Factor multimers, resulting in occlusive microvascular thrombi. TTP occurs either congenitally (cTTP, autosomal recessive), or as an acquired event (aTTP), due to development of anti-ADAMTS13 antibodies. Therapeutic plasma exchange (TPE) remains highly effective therapy; it is often used in conjunction with other therapies including corticosteroids, rituximab, and caplacizumab. We report a retrospective study of individuals admitted between January 2020 and December 2023 to "Annunziata" Hospital (CS, Italy), affected by thrombotic microangiopathy (TMA), with ADAMTS13 activity assessment.Methods: A total of 57 patients were screened for ADAMTS13 testing (54% female, mean age at the first acute episode 56.3±21.0 years, range 3-93; 46% male, mean age at the first acute episode 54.3±21.4 years, range 1-84). Diagnosis was performed by integrating clinical information with laboratory results (blood count, schistocytes, elevated LDH, haptoglobin, serum bilirubin etc.). Plasma ADAMTS13 activity was determined using ELISA chromogenic test "Technozym Adamts13 activity" (Technoclone).Results: Of the 57 analysed patients, 22 (38%), mean age 61.0±17.0 years, range 30-93, 68% female, presented with low ADAMTS13 activity; of those, 54% resulted with ADAMTS13 activity <10% (<0.1 IU/mL) (N=20 females (83%), age range 30-75; N=2 males, age range 35-39 years). Persistently low plasma ADAMTS13 activity was observed in 50% of the followed up patients (N=16). All subjects with ADAMTS13 activity <10% (TTP diagnosis) were referred for TPE. No deaths were documented from TTP-related mortality.Conclusions: Availability of ADAMTS13 testing is very effective in supporting a timely diagnosis of TTP and in allowing rapid use of life-saving therapy. Unfortunately, to date, these tests are not achievable in a homogeneous way throughout the national territory. The creation of a TTP registry in Calabria could constitute a valid tool to optimize identification and management of patients with TTP.

EP104

Importanza del test di miscela nella valutazione dello stato di anticoagulazione:descrizione di un caso

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Il test di miscela rappresenta un importante strumento di indagine per stabilire la causa dell'allungamento di PT e aPTT.E' stato sottoposto alla nostra attenzione un paziente di 87 anni giunto al Pronto Soccorso per la presenza di ematomi alle mani e agli avambracci e sanguinamenti dal cavo orale.Il paziente, affetto da fibrillazione atriale non valvolare, riferiva di non aver subito traumi e di aver assunto il giorno prima una sola compressa di Xarelto 20 mg.

Materiali e metodi.Il PT, l'aPTT, il test cromogenico anti-Xa per misurare la concentrazione di rivaroxaban e il dosaggio dei fattori sono stati eseguiti con i reagenti della Ditta Werfen; l'emocromo sull'analizzatore della serie XN (Sysmex) e il dosaggio della creatinina sierica sullo strumento Cobas C702 (RocheDiagnostics).

Risultati. I risultati degli esami erano i seguenti: PT= non determinabile nel tempo di lettura di 320 sec., aPTT=11.4 ratio, Fibrinogeno=535 mg/dL, Hb=8,1 g/dL, creatinina=2,62 mg/dL, markers epatici nella norma. Dato che una singola pasticca di Xarelto non giustificava un allungamento così importante di PT e aPTT, abbiamo eseguito un test di miscela 1:1 con pool di plasmi normali. PT Mix = 1,04 ratio, aPTT Mix =1,00 ratio. L'incubazione a 37°confermava la correzione suggerendo una carenza fattoriale. Pur supponendo che non si trattasse di un sovradosaggio di rivaroxaban,abbiamo dosato il farmaco che aveva una concentrazione di 375 ng/mL,superiore ai livelli attesi ma non da giustificare i risultati ottenuti. Controllando nello storico del paziente, abbiamo verificato che fino a tre mesi prima era in terapia con Coumadin e siccome soffriva di demenza senile, abbiamo sospettato che ne avesse assunto una dose elevata. Abbiamo pertanto dosato i fattori vitamina K dipendenti: FII=51%, FVII=49%,FIX=65%, FX=24% . Il test del parallelismo per i fattori carenti ha confermato il deficit fattoriale. Il paziente è stato quindi sottoposto a trattamento con Konakion.Il giorno successivo i familiari hanno rinvenuto nell'abitazione del paziente scatole vuote di Coumadin che hanno confermato il nostro sospetto.

Conclusioni. Il test di miscela rappresenta un'utile strategia per individuare l'eziologia di una coagulopatia, e per indirizzare il clinico verso il percorso diagnostico-terapeutico più idoneo.

EP105

Interaction between clinical departments and haemostasis laboratory: clinical case of an anticoagulated patient in a critical area

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Background: The introduction of direct oral anticoagulant drugs (DOAC) into clinical practice produced a revolution in the prevention and treatment of thrombotic events in different clinical contexts. However, the evidence on the use of DOACs in critically ill patients is very limited. Taking into consideration the paucity of available scientific evidence and the particular heterogeneity of critically ill patients hospitalized in intensive care units (ICU), the use of DOACs needs to choose the safest and most effective anticoagulant strategy, based on the characteristics of the individual patient.

Methods :Patient A.P. with anemia and atrial fibrillation, treated with apixaban 10mg /die . Mild long PT (prothrombin time) and APTT (partial thromboplastin time) was in pathological history before anti factor X (apixaban) therapy, withouth any evaluation required. In september 2024 hospitalization for chest pain in cardiology. Cardiocirculatory arrest . Admission to intensive care. Worsening of renal and hepatic function.worsening of anemia and coagulation function. (PT <10 %;INR > 10; APTT :1.6 ratio).

RESULTS: Apixaban is discontinued . Dosing Apixapan is performed 48 hours after discontinuation (93 ng/ml) . Mix test and Factor X dosage test are performed. The mix test corrects for severe Factor X deficiency (16%).All anticoagulants are discontinued but the patient died.

CONCLUSIONS : Direct anticoagulant drugs (DOACs), showed an important improvement in anticoagulant therapy. The predictability of the dose-response relationship, means that their use in critically ill patients, including those with significant hepatic and renal impairment, must be carefully evaluated. Before starting anticoagulant treatment, laboratory tests are strongly suggested : complete blood count, PT, aPTT, fibrinogen, ALT, AST, creatinine, to allow a precise and safe analysis of e indications and contraindications to treatment.

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EP106

Dosage of direct-acting oral anticoagulants (DOAC) : the experience in the emergency laboratory from 2020 to 2023

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Background : DOACs (Direct Oral Anti Coagulants) showed efficacy and safety also in the post-marketing phase of the trial. The four molecules introduced (dabigatran, apixaban, rivaroxaban and edoxaban), showed, even in the context of the real world, significant reduction in thromboembolic events, and therefore the mortality and morbidity related to them

Methods : For the dosage of DOACS, 2 types of tests are available:1) qualitative tests such as prothrombin time (PT) and activated partial thromboplastin time (aPTT); 2) quantitative tests such as dilute thrombin time (dTT), assessment of activated factor X (anti-FXa) activity and ecarin time (ECT). Qualitative tests are available in all laboratories, are quick to perform and in emergency situations offer indications on the patient's coagulation status. These tests give no indication of the plasma concentration of the drug. Quantitative test can be used to evaluate the plasma concentration of the drug by determining the antithrombin effect for dabigatran and anti-FXa for rivaroxaban, apixaban and edoxaban

Results : Since 2014, the Clinical Pathology Laboratory has introduced the determination of DOACs both in routine and in emergency, with particular attention to the optimization of the procedure that has been shared with clinical doctors. In summary, the lab must be provided with the following informations before sending the sample: type of drug, other anticoagulants and, when possible, the time elapsed since the last administration and blood draw. The period from 01/01/2020 to 31/12/2023 was examined. A total of 452 DOAC determinations were performed: 65 for Dabigatran, 63 for Rivaroxaban, 183 for Apixaban and 78 for Edoxaban. The most frequent requests were in urgency (58%) compared to routine (42%).

Discussion and Conclusion :The results confirm the wide ranges obtained for these drugs, which are affected both by the time of sampling compared to the last intake, and by the wide inter-individual variability. The optimization of the protocol for DOAC's testing allowed to get the dosage of drug within 40 minutes from the arrival of the sample in the laboratory. The quantitative dosage of DOACs is obtained in less than 1 hour / h24 which has proven useful in the management of critically ill patients treated with these drugs.

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EP107

The combination of two automated assays for diagnosis of heparin-induced thrombocytopenia (HIT) is necessary?

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Introduction: HIT is an immune-mediated prothrombotic condition characterized by a decrease in platelet count and increased thrombotic risk caused by anti-platelet factor 4 (PF4)/heparin complex antibodies (Ab). HIT appears within 5 to 14 days after heparin (H) administration with incidence that depend on the duration and type of H exposure and patient population. Diagnosis of HIT is based on clinical evaluation and the presence of Ab. Aim of our study was to evaluate whether the combined use of two automated assays for diagnosis of HIT provides a diagnostic advantage over the use of a single assay.
Material and Methods: We extracted from the laboratory informatic system 229 requests for detection of Ab against PF4/H complexes. IgG Ab and total Ab against PF4/H complex were measured with two different methods: IgG Ab with a chemiluminescent immunoassay (HemosIL AcuStar HIT-IgG, Instrumentation Laboratory (IL), Milano, Italy) on the ACL ACuStarTM (IL) and total Ab with a latex enhanced immunoturbidimetric assay (HIT-Ab-(PF4-H), IL) on the ACL-TOP (IL). Qualitative concordance of the results obtained with the two assays was explored with the weighted kappa coefficient (K). Concordance with final diagnoses was analyzed when results obtained with the two methods were discordance.
Results: 206 samples tested negative for both methods, 23 samples tested positive for at least one method and 9 samples tested positive for both methods. 14 samples positive for total Ab were negative for IgG Ab. No sample was positive only for IgG Ab. The grade of concordance between the two different assays shows a K of 0.536 which represents a moderate agreement. Concordance with final diagnoses was analyzed when the results obtained with the two methods were discordance, e.g., positive for only one method. The 9 samples positive for both methods were associated with final diagnosis of HIT as well as 4 samples positive only for total. The remaining 10 samples with positivity only for total Ab were not associated with HIT.
Discussion and conclusion: Our study shows that seem not be necessary to perform both assays. The total Ab assay seems to be sufficient as it shows higher sensitivity although at the expense of lower specificity. Further studies are needed to confirm these conclusions.

EP108

THROMBOTIC THROMBOCYTOPENIC PURPURA TREATMENT: ADVANTAGES OF A SHARED PROCEDURE FOR THE PATIENT MANAGEMENT

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a life-threatening pathology characterized by severe deficiency of ADAMTS13 activity which requires rapid diagnosis and whose treatment includes an expensive and invasive procedure, therapeutic plasma exchange (TPE), associated with significant clinical risk for the patient.Our study aims to evaluate how the setup of a specific TTP patient management procedure shared between biochemistry laboratory, immuno-transfusional medicine service and clinical medicine ward pivoted around our on-demand HemosIL® ADAMTS13 Activity assay, affects costs, Turn Around Time (TAT) and helps to reduce clinical risk for the patient in the diagnosis of TTP.Methods: 4 TTP positive patients plasma samples and 8 TTP negative patients plasma samples have been tested to compare costs of ADAMTS13 assay, combined with TPE, performed by an external laboratory and costs of the newly introduced in-house assay. Results: The use of our laboratory ADAMTS13 Activity assay has allowed to reduce waiting times for diagnostical results from 3 days to only 2 hours, thus improving significantly the TAT and lowering the possibility to jeopardize patient's clinical condition with an unnecessary TPE. From a strict economical point of view: sending a negative sample to an external laboratory costs 5463,05€ in total, including first TTP test (350€) and three days of TPE (5113,05€), while the in-house assay for a negative patient costs 800€, saving 4663,05€. It is noteworthy that costs for each positive patient using in-house assay are slightly higher than external laboratory costs (5913,05€, in-house assay vs. 5463,05€, external lab). Nonetheless, this costs are cushioned by the presence of negative patients in our cohort (8 negative patients on a total of 12 patients) allowing us to save 35504,40€ in total.Discussions: Clinical diagnosis of TTP is a challenge due to the lack of specific clinical criteria to unequivocally identify it. In this perspective, setting up a specific TTP patient management procedure and the use of ADAMTS13 Activity as in-house assay proved to be a viable and cost-effective way to quickly assess differential diagnosis between TTP and other thrombotic microangiopathy, allowing a correct treatment of the patients.

EP109

Variabilità inter-individuale della concentrazione plasmatica degli anticoagulanti orali diretti (DOAC) e correlazione con i test di screening della coagulazione e con la funzionalità renale

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Il dosaggio dei DOAC è consigliato nella pratica clinica solo in specifiche condizioni: la loro concentrazione mostra infatti un'elevata variabilità interindividuale, suggerendo quindi una possibile relazione con il rischio trombotico e/o emorragico. A causa del loro meccanismo d'azione i DOAC interferiscono in modo variabile su PT e aPTT.
Scopo. Valutare la variabilità interindividuale della concentrazione plasmatica dei DOAC in pazienti in trattamento e la sua correlazione con PT, aPTT e creatinina.
Metodi. In campioni di plasma citratato di 100 pazienti (70M, 30F, età mediana di 76 anni - range interquartile 63-84 anni) in trattamento con DOAC (29 con edoxaban, 18 con dabigatran, 28 con rivaroxaban, 25 con apixaban) sono stati misurati i livelli plasmatici di dabigatran (test dTI - diluite thrombin time),rivaroxaban, edoxaban ed apixaban (test cromogenico anti-Xa -Werfen) utilizzando specifici calibratori per ciascuno. La creatinina sierica è stata misurata sul Cobas C702 (Roche Diagnostics).La variabilità interindividuale è espressa come CV%.
Risultati. Abbiamo evidenziato un'elevata variabilità interindividuale per tutti i farmaci, non correlata ai livelli di creatinina. I CV% risultano più bassi al picco che a valle per dabigatran (52% al picco e 122% a valle), apixaban (43% al picco e 76% a valle), rivaroxaban (76% al picco e 136% a valle), mentre per edoxaban il CV% è 78% al picco e 47% a valle. In 4/100 pazienti le concentrazioni a valle sono inferiori al limite di rilevabilità. Le concentrazioni di dabigatran e di edoxaban correlano significativamente sia con il PT (rispettivamente r=0.85;p<0,05 e r=0,68;p<0,05) che con aPTT (r=0,76;p<0,05 e r=0,62;p<0,05); le concentrazioni di rivaroxaban mostrano una correlazione significativa con PT (r=0,83) ed una tendenza alla significatività con aPTT (r=0,51;p=0,05).Apixaban esercita un effetto moderato sul PT (r=0,71;p<0,05) mentre non c'è correlazione con l'allungamento dell'aPTT (r=0,36;p=0,08).
Conclusioni. L'elevata variabilità interindividuale riscontrata per tutti i DOAC, anche se valutata in un numero limitato di pazienti, suggerisce, in accordo con recenti dati della letteratura, l'utilità clinica di dosare questi farmaci almeno in pazienti ad elevato rischio trombotico e/o emorragico.

EP110

Interferenza degli anticoagulanti orali diretti (DOAC) sui test per la diagnostica del Lupus Anticoagulant

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I DOAC influenzano in modo variabile i test globali dell'emostasi, ma in modo significativo i test per la diagnostica del Lupus Anticoagulant (LA).
Scopo. Valutare l'interferenza causata dai DOAC sui test per la determinazione del LA (SCT e dRVVT) e dimostrare l'efficacia del carbone attivo nel rimuovere la presenza dei DOAC dai campioni.
Metodi. Sono stati analizzati campioni di plasma citratato di 100 pazienti (70 M, 30F, età mediana di 76 anni-range interquartile 63-84 anni)in trattamento con DOAC (29 con edoxaban,18 con dabigatran,28 con rivaroxaban e 25 con apixaban); il dosaggio dei DOAC e la ricerca del LA sono stati eseguiti sui campioni non trattati e successivamente su quelli pretrattati con DOAC-Stop (Haematex Research). Sono stati misurati i livelli plasmatici di dabigatran (test dTI-diluite thrombin time), rivaroxaban, edoxaban ed apixaban (test cromogenico anti-Xa, Werfen) utilizzando specifici calibratori per ciascuno. La diagnostica del LA è stata eseguita come da linee guida ISTH 2020 utilizzando i due test integrati dRVVT (dilute Russel Viper Venom Time) ed SCT(silica clotting time). La correlazione tra le concentrazioni del farmaco e i valori di SCT e dRVVT è stata calcolata mediante correlazione per ranghi di Spearman .
Risultati.Dopo trattamento con DOAC-Stop le concentrazioni misurate dei farmaci erano al di sotto del limite di quantificazione ad eccezione di un campione di edoxaban che aveva mantenuto lo stesso dosaggio; 15/28 pazienti in terapia con rivaroxaban sono risultati falsi positivi per il dRVVT; 1/25 in terapia con apixaban è risultato falso positivo per il dRVVT; 8/29 pazienti in terapia con edoxaban erano falsi positivi per il dRVVT e 2/18 pazienti in terapia con dabigatran erano falsi positivi per l'SCT.
Conclusioni: L'interferenza dei DOAC sui test del LA può determinare risultati falsi positivi e falsi negativi e di conseguenza una non corretta classificazione dei pazienti nell'iter diagnostico della sindrome da anticorpi antifosfolipidi. Grazie all'esistenza di sostanze assorbenti sarebbe auspicabile che le informazioni relative allo stato di anticoagulazione del paziente fossero inserite nella richiesta per il LA per evitare dispendio di tempo, di costi e soprattutto una errata interpretazione dei risultati di laboratorio.

EP111

Assessment of hemostatic imbalance in elite basketball players before and after a high-intensity exercise

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Introduction: While athletes are commonly considered to have a lower risk of develop venous thromboembolism (VTE) compared to the general population, they are not completely immune to thrombotic events. Hemoconcentration, induced by exertion, immobilization following injuries, frequent long-distance flights, dehydration and use of oral contraceptives in female athletes, are some of sport-related conditions causing an increased hypercoagulability tendency. Exercise can affect the haemostatic system, promoting the formation of thrombus through a temporary increase in coagulation, platelet aggregation and fibrinolytic activity. We evaluated the hemostatic balance in elite basketball players pre and post training, to investigate the coagulation changes occurring during intense sport activity. Methods and Results: Plasma from 12 elite basketball players was collected before and after an intense training session. First level coagulation assay and thrombophilia screening, were performed to evaluate the effect of exercise on the haemostasis. Interestingly, soon after a strenuous physical activity, a significant increase in Factor VIII (FVIII, r.v 50-130%) (median 218.0, IQR 171.5-240.0 vs 104.0, IQR 89.0-118.0; p=0.038); D-Dimer (r.v. 0-500 ng/mL for <50 years), (median 487.0, IQR 356.5-579.0 vs 239.0, IQR 202.0-294.0; p=0.006) and von Willebrand Factor (vWF, r.v 50-120%) (median 145.6, IQR 116.5-174.8 vs 99.7, IQR 91.0-105.9; p=0.021) was observed on post-training plasma compared to pre-training, respectively. Conclusion: Although it is acknowledged that physical activity induces many positive metabolic changes, our data highlighted a possible hemostatic distress during exercise, confirming that acute endurance training can lead to a hypercoagulable state, as is supported by the increased FVIII, D-Dimer and vWF levels. This is likely due to increased venous blood flow directly affecting the vascular walls due to laminar shear stress inducing the release of tissue factor (TF) on its endothelial membrane, resulting in a triggering of blood coagulation. In athletes, this hypercoagulability tendency associated with the acquired and environmental conditions may trigger the transient risk of cardiovascular complications, questioning the safety of strenuous activity.

EP112

The thrombin activation fragment F1+2 assay: possible hypercoagulable state in patients with a history of lupus anticoagulant positivity

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Introduction

It is well known that there is a strong association between increased thrombotic risk and triple positivity, defined by the presence of lupus anticoagulant (LAC) and pathological levels of anti-cardiolipin and anti-ß2-glycoprotein I antibodies (Ab). The search for the F1+2 activation peptide, which results from the cleavage of prothrombin by the prothrombin complex formed by factor Xa, factor V, phospholipids and calcium ions, is promising for the implementation of thrombotic risk assessment.

Aim of the study

The F1+2 fragment assay allows us to indirectly quantify the thrombin formed, identifying itself as an important marker of hypercoagulability and supporting a possible thrombosis condition in addition to triple positivity. For this reason, we have evaluated a possible hypercoagulable state, taking into account the F1+2 factor, which could serve as support for the therapeutic approach in patients with different strength of LAC and high thrombotic risk.

Methods

A total of 115 patients were selected and assessed for aPTT prolongation in relation to LAC tests (DRVVT and SCT), to anti-cardiolipin and anti-ß2-glycoprotein Ab, and divided into mild (PL), moderate (PM) and severe (PF) LAC, including a control population with negative LAC. D-dimer and F1+2 fragment were measured in each patient.

Results and discussion

The mean F1+2 value in the LAC-negative patient group is 225 pmol/L (reference range 70-230 pmol/L) and increases gradually in the PL (235 pmol/L) and PM (288 pmol/L) LAC patient groups. Surprisingly, the F1+2 fragment decreases in the PF LAC group. This is due to the greater use of anticoagulants and antiaggregants in these patients. However, in all categories of LAC positivity, D-dimer remains constant and below the negative predictive value cut-off (<500 ng/ml FEU), confirming the use of antithrombotic treatment.

Thus, the F1+2 fragment assay appears to better follow the trend in the strength of LAC positivity and to be more sensitive to anticoagulant treatment than the D-dimer assay. Therefore, the F1+2 assay could be an additional parameter to assess the strength of LAC and the efficacy of anticoagulant treatment, becoming an important tool in the diagnosis, monitoring and evaluation of acquired and inherited coagulation disorders.

EP113

Strategies for a correct interpretation of the lupus anticoagulant test: need for harmonization

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Thrombophilia, a pro-coagulant condition, can be inherited or acquired. Antiphospholipid syndrome is the highest risk acquired form of thrombophilia, and it is characterized by the presence of Lupus Anticoagulant (LA), beta-2-glycoprotein and anticardiolipin antibodies.
LA assay can be difficult because often patients are taking anticoagulant therapy, such as warfarin, heparin or DOACs (direct-acting oral anticoagulants). They all interfere in LA assay in different ways but the stop of therapy is very difficult and not safe for patients. In this order, trace timing and type of anticoagulant therapy is fundamental for a correct LA test.
The aim is to find strategies for a correct LA test interpretation and to identify interferences from anticoagulants on the test.
An anamnestic form was administered to patients, before collecting samples, to find out which anticoagulant therapy they are taking and the time of the last intake.
For LA detection we used dRVVT and SCT HemosIL™ (Werfen) with respectively screen and confirm reagents. DOAC Stop™ reagent (Werfen) was used to remove specific anticoagulants.
To interpret the results, we created a flow diagram based on literature and documented professionals' experience.
Our proposal of LA flow diagram is based on these steps: assay perform, interpretation of results using information collected on the form, re-test in order to remove interference from anticoagulant.
If the LA test is negative, literature suggests that, if it is intake specific anti-Xa, this can mask a weakly positive LA. In this case treatment with DOAC Stop and revaluation of results are needed.
If the LA test is positive, we have to consider the intake of anticoagulant therapy. If the patient takes anti-Xa the sample is processed with DOAC Stop pretreatment. In case the patient takes Warfarin, the sample is diluted with normal plasma to mitigate the effect of the drug. Removing the interference is not always effective.
The intake of anticoagulant declared in the form allows you to quickly interpret the results and decide whether to repeat the test after adequate treatment of the sample.
The informatic management of the medical history and the interpretative diagram represents the future evolution allowing us to proceed with confidence in managing the results.

EP114

L'intelligenza artificiale nella gestione dei disturbi della coagulazione: diagnosi, trattamento personalizzato, prevenzione e sfide future

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L' intelligenza artificiale (IA) sta rivoluzionando il campo della medicina, in ogni ambito e in campo laboratoristico risulta avere un impatto significativo, tra le altre cose, sulla gestione dei disturbi della coagulazione. L'IA può analizzare grandi volumi di dati clinici e immagini, come tromboelastogrammi e immagini radiologiche, per identificare con maggiore accuratezza e rapidità i disturbi della coagulazione. Algoritmi di machine learning possono inoltre estrarre informazioni da dati genetici e molecolari per classificare i sottotipi di malattia e predire il rischio di eventi tromboembolici o emorragici. L'IA può aiutare a sviluppare piani di trattamento personalizzati per i pazienti con disturbi della coagulazione, considerando fattori individuali come il profilo genetico, lo stile di vita e la storia medica. Questo approccio può ottimizzare l'efficacia dei trattamenti e ridurre al minimo gli effetti collaterali. L'IA può accelerare il processo di scoperta di nuovi farmaci e lo sviluppo di terapie innovative per i disturbi della coagulazione. Algoritmi di intelligenza artificiale possono analizzare grandi dataset per identificare nuovi bersagli molecolari e progettare farmaci più efficaci e sicuri. L'IA può essere utilizzata per prevedere il rischio di eventi tromboembolici o emorragici nei pazienti con disturbi della coagulazione, permettendo interventi preventivi mirati. Questo può tradursi in una riduzione significativa della morbilità e della mortalità. Nonostante il potenziale dell'IA, esistono ancora sfide da affrontare per la sua implementazione diffusa nella gestione dei disturbi della coagulazione. Tra queste, la necessità di dati di alta qualità, la garanzia di trasparenza e interpretabilità dei modelli di IA e la definizione di chiari quadri normativi ed etici. L'intelligenza artificiale ha il potenziale per rivoluzionare la gestione della clinica dei pazienti, tra cui i disturbi della coagulazione, migliorando la diagnosi, il monitoraggio, il trattamento personalizzato, la previsione e la prevenzione di queste patologie. La continua ricerca e sviluppo, unita a una collaborazione tra esperti di medicina e informatica, sono fondamentali per realizzare appieno questo potenziale e migliorare la qualità della vita dei pazienti con disturbi della coagulazione.

EP115

Comparative Analysis of the PFA-100® System (Siemens Medical Solutions, USA) and PL-chip TTAS (Zacros, Japan) to evaluate primary hemostasis defects in Preoperative Patients

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Aim of this study was to evaluate the agreement between Epinephrine (Epi) and Adenosine di-phosphate (ADP) PFA-100® Test Cartridges and a microchip flow chamber system T-TAS PL-chip in Mauriziano hospital preoperative patients. The evaluation sought to assess whether the TTAS PL-chip, a novel in vitro diagnostic system, that passes whole blood through a collagen coated microcapillary bed at arterial shear stress to measure total thrombus formation process, is a reliable alternative to the PFA-100® in primary hemostasis defects investigation.44 consecutive Mauriziano's preoperative patients with PFA-100® and complete blood count (CBC) were analyzed. Patients underwent testing using Epi and ADP Test Cartridges on the PFA-100® and PL-chip on T-TAS. Cut-off values for positivity were set at >118 seconds for ADP and >165 seconds for Epi PFA-100® cartridges, and <260 for PL-chip TTAS. PFA was considered positive if either PFA-100® ADP and/or PFA-100® Epi were positive (PFA Total). Bland-Altman and Pearson statistical analysis were performed to compare PFA-100® and PL-chip tests continuous values, focusing on OT (Occlusion Time), OST (Occlusion Start Time) and AUC PL-chip test results.9/44 patients (20.5%) had thrombocytopenia. Among these, 3 tested positive for PFA Total, and 4 for PL-chip TTAS. ADP PFA-100® and AUC PL-chip concordance was 81.8% (K = 0.650). Epi PFA-100® and AUC PL-chip concordance was 68.2% (K = 0.391), while PFA Total and AUC PL-chip showed 77.3% concordance (K = 0.566).Bland-Altman plots and Pearson correlation analyses demonstrated significant agreement and correlation between OT PL-chip and ADP PFA-100® ($r = 0.85$, $p < 0.001$) and Epi PFA-100® ($r = 0.78$, $p < 0.001$) and OST PL-chip and ADP PFA-100® ($r = 0.82$, $p < 0.001$) and Epi PFA-100® ($r = 0.75$, $p < 0.001$). TTAS PL-chip showed a high degree of agreement with the PFA-100® system, particularly with the ADP cartridge. This suggests that the PL-chip TTAS is a reliable alternative to evaluate impaired primary hemostatic function, for preoperative screening. Its comparability with the PFA-100® highlights its potential to provide enhanced diagnostic capabilities.

EP116

Appropriatezza diagnostica nella richiesta del test LAC: esperienza del laboratorio di Lucca

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La sindrome da anticorpi antifosfolipidi (APS) è un raro disordine autoimmune associato a trombosi arteriosa e venosa, aborti ricorrenti, trombocitopenia, microangiopatia trombotica. La diagnosi di APS richiede almeno un criterio clinico e la presenza persistente di anticorpi anti-fosfolipidi: lupus anticoagulant (LAC) e/o anticorpi anticardiolipina e/o anti ß2 glicoproteina I (confermati a distanza di 12 settimane). Secondo le linee guida ISTH il test del LAC dovrebbe essere eseguito solo in caso di alta probabilità di APS o nei soggetti con APTT allungato, per evitare dei falsi positivi a causa della scarsa specificità del test. Per verificare l'appropriatezza delle richieste di LAC pervenute nel nostro laboratorio, siamo andati a rivedere tutti i risultati ottenuti dal 1 gennaio 2024 ad oggi. L'esperienza del nostro laboratorio mostra la difficoltà di interpretazione dei risultati del test LAC. Abbiamo osservato che su 354 test eseguiti, ben l'80% delle richieste ricevute mancava di indicazione clinica, e nel 40% il LAC era richiesto senza altri esami coagulativi. Tra i 51 campioni risultati positivi: il 37% risultava fortemente o moderatamente positivo, mentre il 63% dei campioni mostrava una debole positività. Tra questi ultimi: il 70% presentavano APTT normale, e il 30% non aveva la richiesta di APTT. Per verificare la presenza di eventuali sostanze interferenti con il test del LAC, abbiamo trattato tutti i campioni debolmente positivi, con il reattivo doac stop, il quale ha la funzione di far precipitare il farmaco DOAC se presente nel campione. La ripetizione dei test del LAC dopo il trattamento, ha confermato la presenza dei DOAC nel 40% dei campioni. Abbiamo inoltre eseguito il dosaggio del tempo di trombina per escludere la presenza di eparina non frazionata nei pazienti interni. Per 5 pazienti esterni abbiamo contattato il medico curante. E' emerso che nel 100% dei casi era stato richiesto il dosaggio del LAC in pazienti che non avevano sospeso il trattamento prima di eseguire il prelievo. In conclusione dai dati mostrati nella nostra esperienza, emerge l'importanza dell'appropriatezza prescrittiva nel fornire una risposta corretta per il test LAC, ed evitare risultati falsamente positivi, che potrebbero sottoporre il paziente ad una terapia anticoagulante non necessaria, o alla negazione di interventi chirurgici per il possibile rischio pro trombotico legato alla positività al LAC.

EP117

Studio di comparazione tra misurazione meccanica viscosimetrica e ottica turbidimetrica nella rilevazione di parametri coagulativi

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In ambito di test coagulativi è sempre maggiore la richiesta di eseguire dosaggi analitici rapidi ed affidabili a scopo di screening, di monitoraggio terapeutico e per la diagnosi di coaugulopatie. Tale bisogno porta alla necessità di valutare sistemi analitici altamente automatizzati e integrabili in flussi operativi ad elevata automazione. Presso l'Ospedale San Raffaele di Milano sono stati confrontati due modelli strumentali, Stago STA-R Max con principio analitico meccanico-viscosimetrico, e Roche cobas t711 con sistema ottico-turbidimetrico, per valutare parametri di coagulazione quali tempo di protrombina (PT), tempo di tromboplastina parziale attivata (aPTT), fibrinogeno (FG), D-dimero (XDP) e antitrombina (ATIII).

La valutazione comparativa è stata eseguita con campioni di donatori sani (n=61), campioni dosati in routine ospedaliera (n=438) e campioni in TAO (n=102). I campioni sono stati raccolti in provette addizionate con Na Citrato 0.109M e centrifugati a 2500g per 10 minuti. Il PT è stato testato con STA-NeoPTimal e PTRec in 601 campioni (61+438+102) e il aPTT con STA-CKPrest e aPTTScreen in 499 campioni (61+438). I valori dei donatori sono stati usati per il calcolo di MN-PT, MN-aPTT, PT-ratio, aPTT-ratio e INR. Fibrinogeno, XDP e ATIII sono stati testati rispettivamente in 194, 93 e 79 campioni della routine. La valutazione statistica è stata eseguita mediante regressione lineare e con i test di Cohen Kappa (CK) e Kendall Tau-B (KTB).

I risultati ottenuti hanno evidenziato una buona correlazione per tutti gli analiti nelle relative popolazioni analizzate (r>0.91) tranne per aPTT-ratio apparsa discreta nei donatori (r=0.65) e nei campioni di routine (r=0.87). I coefficienti CK e KTB di concordanza erano discreti per PT-ratio MN-PT (0.50±0.04 CK, 0.56±0.03 KTB) e aPTT MN-aPTT (0.53±0.06 CK, 0.59±0.05 KTB), buoni per INR MN-PT (0.69±0.08 CK, 0.73±0.06 KTB) e ottimi per FG, XDP e ATIII (FG 0.80±0.04 CK, 0.90±0.02 KTB; XDP 0.87±0.04 CK, 0.92±0.03 KTB; AT 0.88 ± 0.05 CK, 0.90 ± 0.04 KTB).

In base ai risultati ottenuti si conclude che la tecnologia ottica-turbidimetrica mostra una soddisfacente correlazione per i test oggetto di valutazione rispetto a quella meccanica-viscosimetrica in uso. Le correlazioni sono elevate per PT, FG, XDP e ATIII e minori per aPTT. I risultati da noi ottenuti sono in linea con quelli della letteratura.

EP118

The etiopathogenesis of Acquired von Willebrand Syndrome in patient with MGUS highlighted from Pharmacokinetic studies

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Introduction: Acquired von Willebrand Syndrome (AVWS) is a rare disorder characterized by a bleeding diathesis, with symptoms ranging from mild to severe. It differs from the congenital von Willebrand disease (vWD) due to the absence of personal and family history of hemorrhagic disorders, a late onset bleeding tendency and the frequent association with hematological malignancies, autoimmune or cardiovascular diseases. Monoclonal gammopathy of undetermined significance (MGUS) is the most frequently reported condition associated with AVWS. Laboratory investigations can be challenging and discerning between neutralizing antibodies, able to inhibit the von Willebrand Factor (VWF) activity and those forming immunocomplexes with the VWF, is crucial since neutralizing antibodies are associated with a more severe bleeding pattern. Here we report a case of a 72-year-old female, with no previous bleeding diathesis, suffering from MGUS IgG and hemorrhagic episodes following a robotic cholecystectomy. Methods and Results: First level hemostasis assays highlighted a prolongation of activated Partial Thromboplastin Time (aPTT, ratio 1.49), with normal Prothrombin Time (PT) and Fibrinogen levels. Mixing assay for aPTT excluded the presence of inhibitors, suggesting a factor deficiency. The intrinsic pathway coagulation factors level were all normal, except for low Factor VIII level (FVIII) (17%), while VWF:Antigen (VWF:Ag), VWF: Ristocetin Cofactor (VWF:RiCof) and VWF:Collagen Binding (VWF:CB) showed a substantial reduction (respectively 15.5%, 9.6% and 11.1%). Following these results and due to the patient's personal and family history, the AVWS was strongly suspected. Post-infusion pharmacokinetic studies at 1 hour, 4 hours, 6 hours and 24 hours after FVIII/VWF concentrates administration and two days/time IVIG infusion, confirmed the presence of neutralizing antibodies and those accelerating VWF plasma clearance. Conclusion: This report confirmed the effectiveness of IVIG treatment in patients with MGUS IgG and highlighted how mixing and pharmacokinetic studies can play an important role in the identification of anti-VWF antibodies etiopathogenesis, thus confirming the presence of non-neutralizing antibodies even when an ELISA test is not available.

EP119
Piastrine grigie sullo striscio di sangue periferico

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Il caso riguarda una paziente di 58 anni con polmonite batterica trattata con piperacillina-tazobactam; l'esame emocromocitometrico eseguito su un campione di sangue intero raccolto in K2-EDTA (EDTA) su XN9100 (Sysmex, Kobe, Giappone) mostrava riduzioni numeriche delle piastrine senza alterazioni nei citogrammi relativi alle popolazioni leucocitarie ed eritrocitaria. La piastrinopenia mostrava una variabilità tra 110 a 30 x109/L con segnalazione di aggregati piastrinici confermati mediante esame microscopico. All'osservazione microscopica dello striscio di sangue periferico con colorazione May-Grunwald e Giemsa si evidenziava la presenza di elementi piastrinici agranulati, di colore grigio pallido e di volume aumentato nei campioni di sangue con segnalazione di aggregati piastrinici raccolti in EDTA. Ad una ulteriore verifica con doppio prelievo in EDTA e in sodio-citrato per il conteggio delle piastrine e valutazione morfologia piastrinica, lo striscio in EDTA ha confermato la presenza di piastrine degranulate anche nei giorni successivi. Il campione raccolto in sodio citrato ha mostrato solo la presenza di aggregati piastrinici con morfologia piastrinica normale. L'integrazione dei dati ottenuti, sia bibliografici che relativi agli esami di laboratorio eseguiti sulla paziente, poneva il sospetto della coesistenza di due condizioni: la sindrome piastrinica pseudo grigia indotta da EDTA (PGSP) e l'agglutinazione piastrinica citrato/ EDTA dipendente. La PGSP indotta da EDTA è una condizione rara e scarsamente conosciuta, è un fenomeno in vitro, che sembra essere mediato da un anticorpo che, in presenza di EDTA, induce il rilascio del contenuto dei granuli alfa e delta-piastrinici, dunque non associato ad alterazione della funzionalità piastrinica confermata con il test PFA-200. In conclusione, l'osservazione di diversi strisci di sangue periferico eseguiti con due diversi anticoagulanti (EDTA, sodio citrato) ci ha permesso di identificare correttamente una condizione innocua, distinguendola dalla vera sindrome piastrina grigia, un disturbo molto più grave.

EP120
Evaluation of acceptability of hemolysis cut-offs proposed on Werfen ACL-TOP 750 CTS

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Introduction. Hemolysis is one of the most common causes of preanalytical non-conformity. In vitro hemolysis can result from improper sample collection, such as poor venous access, excessive shaking of the blood specimen, or inadequate sample transportation or storage. The ACL TOP 750 CTS (Instrumentation Laboratory, Bedford, CA, USA) automated hemostasis testing systems performs hemolysis checks by measuring optical absorbance to determine a range of hemoglobin concentrations. The aim of our study was to evaluate whether the limits proposed by the manufacturer are acceptable for routine analysis by hemolyzing manually erythrocytes rather than adding free hemoglobin, which should not interfere with coagulation methods.
Methods. On 10 citrated plasma samples referred to the laboratory we conducted an initial analysis of the samples (baseline). Subsequently, using 1mL insulin syringes with a G25X needle we mechanically hemolyzed the samples and repeated the tests. The hemolysis and processing process was repeated 4 times, during each of which 10 U of the cellular part of the blood from centrifuged tubes was withdrawn via syringe for 4 repetitions. We executed as tests: prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin activity (AT), D-dimer (DD), and fibrinogen (Clauss method), on ACL TOP 750 CTS. To evaluate clinically significant differences, we compared the initial result for each analyte to the hemolyzed results at various levels using reference change values (RCVs) calculated with the tool available on BiologicalVariation.eu.
Results. In the initial run without induced mechanical hemolysis, the H index ranged from 28 mg/dL to 104 mg/dL. By the third run, it increased to a range of 60-282 mg/dL. In the fourth run, one sample showed an H index of 457 mg/dL, while the remaining nine samples exceeded 900 mg/dL. Until the third run, all parameters exhibited variations lower than their respective reference change values (RCVs) in both increase and decrease, and the instrument successfully provided results in all cases.
Conclusion. The cut-off values provided by the manufacturer are accurate, and hemolysis interference did not generate altered results in the principal coagulation tests, except at extremely high levels of hemolysis.

EP121

Respiratory virus and SARS-CoV-2 virus circulation in the provinces of Lucca and Massa Carrara during the 2022/2023 and 2023/2024 flu seasons

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Introduction: Influenza epidemics remain a serious public health problem and a significant source of direct and indirect costs both for the implementation of control measures and for the management of cases and complications of the disease. Older people, young children, pregnant women and people with chronic diseases are the groups at greatest risk for developing severe forms, but the entire population can be subject to significant complications, including pneumonia, myocarditis and encephalitis, which can result in unfavorable outcomes. In the 2022/2023 influenza season the majority of infections were caused by the A/H3N2 influenza virus, while the A/H1N1pdm09 influenza virus was attributed to influenza infections in the 2023/2024 season. The aim of our study is to analyze and compare the circulation of influenza viruses and the SARS-CoV-2 virus in the 2022/2023 and 2023/2024 influenza seasons received at the Lucca Analysis Laboratory. Materials and methods: A retrospective single-center observational study was conducted of adult and pediatric patients who accessed hospital facilities in the province of Lucca in the influenza periods from September to April 2022 and 2023 and from September 2023 to April 2024. The subjects of the study were selected taking into account the provisions issued by the General Directorate of Health Prevention of the Ministry of Health for the 2023/2024 season. Results: The 2022/2023 influenza season was characterized by the circulation of the A/H3N2 influenza virus with a peak of positive samples from week 42/2022 to week 52/2022. The influenza A/(H1N1)pdm09 virus is responsible for influenza infections in the 2023/2024 season with a peak of positive samples between 52/2023 and 3/2024 weeks. Conclusions: To contain annual influenza epidemics, it is important to activate global surveillance programs to detect virological, epidemiological and clinical changes associated with circulating influenza viruses. The evaluation of the relationships between the vaccination status of the population and the viruses circulating in specific geographical areas becomes an interesting target in light of the new IT availability, for an effective management of epidemiological surveillance programs of the Italian population.

EP122

Frequency and predictors of shoulder pain related to vaccine administration after BNT162b2 bivalent vaccination

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Background: Shoulder pain after intramuscular vaccination has a rather heterogeneous prevalence, depending on many factors such as type of vaccine, injection modality, arbitrary sensation and possible onset of complications. In order to garner additional information on this important aspect insubjects receiving the BioNTech/Pfizer BNT162b2 bivalent formulation (ancestral-BA.4/5), we conducted a standardized study to define the prevalence and correlates of shoulder pain after administration of this vaccine.Methods: The study population consisted of 68 healthcare workers (45±13 years of age; 52.9% men; body mass index: 25.3±4.1 kg/m2, 91.2% had received at least three previous vaccine doses, and 38.2% reported never having been infected with SARS-CoV-2) from Peschiera del Garda Hospital (Verona, Italy). All recruited subjects received an intramuscular administration of the bivalent booster vaccine BNT162b2. One month after vaccine administration, a specific questionnaire was administered to all vaccinated subjects to assess post-vaccination side-effects. Statistical analysis was performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK). All subjects gave written informed consent to participate in the survey. The study was conducted in accordance with the Declaration of Helsinki, and its protocol was approved by the Ethics Committee of the Provinces of Verona and Rovigo (59COVIDCESC; November 8, 2021).Results: Overall, the rate of patients reporting shoulder pain within 48 hour after administration of the bivalent vaccine BNT162b2 was 66.2% (45/68). In multivariate linear regression analysis, shoulder pain was not associated with any of the variables tested, thus including sex (p=0.284), age (p=0.723), body mass index (p=0.159), number of prior vaccine doses (p=0.941), and number of prior SARS-CoV-2 infections (p=0.487).Conclusion: The results of this epidemiologic survey suggest that the incidence of shoulder pain after administration of a booster dose of the bivalent vaccine BNT162b2 was consistently high, but no significant clinical or demographic predictors of occurrence could be identified.

EP123

Valutazione del programma di screening per la ricerca del virus dell'epatite C (HCV) nella AST Pesaro-Urbino

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BACKGROUND

L'infezione da virus dell'epatite C (HCV) è la principale causa di malattia cronica del fegato nel mondo. In Italia si stima che circa l'1% della popolazione conviva con una forma cronica di epatite C. Nel mese di luglio del 2023 è stata attivata in tutte le Aziende Sanitarie Territoriali (AST) della Regione Marche, la campagna di screening promossa dal Ministero della Salute, per l'eliminazione del virus HCV responsabile dell'epatite C. Lo screening rivolto alla popolazione iscritta all'anagrafe sanitaria nata dal 1969 al 1989, è completamente gratuito e prevede un prelievo di sangue venoso su cui effettuare l'esame sierologico per la ricerca degli anticorpi anti-HCV. Se positivo, si procederà all'esecuzione del test molecolare (HCV-RNA). In caso di conferma il paziente sarà indirizzato alla struttura più idonea per ulteriori indagini.

OBIETTIVO DELLO STUDIO

L'obiettivo è quello di estendere il programma di screening ad una popolazione sempre più ampia al fine di aumentare il numero di casi diagnosticati e, soprattutto, di raggiungere i soggetti inconsapevoli dell'infezione che rappresentano il serbatoio per la trasmissione del virus. In assenza di un vaccino efficace, l'eliminazione del virus può essere ottenuta solo attraverso trattamento con i nuovi farmaci antivirali ad azione diretta (DAA) in grado di ridurre completamente la carica virale. Un programma di screening efficace permetterà di individuare le infezioni ancora non conosciute, il cosiddetto "sommerso" al fine di aumentare la possibilità di una diagnosi precoce, avviare subito i pazienti al trattamento farmacologico per evitare complicazioni maggiori ed interrompere la circolazione del virus impedendo nuove infezioni.

MATERIALI E METODI

Lo screening è stato realizzato mediante l'utilizzo del test LIAISON XL MUREX HCV-Ab (DIASORIN), con tecnologia in chemiluminescenza (CLIA) per la determinazione degli anticorpi contro il virus HCV in campioni di siero o plasma umano. Il test di approfondimento (HCV-RNA) è stato realizzato mediante utilizzo del test DUAL PROBE per la determinazione della carica virale dell'HCV su COBAS 6800 (ROCHE).

RISULTATI

Lo screening, condotto nella AST Pesaro-Urbino tra agosto 2023 e maggio 2024, ha coinvolto 5073 soggetti, di cui 2068 (41%) maschi e 3005 (59%) femmine. Dei 5073 soggetti sottoposti al test, 46 (23 maschi e 23 femmine) sono risultati debolmente positivi. Tuttavia, in solo 5 soggetti (3 maschi e 2 femmine) di questi è stata confermata la positività attraverso il test di approfondimento (HCV-RNA). Questo ha permesso di ottenere una prevalenza dell'infezione viremica di circa 0,1%

CONCLUSIONI

I risultati ottenuti, nonostante siano relativi ad un periodo di tempo non troppo ampio, indicano che il programma di screening ha permesso di avere un'aggiornamento dell'infezione da HCV nella AST Pesaro-Urbino. I dati indicano una bassa prevalenza di infezione (0,1%)

EP124

Prevalenza dell'infezione da Epatite C in una popolazione di pazienti oncologici

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INTRODUZIONE-SCOPO

Il virus dell'epatite C (HCV) è un virus a RNA che infetta circa 170 milioni di persone nel mondo rappresentando una delle principali cause di epatite cronica, cirrosi e carcinoma epatocellulare. L'obiettivo di questo studio è stato quello di valutare la prevalenza di HCV in una popolazione di pazienti oncologici.

METODI

Da giugno 2021 a giugno 2024 sono stati analizzati campioni di sangue di pazienti afferenti all'Istituto Nazionale dei Tumori IRCCS "Fondazione G. Pascale". I campioni sono stati testati per la ricerca degli anticorpi anti-HCV mediante l'utilizzo del test immunologico in elettrochemiluminescenza (ECLIA) su strumentazione Cobas e801 (Roche). Tutti i campioni risultati positivi sono stati successivamente analizzati mediante tecnica Real Time RT-PCR per la rilevazione e quantificazione dell'RNA del virus con kit RealTime HCV (Abbott) su strumentazione m2000.

RISULTATI

Nel periodo di osservazione sono stati analizzati 27.535 campioni. Sono risultati positivi al test ECLIA 988 campioni con una prevalenza del 3,6%: 224 (4,1%) nel 2021, 329 (3,7%) nel 2022, 279 nel 2023 (3,2%) e 155 (3,5%) nel 2024. 49 di questi campioni sono risultati positivi all'analisi molecolare per la ricerca dell'HCV RNA. La prevalenza di HCV è risultata essere maggiore all'aumentare dell'età (età media di soggetti negativi 57,59±16,10 vs età media di quelli positivi 70,27±11,08, $p < 0,0001$), e maggiore negli uomini rispetto alle donne (4,3% vs 3,1%, $p < 0,0001$). Per verificare il peso di queste variabili, è stata eseguita una regressione logistica binaria che ha mostrato come unica variabile significativa l'età (OR 1,066, IC 1,060 – 1,072, $p < 0,0001$).

CONCLUSIONI

Dal nostro studio si conferma, anche per la popolazione oncologica, che i pazienti in età avanzata presentano un rischio maggiore di sviluppare un'infezione cronica da HCV. È quindi di fondamentale importanza rilevare precocemente le infezioni per avviare i pazienti al trattamento antivirale ed evitare le complicanze di una malattia epatica avanzata. I pazienti oncologici con pregresse infezioni da HCV dovrebbero, inoltre, essere sottoposti ad un attento monitoraggio in quanto esposti ad un maggiore rischio di epatotossicità e riattivazione virale durante il trattamento antitumorale.

EP125

Infezione da BK polyomavirus in pazienti sottoposti a trapianto di rene

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Il BK polyomavirus (BKV) è un virus ubiquitario responsabile di infezioni spesso asintomatiche con sieroprevalenza superiore al 90% nella popolazione adulta. Dopo l'infezione primaria, BKV persiste principalmente nell'urotelio e nelle cellule tubulari ma nei pazienti trapiantati di rene può riattivarsi causando gravi complicazioni come la nefropatia associata a BKV che può esitare anche nel rigetto dell'organo. Il virus è rilevabile inizialmente nelle urine, successivamente nel sangue: l'assenza di BKV nelle urine ha un valore predittivo negativo del 100%. I centri presso cui vengono seguiti i pazienti trapiantati di rene, adottano protocolli di screening diversi, alcuni monitorando viruria e viremia, altri solo la viremia, sottostimando così la replicazione del virus nella sua fase iniziale a livello urinario: infatti quasi l'80% dei pazienti con urine positive può sviluppare una viremia, condizione severa che richiede un intervento terapeutico immediato. Utilizzando una RT-PCR quali/quantitativa, abbiamo condotto uno studio prospettico su 57 pazienti trapiantati, monitorando la carica di BKV su urine e sangue presso il Laboratorio Analisi della ASST MELEGNANO E MARTESANA nel periodo di gennaio 2023-giugno 2024. Il gruppo di 57 pazienti era costituito da 34 uomini (età media 60,6 anni) e 23 donne (età media 62,7 anni). 17 pazienti avevano positività solo nelle urine, 3 pazienti solo nei campioni di sangue, 4 pazienti sia nel sangue che urine. 8 pazienti avevano un'elevata carica virale nelle urine ma nessuno aveva una viremia alta (superiore a 10.000 copie/ml), dato quantitativo da tenere in considerazione poiché l'eliminazione di BKV dopo la riduzione dell'immunosoppressione è più facile quando la carica virale nel sangue è <10.000 copie/ml. Per alcuni pazienti è stato analizzato il sedimento urinario alla ricerca di decoy cells, ma solo per un paziente è stato possibile osservarle.

Lo screening post-trapianto che comprenda la rilevazione nel sangue e nelle urine del virus BK, l'osservazione delle decoy cells, è di fondamentale importanza nel paziente trapiantato per anticipare la diagnosi di nefropatia e iniziare il trattamento precoce con la riduzione del regime immunosoppressivo e la terapia antivirale.

EP126

SCREENING DI POPOLAZIONE PER INDIVIDUARE INFEZIONI DA VIRUS DELL'EPATITE C (HCV): I PRIMI SEI MESI DELL'AST - ASCOLI PICENO

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Premessa: per raggiungere l'obiettivo di eliminare il virus dell'epatite C entro l'anno 2023 (WHO Hepatitis Strategy) è stato organizzato lo screening di popolazione, attualmente rivolto alla coorte dei nati tra il 1969 e il 1989, per identificare e trattare la quota di casi non noti di infezione.

Metodi: la ricerca degli anticorpi anti – HCV è stata effettuata con una singola determinazione, utilizzando un metodo immunochemiluminescente (CLIA) automatizzato su strumenti Liaison® XL (Diasorin, Saluggia VC). Sono stati classificati come "non reattivi" i campioni in cui il rapporto, tra il segnale analitico ottenuto e quello misurato per un calibratore corrispondente al livello soglia (indice), sia risultato inf. a 1,0. Nei campioni risultati positivi alla ricerca degli anticorpi, con valore dell'indice pari o superiore a 1,00, è stata ricercata la presenza del virus dell'epatite C mediante un test molecolare, basato su tecnica di real-time PCR (Cobas®HCV, Roche Diagnostics CH).

Risultati: AST di Ascoli Piceno, nei primi 6 mesi di attivazione dello screening, ha invitato 30.227 soggetti, 2.710 (9,0%) hanno aderito, 2.096 donne (77,3%) e 614 uomini (22,7%) con età media di 46,2 ± DS 6,17 anni. Lo screening sierologico, relativo alla coorte dei soggetti valutati, ha evidenziato la positività nell'1,0 % dei casi, in linea con i dati nazionali riportati in letteratura.Tra i 27 pazienti risultati positivi alla ricerca di anticorpi, 15 sono risultati debolmente positivi (Indice compreso tra 1,1 e 12,0 e nessuno di essi ha mostrato positività per la ricerca di HCV RNA, mentre in 4 casi (14,8%) il genoma virale è stato rilevato e quantificato (media 2.123.250 UI/mL ± DS 2.107.739).

Conclusioni: dai dati ottenuti, in considerazione della percentuale di campioni risultati positivi per la ricerca di anticorpi anti-HCV, si evidenzia che la sensibilità del metodo in uso risulta adeguata per la finalità dello screening. La conferma dei campioni positivi con metodo molecolare consente l'immediata individuazione dei pazienti che devono essere avviati al trattamento antivirale.Una maggiore efficacia dello screening può essere incrementata con una maggiore sensibilizzazione della popolazione per raggiungere una percentuale più elevata di adesione che, al momento, risulta limitata.

EP127

IL RUOLO PREDITTIVO DELL'ADRENOMEDULLINA E DELLA COPEPTINA NELLE ENDOCARDITI INFETTIVE

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Background: L'Endocardite Infettiva (EI) è un processo flogistico a carico dell'endocardio sostenuto da agenti microbici ad elevato rischio di morbilità e mortalità¹. Nella pratica clinica, l'utilizzo di biomarcatori specifici in grado di monitorare l'andamento della EI e la sua possibile evoluzione risulta di grande utilità per il clinico. Nel moderno approccio diagnostico, oltre al dosaggio di Proteina C Reattiva (PCR) e Procalcitonina (PCT), è stato proposto il dosaggio della MR-proAdrenomedullina (MR-proADM) e della Copeptina (COOP). Obiettivo dello studio: Il nostro studio retrospettivo osservazionale ha analizzato il possibile ruolo dei valori di COOP e MR-proADM in pazienti con EI definita, al fine di valutare il possibile significato prognostico dei marcatori utilizzati e il loro eventuale contributo al monitoraggio della progressione della malattia. Metodi: Lo studio ha arruolato 196 pazienti afferenti presso l'Unità di Medicina Infettiva e dei Trapianti presso l'AORN Monaldi di Napoli, nell'intervallo di tempo compreso tra 2007 e 2019. L'età media dei pazienti in esame era pari a 62,3 anni di cui 139 maschi e 57 femmine. Tutti sono stati sottoposti alla raccolta dei dati clinici anamnestici ed ecocardiografici al ricovero (T0) e ad un prelievo ematico. Risultati: L'analisi dei dati ha mostrato valori medi di PCR (6.1 mg/dL) e di PCT (0.72 μg/L). È risultato evidente che i pazienti che mostravano valori più elevati di ADM al T0 (2.3 nmol/L) presentavano un maggiore rischio di sviluppare una compromissione multiorgano a breve termine, rispetto a quelli con valori al T0 più bassi. Quelli con valori più elevati di COOP al T0 (12.5 pmol/L) presentavano un maggiore rischio di sviluppare un evento avverso entro 1 anno, rispetto ai pazienti con valori al T0 più bassi.Conclusioni: il dosaggio della ADM e della COOP, in pazienti affetti da EI, arricchisce la valutazione del quadro clinico del paziente, proponendosi come validi marcatori prognostici. Bibliografia: 1. Murdoch et al. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the international collaboration on endocarditis prospective cohort study. Arch Intern Med. 2009.

EP128

Evaluation of MR-proADM and MDW for early diagnosis of sepsis

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Background

Sepsis early diagnosis is still a concern because no specific gold-standard diagnostic test exists. Clinical criteria are used for diagnosis but laboratory biomarkers as CRP and PCT are traditionally request. Few laboratories measure MR-proADM (Mid-Regional proadrenomedullin) and/or "new" parameter MDW (Monocyte Distribution Width). The aim of this study is to compare MR-proADM and MDW with CRP and PCT to evaluate their usefulness to predict sepsis in patients in Intensive Care Units (ICU).

Methods

Sepsis risk of 32 selected patients was evaluated with SOFA score, temperature >38°C, white blood cell count and positive culture. After complete blood count (CBC), EDTA plasma samples with MDW≥19 (detected 48 and 24 h before and after the positive culture: t-2, t-1, t+1, t+2) were collected and centrifugated to measure CRP, PCT and MR-proADM; 20 patients were excluded, 12 patients (3 females and 9 males, mean age 63±18) were included with a total of 66 collected samples. CBC and CRP were analyzed respectively on DxH900 and AU5800 with immunoturbidimetric method (Beckman Coulter); PCT and MR-proADM were measured using CLIA methods on Liaison XL (DiaSorin).

Results

CRP resulted positive in all sample (mean 12,2±8,2 mg/dL); 9 patients had a negative PCT at time t-2, 3 patients resulted positive. In 4 patients MR-proADM values at time t-2 were <0,87 nmol/L, in 8 patients were ≥0,87 nmol/L and subsequently showed a positive culture; 4 patients showed an increase of MR-proADM and MDW levels (3/4 deceased), 2 patients a decrease of MR-proADM and MDW, 1 a gradual increase of MR-proADM and a decrease of MDW (deceased) and 1 showed opposite results. Mortality rate was 50%. Principal Component Analysis showed that the principal component was MR-proADM. The outcome was evaluated by calculating ROC curve with statistical significance ($p<0.05$) for MR-proADM cutoff 0,79 nmol/L (sensitivity and specificity 71% and 64% respectively).

Conclusion

The small number of patients is a study limit. CRP is a non-specific biomarker and PCT may be used for proper antibiotic stewardship. MR-proADM reflects endothelial damage and predicts sepsis 24-48 hours before the MDW cut-off of 20 that have lower predictive value.

EP129

Presepsin: An Essential Biomarker for Early Sepsis Diagnosis and Treatment in the Emergency Department

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Presepsin (sCD14-ST) is a soluble N-terminal fragment of the CD14 differentiation marker protein cluster, which is a soluble form of the lipopolysaccharide (LPS) receptor, a member of the of toll-like receptors that recognize pathogen-associated molecular patterns (PAMPs) and initiate the innate immune response. This protein is directly implicated in the pathogenesis of sepsis in an early stage of interaction between the immune system and the pathogen. Because presepsin (P-SEP) is elevated early during sepsis, it may be useful in the clinical setting as a biomarker for diagnosis and risk stratification of patients suspected of being septic. In this study, we assessed the diagnostic role of P-SEP in the early detection of sepsis and compare it with current available infection biomarkers.
METHODS We assessed a total of 43 consecutive patients who presented to the emergency room with suspected sepsis. P-SEP, CRP, and PCT (BRAHMS) levels were measured in K3EDTA whole blood using Pathfast (POCT). Additionally, plasma samples from the same patients were collected and analyzed with DXI 800 Beckman Coulter for the determination of PCT and CRP. All samples were measured at admission (T0-suspected sepsis at triage) and for 28 patients also T1 (12 hours), and T2 (72 hours).
RESULTS All three biomarkers showed a statistically significant increase in different cut-off intervals (suspected, positive) during the POCT test. P-SEP values additionally, the PCT and CRP values measured by DXI demonstrated overlap with those obtained through POCT. Our results confirmed a strong agreement between the PCT and CRP values measured with Pathfast and those obtained using the Beckman Coulter DXI analyzer. We also used P-SEP as a marker of sepsis, togheter with clinical scores, PCR e PCT, to decide on hospitalization or discharge after the first ED visit. We measuring the rate of rehospitalization to 72 h to prove the safety of this approach.
CONCLUSIONS For the early diagnosis and treatment of sepsis, P-SEP appears to be a sensitive, specific, early, and prognostic biomarker, proving to be superior to other types of markers and, therefore, a valuable tool for ruling in or ruling out sepsis.

EP130

UTILIZZO DELLA PRESEPSINA IN PRONTO SOCCORSO PER LA DIAGNOSI PRECOCE DI SEPSI

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La sepsi è una disfunzione organica conseguente a una risposta sregolata dell'organismo all'infezione; è una condizione frequente, potenzialmente letale e tempo-dipendente; pertanto, la tempestività della diagnosi rappresenta un fattore di vitale importanza affinché le terapie risultino efficaci. Ad oggi, la diagnosi di sepsi è basata su parametri clinici non specifici per questa condizione. La presepsina è un frammento della forma solubile del CD14 immesso in circolo dalle cellule della linea monocitaria in risposta all'infezione, con cinetica più rapida rispetto ai biomarcatori in uso nella pratica clinica come la procalcitonina (PCT).In questo studio abbiamo voluto valutare il potenziale ruolo della presepsina nella diagnosi precoce di sepsi in pazienti adulti afferenti al DEA della Fondazione Policlinico Universitario A Gemelli.Sono stati arruolati 172 pazienti (M 103) con sintomi suggestivi di sepsi, ai quali oltre ai test di laboratorio di routine secondo linee guida utilizzate nel nostro Policlinico in questi casi, era effettuato un prelievo aggiuntivo in K3-EDTA per il dosaggio della presepsina. Dopo centrifugazione i campioni sono stati aliquotati e stoccati a -80°C fino al momento del dosaggio quantitativo eseguito in immunofluorescenza enzimatica mediante TOSOH AIA-360 Automated Immunoassay Analyzer, nel Corelab Alta Automazione del nostro Policlinico.I risultati della presepsina sono stati analizzati retrospettivamente, in rapporto alle diagnosi di dimissione ed ai dati clinici e di laboratorio, in particolare di PCT e proteina C reattiva. La presepsina ha dimostrato una specificità migliore (76%) della PCT (54%) nella diagnosi di sepsi, anche se con sensibilità minore (57% vs 68%), suggerendo la complementarità dei due marcatori. Nei pazienti con PCT negativa, una presepsina positiva ha identificato i pazienti settici con una specificità del 91%; probabilmente grazie alla cinetica più rapida della presepsina ed alla sua capacità di incrementare anche in infezioni atipiche. Dallo studio è emerso che la presepsina permetterebbe un incremento dell'11% nelle diagnosi di sepsi, rispetto al solo utilizzo della procalcitonina.

EP131

NEW TESTS FOR DIAGNOSTIC IMPROVEMENT IN AUTOIMMUNE BULLOUS DISEASES: ANALYSIS OF REQUESTS AND RELATED RESULTS IN A COHORT OF PATIENTS WITH SUSPECTED DBS

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Background Autoimmune blistering dermatoses(ABD)are a heterogeneous group of rare diseases clinically characterized by erosions and blisters on the skin.In ABD the immune system produces autoantibodies(Abs)directed against cell-cell or cell-matrix adhesion molecules.More common ABD entities, pemphigus and pemphigoid,are identified by circulating and tissue-bound Abs against the desmosomal cadherins (DSG1 and DSG3) and dermal-epidermal junction components (BP180 and BP230), respectively.Nowadays, the diagnosis of ABD is based on a combination of various criteria: histopatology of a lesional biopsy, direct immunofluorence of a perilesional biopsy skin, indirect immunofluorescence (IIF on monkey esophagus and BIOCHIP technology) and ELISA assay. The precise identification of the target antigen is crucial for the diagnosis and patient management.Several assays for the serological detection of anti-DSG1/DSG3/BP180/ BP230 Abs have been developed, however no generally accepted gold standard assay exists, and thus a working set for as definitive diagnosis as possible is urgently needed.Method We tested 312 patients suspected or diagnosed for ABD to evaluate the diagnostic performance of 3 different assays: traditional IIF on monkey esophagus, innovative IIF BIOCHIP mosaic consists of an array of six different diagnostic substrates including monkey esophagus,primate salt-split skin,dots of BP180 protein as well as DSG/DSG3 extracellular and transmembrane domains and BP230 C-terminal domain expressed in HEK293 cells) and ELISA systems based on recombinant forms of the immunodominant regions of the target antigens (DSG1/DSG3/BP180).ResultsThe tests profiles required were different between Specialist Departments,General Medicine Departments and Medical Departments: ASA IIF 12%vs78%vs10%, ELISA 57%vs31% vs12% and BIOCHIP 50% vs25%vs25% respectively. We observed different percentages of positivity for the assays (between Specialists, MMG and Medical Departments): ASA-IFI 21%vs21%vs9%; ELISA 40%vs8%vs10%; BIOCHIP 50%vs17% vs16%. The agreement between ASA IIF and Antigen specific test (Ag)(ELISA-BIOCHIP) was 85% (39/46).3/46 patients (6%)were ASA- / Ag+ and mainly represented by BP180 +;instead 4/46 patients(9%)were ASA +/Ag-mainly represented by intercellular substance.

EP132

The BAG3 Biomarker: the Stress-to-Fibrosis Pathway in Systemic Sclerosis

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Systemic sclerosis (SSc) is a complex disease characterized by fibrotic progression affecting skin and internal organs. The key to understanding this autoimmune disease lies in transforming fibroblasts into collagen-depositing myofibroblasts, a process driven by a cascade of cytokines. Our innovative research has identified the extracellular BAG3 protein as a crucial biomarker for monitoring the fibrotic progression of SSc. We found that in patients with the more aggressive form of the disease, diffuse cutaneous SSc (dcSSc), BAG3 serum levels are elevated. This distinguishes them from patients with limited cutaneous SSc (lcSSc) and healthy individuals.

Furthermore, BAG3 levels offer insight into the pulmonary involvement in SSc, as they correlate with interstitial lung disease (ILD). This correlation led us to investigate a potential link between serum BAG3 levels in SSc patients with ILD and their response to Nintedanib therapy. Interestingly, we observed a decline in BAG3 levels in responders to this therapy, while non-responders showed an increase. This finding underscores the diagnostic significance of BAG3.

In a deeper exploration, we isolated fibroblasts from dcSSc patients and found a higher expression of IFITM2 (BAG3R) protein. These cells not only express BAG3 but also secrete it in response to pro-fibrotic cytokine stimulation. In a skin fibroblast cell line, we discovered that Nintedanib can inhibit the TGFβ1-induced BAG3 secretion. Collectively, these latest in vitro findings suggest the need for further investigation into the promising diagnostic role of BAG3 for SSc. Our research positions BAG3 as a dynamic biomarker capable of monitoring fibrosis progression and therapeutic response in SSc. This opens up a new frontier in personalized medicine for this complex disease, highlighting the potential of BAG3 as a guiding light in the diagnosis and monitoring of systemic sclerosis

EP133

Advancements in allergy diagnostic: a current review comparing extractive methods and recombinant tests

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Background: Allergies are abnormal and specific reactions of the immune system triggered by exposure to pollen, environmental agents, food, mites, insect venom, and animal dander. Diagnosis is primarily based on anamnesis, skin prick test (SPT) and in vitro tests that, detecting specific serum IgE (sIgE), allow clinicians to predict the risk of severe allergic reactions. Typically, routine laboratory methods use allergen extracts. The recent availability of techniques using recombinant allergens has represented a new challenge in developing tools to improve allergy diagnostics.

Scope: In order to analyse a broader allergenic profile and allow a better identification of potential cross-reactivities, this review provides a thorough and updated critical appraisal of the most frequently used diagnostic tests for allergies. Specifically, we evaluated the analytical performance, in terms of specificity and sensitivity, by comparing instruments that use extract allergens with those that use recombinant allergens.

Methods: We reviewed recent publications from 2018 to 2024, regarding the characteristics of the following three diagnostic instruments involved in allergy diagnostics: ELISA multiplex test that uses both recombinant and extractive allergens (ALEX2/MAX9K - The Allergy Explorer, Dasit); PHADIA multiplex and monoplex tests that uses recombinant allergens (ImmunoCAP, Thermo Fisher); CLIA monoplex test that uses extractive allergens (IMMULITE 2000 Xpi, Siemens Healthineers).

Results and Conclusion: This review aims to better understand the advantages and limitations of different methods used in allergology diagnostic, including: the ability of each method to identify allergic patients, the capability of method that uses molecular allergens to detect potential cross-reactivities, the importance of a broader allergenic profile obtained from multiplex tests in predicting anaphylactic shock risk. This study indicates that the complementary use of these different techniques, both extractive and recombinant, allows a better patient characterization and a precise diagnosis aimed at optimal therapeutic strategies.

EP134

A new diagnostic algorithm for positive prick test and very low IgE titre sensitisation to Dermatophagoides

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Introduction

Dust mites are one of the most common causes of allergy worldwide. The clinical laboratory plays a key role in the diagnosis of Dermatophagoides allergy. In addition to the prick test, clinical suspicion can be confirmed by IgE positivity to the extractive allergens of Dermatophagoides pteronissinus (d1) and/or Dermatophagoides farinae (d2) and by allergen profile analysis using major molecular allergens of groups 1, 2 and 23. This is crucial for the selection of patients suitable for specific immunotherapy (SIT).

Aim of study

The objective of this work is to investigate a possible diagnostic algorithm for those cases of suspected Dermatophagoides allergy in which, after a positive prick test, IgE to the extractive allergen d1 is present at very low/ negative titre, with the aim of reducing the number and cost of molecular profiling.

Methods

In our laboratory, 87 serum samples from patients with a positive prick test result for Dermatophagoides and a very low/negative IgE titre (IgE <0.25 kUI/L) to the extractive allergen d1 were analysed for IgE directed against major molecular allergens Der p 1, Der p 2 and Der p 23. An IgE titre >0.10 kUI/L was considered positive. The ImmunoCAP system with automated Phadia 250 instrument (ThermoFisher Scientific) was used for the anti-allergen IgE assays.

Results and discussion

Of the 87 serum samples analysed, 49 samples showed IgE levels for d1 below the positivity cut-off. These patients were negative for all major molecular allergens tested.

In 70% of patients with an 0.10-0.25 kUI/L IgE titre for d1, no sensitisation to molecular allergens could be demonstrated. Only 11 patients were positive for one of the major molecular allergens. The most common of these was Der p 23 with a positivity rate of 73% (7/11). Only one patient was found to be simultaneously sensitised to another molecular allergen. The results obtained show that in the presence of very low titre positivity for IgE to Dermatophagoides, the molecular allergen most frequently positive is Der p 23. For this reason, our laboratory proposes a diagnostic algorithm that, in the case of an 0.10-0.25 kUI/L IgE titre for d1, includes only the Der p 23-specific IgE test. Only if this is negative, specific IgE tests against Der p 1 and Der p 2 are performed.

EP135

A strange case of Kikuchi-Fujimoto necrotizing lymphadenopathy: role of the laboratory in the differential diagnosis

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DESCRIPTION Kikuchi-Fujimoto disease is a rare disease with greater prevalence in the Asian population with unknown etiopathogenesis and a benign course, characterized by lymphadenopathy of the cervical region, usually accompanied by fever mild, night sweats, weight loss, nausea and vomiting. Important for therapeutic purposes is a correct diagnosis between : SLE, malignant lymphoma or adenocarcinoma.
METHOD The patient C.J, 39 years old, was hospitalized at the eye clinic in Parma for: hypovisus, conjunctivitis, Pucker retinopathy, sporadic mild fever, asthenia, lateral-cervical lymphadenopathy and significant weight loss. Laboratory tests showed: leukopenia, anemia, increased VES, hypergammaglobulinemia, cryoglobulinemia, proteinuria, ANA+ ENA+ ASMA+ and RF+. Possible differential diagnoses were: SLE, sarcoidosis, tuberculosis or leishmaniasi. Following the Laboratory results reported different autoantibodies positivies compatible with SLE, but the diagnosis of SLE was excluded for lack of clinical criteria indicative of the pathology. The haemochromocytometric test showed the presence of particular cellular elements for possibile parasites/Leishmania infection. Further specific assays to research the parasites showed a negative results and also the bone marrow biopsy was negative. A subsequent lymph node biopsy revealed lymphadenopathy Kikuchi-Fujimoto necrotizing, with somministration of symptomatic treatment. The patient was subjected to a monthly follow-ups, but showed persistent high proteinuria and development additional clinical symptoms; so a subsequent renal biopsy showed a overlap clinical picture between SLE and Kikuchi-Fujimoto lymphadenopathy disease.
CONCLUSIONS A positive interrelationship between clinicians and laboratorians is actually able to facilitate the resolution of complex clinical case and can,in some cases, allowed to predict future clinical developments. In this case, the positivities for ANA and ENA tests allowed an early diagnosis (thanks to a monthly follow-up) of SLE that was initially excluded.

EP136

THE CORRECT LABORATORY APPROACH TO THE EARLY DIAGNOSIS OF SYSTEMIC SCLEROSIS: OUR PRELIMINARY CONSIDERATION

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Introduction. In the diagnosis of systemic sclerosis (SSc), the search for circulating antinuclear antibodies (ANA) is central for a correct classification and management of the disease. In addiction to classical ACA and anti-Scl70, new autoantibodies having an important role in predecting possible organ involvement, prognosis and monitoring.
Methods. We enrolled 188 patients recruited by the Rheumatology Unit, AOU, University of Modena, Italy (approval Ethical Committee) during 2021-2022 years. We divided them in 2 groups: group 1 of 78 ACA positivy and group 2 of 65 anti-Scl70 positivity. We performed Hep 2000 ANA IIF pattern (Immunoconcepts and Image Navigator Automated Microscope), Elia CTD screen and single specificity antibodies by ImmunoCap FEIA ThermoFisher Scientific. We investigated within each of these 2 groups to detect the presence and frequency of other antibodies because the subsets have different clinical features distinguishing predominating types of circulating ANA. All patients were tested for 3 PMAT: panel 1 CTD Essential, panel 2 CTD Comprehensive, panel 3, Myopathy RUO, by Aptiva Inova Diagnostics, San Diego, USA.
Results. Our first analysis shows the simultaneous presence of multiple antibodies. In particular: in group 1 we had 23 patients with BICD2, 22 Ro52, 8 Ro60, 5 RNP, 3 PM-Scl, 2 SSB, 2 MDA5, 1 NXP-2, 1 Rpp25, 1 Rpp38, 1 Ku, 1 RNApol3. In group 2 we had 22 patients with Ro60, 8 RNP, 5 SSB, 4 BICD2, 4 Rpp38, 4 Ro52, 4 Ku, 3 PM-scl, 3 Fibrillarin, 2 RibP, 1 HMGCR, 1 SRP54, 1Rpp25, 1 RNApol3. We further analysed antibody associations and clinical aspects of patients, organ damage and distinct prognosis.
Conclusion. Despite availability of validated and standardized immunoassays for the detection of classical markers of SSc, the search for new autoantibodies is very important to fill the diagnostic gap particularly in seronegative SSc patients, thanks to their specificty, high predictive value and utility and to define the best therapy for specific subsets. For this purpose, the autoimmunity laboratory plays a crucial role: through the union of different technologies and the harmonization of results, it creates specific diagnostic algorithms useful for the diagnosis and monitoring of SSc

EP137

APPROPRIATEZZA DIAGNOSTICA DI LABORATORIO NELLA MALATTIA CELIACA: L'ESPERIENZA DELL'AST ASCOLI PICENO

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INTRODUZIONELa malattia celiaca (MC) è una patologia immuno-mediata che si manifesta in persone geneticamente suscettibili, in seguito all'introduzione di alimenti contenenti glutine. Le diverse Linee Guida concordano su un approccio sequenziale alla diagnosi, incentrato sulle determinazioni sierologiche come test di prima linea nei pazienti ad alto rischio, seguite da biopsia duodenale se necessaria. In particolare viene indicata la determinazione di IgA totali e Ab anti transglutaminasi IgA (tTGA) come test di ingresso e successiva conferma con Ab anti endomisio (EMA) in caso di positività di tTGA. La determinazione degli Ab anti transglutaminasi IgG (tTGG), degli anticorpi anti gliadina deaminata IgG (DGP IgG) ed EMA IgG è da effettuare in presenza di deficit di IgA totali.MATERIALI E METODISono state esaminate le richieste sierologiche per MC pervenute nell'anno 2023, presso l'U.O. Patologia Clinica dell'AST ASCOLI PICENO. La determinazione degli anticorpi anti tTGA, tTGG, DGP IgG e IgA è stata eseguita con metodica in chemiluminescenza (CLIA, QUANTA-FLASH®, Werfen, Milano), con utilizzo di Ab coniugato con isoluminolo e rilevazione della luce prodotta come RLU, proporzionali alla quantità di anticorpi presenti nel siero. Gli anticorpi anti endomisio (EMA) sono stati determinati con metodo in Immunofluorescenza Indiretta (IFI, QUANTA LYSER 3000, Werfen, Milano) usando esofago di scimmia ricco di transglutaminasi tissutale, rilevate tramite legame con un Ab secondario coniugato con isotiocianato di fluorescina. RISULTATIIn totale sono stati effettuati 9794 esami sierologici. L'80% delle richieste di tTGA non era accompagnata da una richiesta di IgA totali, solo il 5% dei pazienti aveva tTGA positive ed il 70% dei pazienti aveva una richiesta contemporanea di tTGA e tTGG. Il 56% delle richieste di EMA erano associate alla richiesta di tTGA ed il 3% dei pazienti aveva EMA positivi. Per il 52% dei pazienti è stato richiesto tutto il pannello sierologico.CONCLUSIONII dati in nostro possesso evidenziano come nella richiesta di esami sierologici esista un'alta percentuale di inappropriatezza, con conseguente ritardo nella diagnosi e spreco di risorse. L'utilizzo di test reflex potrebbe risultare efficace nel miglioramento dell'appropriatezza diagnostica della MC. Gandini A, Gededzha MP, De Maayer T, Barrow P, Mayne E. Diagnosing coeliac disease: A literature review. Hum Immunol. 2021;82:930-936.Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, Shamir R, Troncone R, Auricchio R, Castillejo G, Christensen R, Dolinsek J, Gillett P, Hróbjartsson A, Koltai T, Maki M, Nielsen SM, Popp A, Størdal K, Werkstetter K, Wessels M. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 2020;70:141-156Linee Guida diagnosi e follow-up della celiachia. Gazzetta Ufficiale della Repubblica Italiana n. 191 del 19 Agosto 2015

EP138

Clinical significance of high titer of antinuclear antibody patterns in autoimmune liver diseases

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Introduction. Autoimmune liver diseases (AILD) are chronic liver conditions without infection, including autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC). Autoimmune serology, especially antinuclear antibodies (ANA) testing, is crucial for diagnosing AILD. Recent advances in automated immunofluorescence microscopy have improved data collection on ANA staining, offering new insights into ANA patterns in AILD. This study aims to investigate the significance of specific ANA patterns in diagnosing AILD.Methods. We conducted a retrospective observational study at the University Hospital of Palermo, Policlinico "Paolo Giaccone." The ANA staining pattern was identified by indirect immunofluorescence on human epithelial type 2 cells. Among 3,499 patients with suspected AILD, 396 patients with an ANA titer of ≥1:320 were retrospectively reviewed for clinical, laboratory, and immunological data.Results. We found that the AC1 pattern is predominantly associated with AIH (23%, $p = 0.006$); no patients with PBC have AC1 pattern ($p = 0.01$). Similarly, the AC4 and AC5 patterns are linked to both AIH ($p = 0.002$), PBC ($p < 0.0001$), and ALD ($p = 0.02$). Additionally, the AC6-AC7, AC11-AC12, AC21 patterns are strongly associated with PBC ($p = 0.002$, $p = 0.003$, $p < 0.0001$, respectively). A strong association was also observed between AC21 pattern and CCH ($p = 0.0002$). Of note, patients with the AC21 pattern have significant association with PBC (98%) and higher levels of alkaline phosphotase (150 vs. 110.0 IU/L, 162 vs. 110.0 IU/L $p < 0.01$ and $p < 0.001$, respectively) and gammaglutamiltransferase (149 vs. 75 IU/L, $p < 0.001$) compared to the AC4 pattern.Conclusion. Our study highlights the different distribution of ANA patterns among patients with liver diseases, providing valuable insights into their diagnostic and prognostic implications.Future studies with larger cohorts and longitudinal designs are needed to further validate these associations and elucidate the underlying molecular mechanisms.

EP139

Comparison between three quantitative fecal calprotectin assays in patients with active inflammatory bowel disease

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[2]

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Background:Fecal calprotectin is a biomarker for monitoring inflammatory bowel disease (IBD) activity. Our objective is to evaluate three methods based on three different method principles:1) latex agglutination test (Eiken Chemical-Dasit); 2) Feia test (the EliA-Phadia Phadia); 3) Elisa test (Chorus-Diesse).In the laboratory economy, we will also take into consideration the usability of the stool extraction device, for use in other methods.

Methods:A total of 210 patients were included in this study; 98 patients with IBD (77 ulcerative colitis and 21 Crohn's disease) and 112 patients with non-IBD. We compared quantitative FC levels in different disease statuses and evaluated the correlation between the FC results of the three FC kits.The diagnostic performance in predicting active IBD was evaluated with reference to different cut-off levels which were almost very similar:1) >50ug/g= positive; 2)<50 µg/g=negative,>50 and 100 µg/g=borderline >100 µg/g= positive;3)<51 mg/kg=negative, => 51 and 119 mg/kg=borderline, >119=positive.

Results:The FC levels in 85 patients with active IBD as defined by endoscopic score were significantly higher compared to the inactive IBD and other diseases (P<0.05). Although the three assays' results correlated (r = 0.642, P < 0.001). The Diagnostic performances in predicting active IBD are comparable as area under the curve (AUC), 0.812, cut-off, 50, sensitivity, 66.4%, and specificity, 83.0% for Eiken-Dasit assay; AUC, 0.826, sensitivity, 69.4%, and specificity 81.9% for Elia-Phadia; AUC 0.843,sensitivity,71.2% and specificity 80.5%. Cut-off level 100, sensitivity, 84.4%, and specificity 61.9% for Elia-Phadia; sensitivity 82.3% and specificity 62% for Eiken-Dasit;sensitivity 80.8% and specificity 63.2% for Chorus-Diesse. FC levels using a cut-off of > 250 µg/g confirmed 85.7% Eiken-Dasit; 86,9% Elia-Phadia; 87,3% Chorus-Diesse.

Conclusion:The results of the three FC assays showed a significant correlation, but the three test results for some concentration ranges are not very interchangeable. With optimized cut-off values, FC tests could be helpful in the diagnosis of IBD and differentiating active IBD from inactive or organic bowel disease. In routine practice, the devices supplied for Eiken-Dasit and Chorus-Diesse are extremely advantageous as they are used to search for occult blood and fecal elastase respectively

EP140

HLA-DQ genotyping in a selected subset of individuals may support precision medicine approaches to the diagnosis of Celiac Disease (CeD)

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BACKGROUND: CeD is a chronic immune-mediated enteropathy caused by dietary gluten with marked autoimmune features. Susceptibility to the disease is strictly dependent on the dose of the predisposing alleles encoding the DQ2 and DQ8 heterodimers. Many studies demonstrated a robust correlation between tTG IgA and EMA IgA positivity and mucosal damage. As a result, the latest ESPGHAN guidelines for the diagnosis of paediatric CeD (2020) support the no-biopsy approach in the presence of elevated tTG IgA (≥10x ULN), positive EMA IgA and HLA predisposing alleles.

AIM: To stratify the risk of carriers of different HLA-DQ alleles of being positive for CeD serology in adults and children, and to estimate the impact of HLA typing in individuals with family history and/or suggestive symptoms on CeD diagnosis and follow-up.

METHODS: The retrospective observational study included all subjects with family history or suspected CeD, whose samples were analysed at the LUM, AUSL of Bologna, between 2021 and 2023, for HLA-DQ, total IgA, tTG and EMA IgA.

RESULTS: Of the 1071 subjects included (531 <18y, 540 ≥18y), 19% (206) were serologically positive, of whom 98,5% expressed DQ2, DQ8 or DQ7. Compared to those with non-permissive HLA (136), DQ2 homozygotes (120) had the highest odds of seropositivity (OR=33.6; 95% CI, 10.1-111.7, p<0.0001). Laboratory evaluation identified the onset of CeD according to ESPHGAN 2020 criteria in 22 children (4%), avoiding duodenal biopsy in one in six seropositive children (140). In adult population, seropositivity was found in 11% of those tested, according to a dose-dependent gradient of DQ risk alleles. Notably, 38% (19) of adult DQ2 homozygotes (50) were tTG/EMA positive or reported to be on follow up, often with associated complications, compared to 1% of DQ7 and 7% of DQ2 Heterozygote carriers.

CONCLUSIONS: Confirming the risk gradient of DQ alleles in the development of CeD, our data suggest that HLA typing, in the presence of suggestive symptoms or family history, allows the identification of a relevant number of individuals at increased risk of CeD and its complications, supporting precision medicine approaches for early and non-invasive diagnosis, and may provide an opportunity to further define genotype-guided preventive strategies.

EP141

Donors blood Troponin I levels compared with other cardiovascular risk factors

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Background and aims

Troponin, a specific marker of cardiac damage and remodeling, was recently established as an independent risk factor for cardiovascular (CV) disease (1). We aimed to measure troponin I levels (TnI) by a high-sensitivity assay on a representative sample of blood donors (BD) to compare TnI CV risk stratification with two other parameters.

Methods

BD serum samples were tested for total, HDL and LDL cholesterol, triglycerides, glucose, C-reactive protein, and TnI levels, which were evaluated by the highly sensitive Abbott Architect assay. TnI levels were stratified for CV risk at ten years by the recommended thresholds for women (low: <4 ng/mL; moderate: 4-10 ng/L; high: >10 ng/L) and men (low: <6 ng/mL; moderate: 6-12 ng/L; high: >12 ng/L) (2). TnI-derived risk scores were compared with the HDL/LDL cholesterol ratio (low: <3; moderate: 3-5; high risk: >5) (3) and with the Framingham CV risk score (4) on a subset of 292 BD for whom all scores were available.

Results

We included 1,117 BD (818 males, 299 females) over a period of 1 year. The mean and median age (50 and 51 years, respectively) did not differ by gender. TnI levels scored 92.0% low risk (women: 96.0%; men: 90.5%; p=0.01), 5.5% moderate risk (3.0% women and 6.4% men) and 2.5% high risk (1.0% women, 3.4% men). On the 292 selected BD, moderate/high risk was 13.4%, 24.0%, and 38.7% by TnI, HDL/LDL ratio, and Framingham, respectively. Among subjects at moderate/high CV risk by HDL/LDL and by Framingham, only 14.3 % and 9.7%, respectively, were scored at moderate/high risk by TnI.

Conclusions

On middle age, asymptomatic subjects 4.0% of women and 10.5% of men were at high or intermediate CV risk by troponin, both figures being significantly lower compared to HDL/LDL ratio and Framingham. Applying TnI measurements on patients with a high/intermediate risk by other methods may allow a more accurate risk stratification and management of people with a confirmed high risk of CV events.

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EP142

Measuring LDL cholesterol (LDL-C): which method is best, and when?

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Background

In 2019 ESC/EAS Task Force proposed new targets for low-density lipoprotein cholesterol (LDL-C), and a revision of CVD (cardiovascular diseases) risk stratification. Even if several direct LDL-C (D-LDL-C) assays are available, many clinicians use some formulas to calculate LDL-C using total cholesterol, triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) concentrations.

Methods

We analysed three groups of 200 patients over the age of 16: group 1 (TG <70 mg/dL), group 2 (TG: 70-300 mg/dL), group 3 (TG >300 mg/dL). D-LDL-C measurement, carried out by AU5800 Beckman Coulter analyser, was compared to LDL-C calculated (C-LDL-C) by three different formulae (Friedewald formula - F, Martin/Hopkins equation – MH, and Sampson-National Institutes of Health equation 2 - SNIH2) performing Passing-Bablok regression (PB) and Bland-Altman analysis (BA). Cohen's k was calculated to verify the correlation of different LDL-C results, dividing patients by five classes of LDL-C concentrations: ≥116 mg/dL, 100-115 mg/dL, 70-99 mg/dL, 50-69 mg/dL and <50mg/dL within each group.

Results

PB showed a proportional and constant systematic error for all comparisons between D-LDL-C and C-LDL-C. BA showed significant bias: 10.4% for D-LDL-C vs F, 13.4% for D-LDL-C vs MH, 10.5% for D-LDL-C vs SNIH2 in group 1; 15.9% for D-LDL-C vs F, 13.3% for D-LDL-C vs MH, 12.9% for D-LDL-C vs SNIH2 in group 2; 62.6% for D-LDL-C vs F, 24.9% for D-LDL-C vs MH, 35.6% for D-LDL-C vs SNIH2 in group 3. The correlation between D-LDL-C and C-LDL-C was excellent in the range 70-115 mg/dL and good in the ranges 50-69 and ≥116 mg/dL for samples with TG ≤ 300 mg/dL, while for LDL-C values < 50 mg/dL we found poor/fair correlation in all groups. In samples with TG > 300 mg/dL, correlation between D-LDL-C and C-LDL-C was good only for LDL-C range 100-115 mg/dL.

Discussion

Our data confirm a significant variability between LDL-C measured and calculated, particularly based on the TG values, with possible misclassification of target values for patients at different CVD risk classes. For this reason, it is necessary working to achieve standardization of direct assays, identifying when it is appropriate to use the calculation, and through which formula, and when is better to use a direct measure.

EP143

HDL subfractions in patients with Familial Hypercholesterolemia

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Introduction. Familial Hypercholesterolemia (FH) is a genetic disease mainly caused by pathogenic variants in three genes: LDLR, APOB and PCSK9. It is associated with increased cardiovascular risk and high levels of LDL-cholesterol (LDL-c). Low levels of HDL-cholesterol (HDL-c) were also observed. We aim to evaluate the distribution of HDL subfractions to better characterize patients with clinical suspicion of FH with (FH/V+) and without (FH/V-) pathogenic variants.
Methods. We analyzed the plasma of 99 patients (72 FH/V + and 27 FH/V-) using the Quantimetrix Lipoprint System. This method allows to separate 10 subfractions of HDL that can be grouped into 3 categories: Large, Intermediate and Small.
Results. We observed a higher proportion of Small HDL in FH/V- (15.9±4.8%) than FH/V+ patients (13.6±5.2%; p=0.042). To better focus on the different proportion of HDL Small and HDL Large we calculated the ratio between these subfractions (HDL S/L). This ratio was significantly higher in FH/V- patients (0.45 (0.33-0.68)) than in FH/V+ (0.32 (0.21-0.52); p=0.030). Considering the whole population, we observed a positive correlation with triglycerides (Spearman coefficient=0.617; p<0.001) and LDL-c/HDL-c ratio (Spearman coefficient=0.338; p=0.001) and a negative correlation with HDL-c (Spearman coefficient= -0.432; p<0.001). The association of HDL S/L ratio with HDL-c and triglyceride levels remains significant at a multivariate linear regression independently from age, sex and presence of pathogenic variants with a β-coefficient of -0.357 for HDL-c and of 0.382 for triglycerides (p<0.001 for both).
Conclusions. Small HDL resulted decreased in FH/V + patients, suggesting an alteration of HDL dimension associated with the presence of a pathogenic variant. This different distribution of HDL subclasses could also explain the different predisposition to cardiovascular diseases due to the presence of pathogenic variants.

EP144

Evaluation of the new Access NT-proBNP on DxI 9000 Access Immunoassay analyzer

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Evaluation of the new Access NT-proBNP on DxI 9000 Access Immunoassay analyzer
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1 DAI Department of Services Medicine, SC Laboratorio Unico, Trieste, Italy
2 Beckman Coulter srl, Milano, Italy Heart failure (HF) is the main cause of mortality worldwide, particularly in the elderly. N-terminal pro-brain natriuretic peptide (NT-proBNP) is now a cornerstone of many clinical guidelines in the diagnosis of heart failure, determined in blood samples generally by immunochemical methods. Aim of this study is to evaluate the new Access NT-proBNP assay on DxI 9000 Access Immunoassay analyzer (Beckman Coulter Inc., Brea, CA, USA) on different samples, compared with the routine method Elecsys proBNP II Cobas on Elecsys (Roche Diagnostics GmbH, Mannheim, Germany, reporting rule-out cut-off = 300 pg/mL). 126 fresh residual anonymized samples, 72 males (M, age 34-102) and 55 females (F, age 5-94) from 41 departments and withdrawal points were analyzed within 4-6 hours from Elecsys determination. Access NT-proBNP (A) and Elecsys proBNP II Cobas (E) assays claims respectively in pg/mL: LoB A=1.1, E=3; LOD A=4.8, E=5; LoQ 20%CV A= 4.8, E=50; linearity A= 35,000, automatic dilution up to 350,000, E=35,000, automatic dilution up to 70,000. Statistical analysis: MedCalc V22.023 (https://www.medcalc.org). Quality Control (QC) verified by 5x5 protocol from SIBioC Guidelines for Laboratory Statistics.E: minimum 14, maximum 35,000; mean 4681, median 1452 pg/mL; A: minimum 20, maximum 42,780, mean 4642, median 1338. Passing Bablok regression: y=0.554 + 0.979x; intercept 95%CI -17.6924 to 6.8540. Bland Altman (differences as %): y=0.4854 + 0.00001952x. Intraclass Correlation Coefficient = 0.9822 (IC 95% 0.9747 to 0.9874. 5x5: level 1 mean 171, CV 2.8%; level 2 mean 545, CV 4.4%, in line with manufacturer claim.Considering Access NT-proBNP rule-in and rule-out cut-off by age classes, results are classified as follow: rule-out 5 <300, 17 between 301 and 900, 38 between 901 and 1800; rule-in 22 ≥900 and 44 ≥1800.Our samples selection well covered the analytical measuring range and the various rule-in and rule-out cut-offs. QC performances confirm the manufacturer claims.Access NT-proBNP comparison with the reference method gave good results in terms of Mountain Plot, Bland Altman, Passing Bablok. NT-proBNP assays showed an acceptable concordance, and their clinical performance was comparable; these results should have a positive impact on workflow, without the need for dedicated sample and analyzers.

EP145

EXPLORING NEW FRONTIERS: CA-125 AND EMERGING BIOMARKERS IN HEART FAILURE RESEARCH

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The Carbohydrate Antigen 125 (CA-125), originally known as a tumor marker associated with ovarian carcinoma, has recently garnered attention within the scientific community for its potential implications in the cardiovascular context, particularly in heart failure (HF). B-type Natriuretic Peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NtproBNP) are pivotal biomarkers in the context of heart failure, providing valuable insights into cardiac function and patient prognosis. The aim of the study was to evaluate the correlation between CA125, BNP, and NT-proBNP in patients with heart failure using the Atellica Solution® platform. METHODS Forty-five samples from patients diagnosed with heart failure were analyzed to determine levels of CA125, BNP, and NT-proBNP. A comprehensive assessment was conducted, involving medical history, physical examination, electrocardiography, telecardiography, and transthoracic echocardiography, to establish the diagnosis.
RESULTS Elevated levels of CA-125 have been observed in some patients with mild HF, and higher CA-125 levels have been associated with worse outcomes, including increased mortality and adverse cardiac events. Serum CA-125, BNP and Pro-BNP levels in patients with HF worse outcomes (CA 125=213.38 U/ml, Pro-BNP=11465.06 pg/ml and BNP=758 pg/ml) were found significantly higher than those in mild autcomes (CA 125=55 U/ml, Pro-BNP=1197.65 pg/ml and BNP=111 pg/ml) ($p<0.001$). There was a statistically significant relationship between CA 125 serum level and HF stage.
CONCLUSIONS In summary, while CA-125 shows promise as a potential prognostic marker in the context of heart failure, its integration into clinical practice demands further evaluation through robust clinical studies and ongoing research efforts. To fully comprehend its utility and applicability, it is imperative to validate its effectiveness across diverse patient populations and clinical settings. Further research is essential to establish standardized reference ranges for CA-125 in heart failure, ensuring consistent and reliable interpretation of its levels. This process will enable clinicians to effectively utilize CA-125 alongside established biomarkers like BNP and NT-proBNP, enhancing diagnostic accuracy and prognostic precision in managing heart failure patients.

EP146

Proteomic profiling of extracellular vesicles from serum. Biomarker discovery for prediction of obstructive critical coronary artery disease

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Acute coronary syndrome is defined as a sudden reduction in blood supply to heart. It includes a wide range of pathological manifestations, such as non–ST segment elevation myocardial infarction (NSTEMI) and unstable angina (UA). These are overlapping entities and only high-sensitivity cardiac troponin analysis allows a better differential diagnosis. However, international guidelines still recommend a routine invasive exam in both NSTEMI and UA patients, resulting in many coronary angiograms with high-risk features, also in absence of obstructive events. Therefore, the identification of patients with obstructive coronary artery disease (CAD) remains an unsolved issue.Recently, circulating extracellular vesicles (EVs) have emerged as potential source of diagnostic/prognostic biomarkers in cardiovascular disease. Here, a profiling of EVs from UA and NSTEMI patients with and without obstructive CAD is described, aiming at identifying candidate predictors of critical CAD. A preliminary proteomic-based study was performed on samples from 36 patients (10 UA, 10 UA with CAD, 8 NSTEMI and 8 NSTEMI with CAD) who underwent coronary angiogram. Our results revealed that protein composition of the samples was quite similar, even though significant differences in the principal component proteins emerged. Proteins differentially enriched between CAD and NO CAD groups were selected (p-value <0.005) and subjected to gene ontology analysis using STRING and ShinyGO software. Results indicated interesting differences in proteins involved in triglyceride metabolism and lipid transport. More specifically, ANG without CAD and NSTEMI with CAD were the conditions in which lipid metabolism and transport functions seemed significantly increased, but with different effectors, thus, suggesting a differential regulation of this metabolic function in the two pathological states. Moreover, a subset of promising putative protein biomarkers for CAD and NO CAD conditions was defined, and their expression level was verified in the complete cohort of 130 patients through western blot and flow-cytometry assays. Results obtained confirmed the significant differential expression of the proteins in presence or absence of critical obstructive events for some of the proposed protein biomarkers.

EP147

Un nuovo metodo di misura ad alta sensibilità (hs) per c-TnI: quale valore aggiunto?

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Introduzione- I metodi ad alta sensibilità per le troponine cardiache rappresentano il "gold standard" biochimico per identificare il danno miocardico e consentono l'adozione di protocolli accelerati (0-1 h, 0-2 h) ottimizzando il tempo di gestione e di permanenza dei pazienti in Pronto Soccorso (PS). Nello studio sono state valutate le caratteristiche analitiche e di ricadute cliniche di un nuovo metodo hs-cTnI CLIA (Mindray) in confronto a metodi ad alta sensibilità da tempo utilizzati: Access hs-cTnI (Beckman Coulter, UOC Medicina di Laboratorio, AOUP); Architect hs-cTnI (Abbott, UOC Medicina di Laboratorio, ULSS7).
Materiali e metodi- Sono stati analizzati i campioni residuali di plasma (LiHe) da 194 pazienti consecutivi ammessi in PS (gennaio-febbraio 2024) con dolore toracico o sintomi suggestivi di patologia cardiaca, con prelievo basale (T0) e dopo 2 ore (T2): 113 presso AOUP (P1) e 81 presso ULSS7 (P2). I valori di hs-cTnI sono stati valutati utilizzando LOD e 99° percentile (ng/L) dichiarati dai produttori: Mindray-LOD <0,7; F 15,3, M 31,3; Beckman-LOD <2,3; F 12, M 20; Abbott-LOD <1,9; F 16, M 34. La variazione cinetica delle concentrazioni -T0 e T2- è stata considerata significativa se >30%.
Risultati- Nelle popolazioni studiate (P1: M 51%, P2: M 59%) il numero di pazienti dimessi (D) e ricoverati (R) in Unità Cardiologiche (UC) è risultato: P1-70 D, 43 R (37% in UC); P2-70 D, 11 R (64% in UC), essendo l'età media (range) dei pazienti ricoverati superiore a quella dei dimessi: P1-D: 59a (18-93), R: 73a (39-97), p=0,00001; P2-D: 57a (19-90), R: 64a (26-92), p=0,00001. A T0 i valori mediani con il metodo in valutazione sono risultati inferiori al cut-off nell'84,3% (P1: 3,35 ng/L) e nel 81,4% (P2: 5,03 ng/L) dei pazienti dimessi in confronto a 71,4% e 75,7% dei valori con i metodi di confronto; nei pazienti ricoverati (P1 e P2) a T2 il 100% dei valori è risultato superiore al cut-off, in confronto a 81,3% e 90,9% dei valori con i metodi di confronto. Nei pazienti ricoverati in UC le cinetiche di rilascio sono risultate positive e concordanti tra metodi (P1 62,5%, P2 57,1%).
Conclusione- La maggiore sensibilità del metodo in valutazione consente, rispetto ai metodi di confronto, un più efficace rule-out dei pazienti con dolore toracico in PS.

EP148

Long Term Effects of Liraglutide on glyco-metabolic parameters, Small Dense Low-Density Lipoproteins and cIMT in patients with type-2 diabetes: 5 years prospective real-world study.

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Background: Liraglutide has several non-glycemic effects, including those on plasma lipids and lipoproteins, contributing to its cardiovascular benefit; however, the long-term effects of liraglutide on CV risk markers are still limited. Here we investigated whether the reduction in glycemic and metabolic parameters, with particular focus on Small Dense Low-Density Lipoproteins and cIMT, could be maintained in T2DM subjects under routine clinical practice.
Methods: Sixty-two patients with T2DM (31 men, 31 women; mean age ± standard deviation 61 ± 9 years) without prior history of a major CV event and naïve to incretin-based therapies were treated with liraglutide (1.2 mg/day) as add-on therapy to metformin (1500–3000 mg/day) for 5 years. Laboratory analyses included the assessment of lipoprotein subclass profile by gel electrophoresis (Lipoprint; Quantimetrix Corp., Redondo Beach, CA, USA). Carotid intima-media thickness (cIMT) was assessed by Doppler ultrasonography. Statistical analyses included the paired t test, Spearman correlation and multiple regression analysis.
Results: The addition of liraglutide to metformin monotherapy resulted in significant reductions in fasting glycemia, hemoglobin A1c, body mass index, waist circumference, total cholesterol, triglycerides and low-density lipoprotein (LDL)-cholesterol, as well as in cIMT. There was an increase in the large LDL-1 subfraction, with a concomitant reduction in atherogenic small dense LDL-3 and LDL-4 subfractions. Correlation analysis revealed a significant association between changes in cIMT and changes in small dense LDL-3 subfraction ($r = 0.501$; $p < 0.0001$). Multivariate analysis, including all of the measured anthropometric and laboratory parameters, revealed that only changes in the small dense LDL-3 subfraction were independent predictors of changes in cIMT ($p < 0.0001$).
Conclusion: Long-term liraglutide treatment in real world settings effectively maintained the reduction of several glyco-metabolic parameters in T2DM subjects as the reduction of cIMT. Our findings are the first to show that the vascular benefit of liraglutide in patients with T2DM is associated with reductions in atherogenic small dense LDL. This effect is independent of glycemic control and body weight reduction and may represent one of the key mechanisms by which liraglutide is able to reduce cardiovascular events.

Keywords: Cardiovascular Risk, Carotid Intima-Media Thickness, Liraglutide, Lipoproteins, Small Dense Low-Density Lipoproteins, Type 2 Diabetes.

EP149

Increased high-sensitivity cardiac troponin I in amateur soccer players and differences with age: a possible biomarker of early cardiovascular risk?

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Background: Cardiac troponins are the reference markers for the diagnosis of acute coronary syndromes. However, recent studies have shown that healthy professional athletes could have a significant increase incardiac troponins after intense physical activity, defined as a reversible myocardial injury. Conversely, there are few studies on the evaluation of cardiac troponins in amateur athletes. The aim of this study was to evaluate cardiac troponin I in amateur soccer players after a soccer game. Methods: For the study, 22 amateur soccer players from the University Sports Centre of the University of Rome Tor Vergata were enrolled. The first team was made up of 11 university students with an average age of 22 years, the second team was made up of 11 university professors with an average age of 54 years. All subjects enrolled underwent a cardiological check-up program before the match. The blood samples were collected before the start of the soccer game, 2 hours and 24 hours from the time the game ended. The two groups had similar training loads throughout the week.Results: The data showed a significant increase of serum troponin concentrations after 2 hours ($p < 0.01$) and then a return to baseline levels after 24 hours. The data demonstrated no significant differences in troponin values at T0 between the two groups, while comparing the values 2 hours after the end of the race, the team of teachers had higher troponin levels than the students ($p<0.05$). No measurements were found above the 99th percentile URLConclusions: Our study demonstrated a significant increase in cardiac troponin I in amateur soccer players, without any evidence of cardiac damage. The interesting data is that the players of the professors' team, with an average age more than double that of the students, had a greater increase after the match compared to the students, with a probable correlation with age, as already known by other studies. The data suggest the importance of measuring cardiac troponin as a possible biomarker of cardiovascular risk in general and not only of myocardial damage. More studies will be necessary to confirm these data and to further understand the pathophysiology and kinetics of cardiac troponin release in response to physical exercise and more generally in the healthy population as an early marker of cardiovascular risk.

EP150

Determination of osteopontin in monitoring retinal damage in metabolic syndrome

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Objective: Metabolic syndrome (MetS) is becoming a public health challenge. MetS is a complex metabolic disorder that affects globally and carries high socioeconomic costs. Many of the individual components of MetS are associated with ocular changes, but it is not yet clear what the association is. It is known that MetS can lead to a condition of diabetes and its consequences such as retinopathy. Osteopontin (OPN) is a phosphoglycoprotein with multiple functions, in fact it carries out a broad spectrum of biological activities. OPN appears to be implicated in diabetic retinopathy. Therefore, we evaluated the implication of OPN in retinal damage and whether this protein can be used as a predictive biomarker of retinopathy in subjects affected by MetS.
Methods: Given the involvement of OPN in retinal damage, the aim of this research was to evaluate OPN expression and its variation over time in a model of MetS induced by 30% fructose consumption for 1, 2 and 3 months. Male CD1 mice aged 8 to 10 weeks were used. Fructose mice were given a 30% fructose solution in drinking water, while control mice were given regular drinking water. ELISA kits, Western blot and immunohistochemical analyzes were used to evaluate the alterations caused by MetS and the involvement of OPN in retinal damage.
Results: Compared to controls, mice treated with 30% fructose showed a time-dependent increase in weight and fluid consumption. By monitoring the animals during MetS in the fructose supplemented group, a time-dependent decrease in glucose regulation and an increase in blood levels of insulin, cholesterol and triglycerides were observed. Furthermore, after one month of fructose supplementation, immunohistochemistry and western blot results showed a time-dependent increase in the expression of Iba-1 and OPN and co-localization of Iba-1/OPN, which demonstrates the correlation between microglia activation and OPN secretion, which seems to be linked to retinal damage. Furthermore, immunohistochemistry and western blot confirmed an alteration of tight junctions over time, of zonula occledens-1 (ZO-1) and Occludin. These alterations confirm the role of OPN as a mediator of inflammation at the site of injury and retinal damage in the MetS.
Conclusions: Given the role of OPN as a mediator of inflammation at the site of retinal injury and damage, it can be stated that the identification of OPN in MetS patients could be used as an early marker of retinal damage, thus preventing complications related to the progression of this pathology

EP151

HbA1c QUANTIFYING BY CAPILLARY ELECTROPHORESIS: THE IMPORTANCE OF A HIGH-RESOLUTION METHOD

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Background: HbA1c is a biochemical marker widely used in monitoring long-term glycemic control in diabetic patients and assessing the risk of complications. To obtain an accurate measurement, it can be quantitatively measured by several examining systems such as capillary electrophoresis (CE), ion-exchange high-performance liquid chromatography (HPLC), immunoassay and enzymatic assays. Nonetheless, the presence of hemoglobin variants (HV) can interfere analytically with HbA1c measurements. Aim: We evaluated the presence of interfering HVs with HbA1c measurements in 7 patient blood samples by CE (Sebia), which have been previously assayed by rapid HPLC.Methods: From January 2024, in our laboratory, the HbA1c measurement has been performed by CE using Capillarys 2 Flex Piercing® as a replacement for the rapid HPLC method used until December 2023. Results: Throughout these five months, the introduction of CE has showed an atypical profile in 7 blood samples that did not allow to obtain a reliable result for the presence of interfering peaks. Previously, these samples were analyzed by rapid HPLC that performed chromatograms without abnormalities, supplying HbA1c quantification. Interestingly, the CE method allowed to highlight the presence of a HV interfering with the HbA1c measurement. The latter could be investigated to provide useful informations to medical practice. Discussion: The increasing prevalence and heterogeneity of genetic variations in hemoglobin in the population requires effective methods to detect the possible presence of such alterations that may compromise the correct assessment of HbA1c. Compared to rapid HPLC, CE presents a very good separation, with a clear isolation of HbA1c from the main component HbA0 and other fractions that usually migrate very close together such as HbA1a, HbA1b, HbF and HbA2. These 7 cases underline the importance of using a high-resolution separative method in the determination of HbA1c to avoid erroneous or overestimated HbA1c determinations due to HVs. In this way, the report could be completed by suggesting these patients a more reliable alternative biomarker like as glycated albumin or fructosamine.

EP152

Method comparison between ion-exchange HPLC and capillary electrophoresis for the measurement of glycated hemoglobin

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Background

Glycated hemoglobin (HbA1c) is a widely used biomarker of long-term glycemic control. In the clinical Laboratory of Baggiovara Ospedale Civile (Modena), we performed a comparison between our current ion-exchange HPLC method (D-100, BioRad) and a new capillary electrophoresis (CE) analytical system (Capillarys 3 Tera, Sebia).

Methods

Whole blood samples analysed for HbA1C measurement and randomly chosen among those accepted in our Laboratory in July 2022 were tested with both our current method and the CE method. Results were analysed with Passing-Bablok regression and Bland-Altman plot. Allowable bias between methods was based on biological variation from the EFLM database (minimum specification: 2.7%). Inter-method agreement for genetic variants intereference was evaluated with Cohen's kappa. Statistical analysis was performed with MedCalc 18.2.1.

Results

Overall, 547 samples from subjects 12-97 years of age (median age 68 years; IQR: 58-78 years) were analysed, 254 from female and 293 from male subjects. Regression analysis revealed a significant difference between the methods, with both a constant and a proportional bias of CE (y) relative to HPLC (x) (intercept: 1.18 mmol/mol, 95%CI 0.52-1.72; slope: 0.95, 95%CI 0.94-0.97). Bland-Altman plot confirmed that CE provided on average lower results than HPLC by 0.99 mmol/mol (95% CI: 0.83; 1.16), with higher differences with increasing concentrations (regression of differences CE-HPLC: intercept -1.09, 95% CI: -1.60; -0.57; slope: 0.04, 95%CI: 0.03; 0.05). At the decisional limit for diabetes diagnosis (48 mmol/mol), the estimated bias was found clinically acceptable when compared to the analytical specification based on biological variation (2.5% vs 2.7%). All 13 cases of beta-chain heterozygosity were correctly identified by both methods as atypical, with a reportable HbA1C concentration. Similarly, in 3 cases of beta-chain homozygosity or compound heterozygosity (2 cases of HbSS and 1 case of HbS/HPFH), both CE and HPLC did not report any (erroneous) HbA1C result. Overall, inter-method agreement was excellent (kappa=1.0).

Conclusions

In conclusion, the CE method gave clinically comparable results to our current and widespread HPLC method, with a very good agreement in detecting genetic variants.

EP153

Indici di stress e stato infiammatorio cronico nel paziente diabetico

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Introduzione

Studi recenti hanno esaminato la relazione tra l'iperglicemia indotta dallo stress, determinata dal gap glicemico tra i livelli di glucosio al ricovero e i livelli medi di glucosio derivati dall'A1c, e la gravità della malattia e/o esiti clinici sfavorevoli. I pazienti diabetici che soffrono di iperglicemia cronica hanno conseguenze identiche a quelle causate dall'iperglicemia indotta dallo stress, tra cui aumento dello stress ossidativo, infiammazione e attivazione delle chinasi reattive allo stress e compromissione della funzionalità renale.

Il rapporto piastrine/linfociti (PLR) è stato recentemente dimostrato come un marcatore infiammatorio durante l'epidemia di Covid-19. Inoltre di recente l'NT-proBNP è stato indicato come fattore di rischio per lo sviluppo di malattie cardiovascolari nei soggetti diabetici con diabete di tipo 2. Lo scopo di questo studio è quello di valutare come il gap glicemico, il livello di PLR e di NT-proBNP, e il rapporto Albumina/Creatinina (A:C), tutti possibili spie di infiammazione cronica nei pazienti diabetici, si correlano tra di loro.

Materiali e Metodi

Per calcolare il gap glicemico è stato, prima, stimato il valore della glicemia media (ADAG), tramite la formula 28.7 x HbA1c(%) – 46.7, poi, il risultato è stato sottratto al valore di glucosio ematico. La popolazione studiata comprendeva 129 soggetti e sono state analizzate le relazioni tra PLR e gap glicemico, NT-proBNP e gap glicemico, e PLR e A:C.

Risultati

Abbiamo osservato che il PLR e NT-proBNP aumentano proporzionalmente ai valori del gap glicemico. Inoltre, i risultati indicano un incremento dell'NT-proBNP con l'aumentare del valore del rapporto albumina/creatinina, indice di funzionalità renale.

Conclusioni

Il gap glicemico riflette uno stato infiammatorio cronico del paziente diabetico e sembra essere correlato con altri indici di infiammazione e stress, quali il PLR, l'NT-proBNP, e il rapporto A:C. Ulteriori studi sono necessari per valutare se il gap glicemico possa essere utilizzato, insieme altri parametri analizzati, come indice di progressione del danno cardiaco e renale provocato dallo stato infiammatorio cronico del paziente diabetico.

EP154

Method Comparison of two HPLC systems for the determination of Glycated Hemoglobin: BioRad D-100 and Tosoh G11

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Background

Glycated Hemoglobin (HbA1c) can be measured with different laboratory techniques that take advantage of the chemical and structural differences existing between HbA1c and other hemoglobin fractions. HPLC techniques are widespread in clinical laboratories. We compared the performance of two ion-exchange HPLC methods for HbA1c measurement.

Methods

Whole blood samples randomly selected from those accepted in our Laboratory in May 2024 were analyzed with our current HPLC method (D-100, BioRad) and with a test HPLC method (G11, Tosoh).Comparison between methods was estimated with Passing-Bablok regression, Bland-Altman plot and Spearman correlation. Allowable bias was based on biological variation from the EFLM database (minimum specification: 2.7%). Inter-method agreement for genetic variants identification was evaluated with Cohen's Kappa. Data analysis was performed with MedCalc 18.2.1.

Results

Overall, 257 samples were analysed with both methods. Passing-Bablok regression analysis showed a positive constant bias of Tosoh (y) relative to BioRad (x) (intercept: 2.0 mmol/mol, 95%CI 2.0-2.0; slope: 1.0, 95%CI 1.0-1.0). The correlation coefficient was 0.98 (95%CI: 0.98-0.99). Bland-Altman plot confirmed G11 provided on average higher results than D-100 (mean of differences 1.59 mmol/mol).At the decisional limit for diabetes diagnosis (48 mmol/mol), the estimated bias was found clinically significant when compared to the analytical specification based on biological variation (4.2% vs 2.7%).28 cases of beta-chain heterozygosity were correctly identified by both methods as atypical, with a reportable HbA1c concentration; 2 more cases were identified only by our current method (D-100). Overall, inter-method agreement was deemed very good (kappa=0.96; 95%CI: 0.91-1.00).

Conclusions

Our data revealed a clinically significant bias between the two methods. This is in concordance with published findings from other authors. Moreover, even if the agreement for the identification of hemoglobin genetic variants was very good, some discrepant results were observed. In conclusion, our comparison highlighted differences that should be carefully taken in consideration by clinical laboratories shifting between these two methods.

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EP155

Association between triglyceride glucose index and diabetic foot in patients with type 2 diabetes mellitus

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Severals studies have elucidated the pivotal role of insulin resistance (IR) as risk factor for diabetic foot (DF). The triglyceride-glucose (TyG) index is a dependable and simple indicator of IR. However, little is known about the link between the TyG and the risk of DF. In this study we investigated the relationship between the TyG index and the prevalence of DF.We carried out a retrospective case-control study on 482 Albanian adult inpatients with T2D. DFU group, including 104 participants with DFU and non-DFU group, including 378 participants without DFU. The TyG index was calculated as ln[(fasting triglycerides (mg/dL)×fasting glucose (mg/dL)/2)]. The continuous variables between groups were compared by the Mann-Whitney U test, and categorical variables were compared by the chi-square test. Receiver operating characteristic curve (ROC) analysis estimated the predictive value of the TyG index for DF. Logistic regression models carried out to evaluate the associations between the TyG index and the risk of DF. The TyG index was significantly higher in the DF group comared to no-DF group ($P < 0.001$). The logistic regression revealed that an increased TyG index was associated with an higher risk of DF after adjusting for potential confounders, Odds Ratio of 82 (95% CI 8.8–765.5, $P < 0.001$). Moreover, ROC analysis indicated the discriminatory ability of the TyG index in DF presence with an area under the curve (AUC) of 0,98 (95% CI 0,970-0,994, $P < 0.001$). High TyG index could be cosidered a risk factor in the decelpment of DFUs. New studies are therefore necessary to confirm our finding to investigate the pathological mechanism involved in this process

EP156

New approach to the analysis of Second Tier Tests in Extended Newborn Screening: direct injection LC-MSMS in the determination of homocysteine, propionylglycine and methylmalonic, methylcitric and ethylmalonic acids.

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Background

In the present work, a new LC-MSMS method with direct injection (FIA) without the use of chromatographic columns has been developed for the analysis of Second Tier Tests in the extended newborn screening (ENS) path of homocysteine (HCY), propionylglycine (PGC) and methylmalonic (MMA), methylcitric (MCA), and ethylmalonic (EMA) acids.

Materials and methods

In each well of a 96 plate, 2 DBS of 3.15mm diameter are punched, added with 80ul of 60%H2O/40%CH3CN extraction solution containing the deuterated standards in a concentration equal to 10umol/L and 10uL of 0.025M solution of TCEP (reducing for HCY). The plate is incubated for 45 min at 37°C, shaken at 600rpm and centrifuged at 2000rpm for 2 min. Then evaporated with N2 for 30 minutes and taken up again with 70uL of 60%H2O/40%CH3CN. It is analyzed with a QSight225 spectrometer in FIA at 0.4 mL/min with 60%H2O/40%CH3CN 0.02% HCOOH eluent. The total analysis time is 1.8 min. 8 calibration points and three controls were used for each analyte. The data is processed with Simplicity 3Q MD software.

Results

Each calibration curve presents linearity with r2 >= 0.99. Normal subjects show values < 1 uM except for endogenous HCY with values between 2 and 9 uM. The LOQ found is 1.0 uM for each analyte. MMA is detected with the specific m/z 55 fragment which excludes the interference of Succinic Acid (SA) evaluated instead with the m/z 73. The presence of SA on an analyzed sample generates a false positive of MMA at 40 uM with m/z 73 that is completely eliminated using the m/z 55 (< 1 uM). A positive sample of 29 uM in MMA detected with a column on XEVO TQD showed a concentration of 27.7 uM with FIA methodology. All negative and positive samples analyzed with Qsight 225 FIA correlate with XEVO TQD and QSigh 225 methods using chromatographic columns.

Conclusions

The results obtained show that the LC-MSMS FIA calibrated method is faster and simpler than classic column methods without loss of sensitivity and analytical reproducibility in the quantitative analysis and is also extendable to other molecules not only involved in the ENS evaluation process.

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EP157

Spinal muscular atrophy newborn screening program in the Campania region (Italy): current limitations and potential perspectives associated with the EonisTM platform.

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Spinal Muscular Atrophy (SMA), one of the leading genetic causes of mortality for children under the age of 2 years, results in the loss of motor neurons that control voluntary muscular movements, thereby leading to progressive muscle weakness/atrophy. The incidence is 1:10000–12000 live births worldwide. More than 95% of SMA cases has homozygous deletion of exon 7 of the SMN1 gene.Three targeted therapies are currently available for SMA. If treated early, patients may achieve motor developmental milestones. Therefore, early diagnosis through newborn screening (NBS) is crucial to improve long-term outcomes (1).

To date, SMA NBS programs are active in 12 Italian regions. In Campania, a pilot study started on April 2023 in Naples, at the CEINGE Institute, Regional Reference Center for NBS, in collaboration with the Neurology Unit of the Santobono-Pausilipon Children's Hospital.

The NBS test is performed with the CE_IVD declared EonisTM platform (Revvity), by using DNA extracted from Dried Blood Spot (DBS), and real time PCR targeting SMN1 exon 7 and RNase P (RPP30) as reference gene. Absence of the amplification curve corresponding to SMN1 is consistent with SMA molecular diagnosis. Carrier status is not detected or reported.In first year of activity, we analyzed 41818 DBS and identified 5 positive newborns; 4 were treated with gene therapy within the first 20 days of life. To date, disease incidence (1:8364) appears significantly higher in our region compared to the average of the other Italian regions (~1:13000), despite adherence to the program, which is on a voluntary basis, is about 90%.
As significant limitation, the test failed to amplify RPP30 in 5/41818 DBS, depending on the presence of a polymorphic missense variant that prevents RPP30 amplification in homozygotes, whereas affects RPP30-related Ct values in heterozygotes. Since unbalanced/absent amplification of the reference gene is not recommended in a diagnostic test, a new primer pair located in a genomic region not affected by common polymorphisms should be used. By solving this bias, the screening program would implement its diagnostic perspective because it could also identify heterozygous carrier of SMN1 deletion and consequently increase the detection of families at procreative risk of SMA.

Study supported by Campania Region and Novartis Gene Therapy.

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EP158

Molecular diagnosis of inherited cardiac diseases in a large population from Southern Italy through next generation sequencing-based analysis

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Inherited cardiac diseases are a wide group of heart diseases, including cardiomyopathies and arrhythmic disorders. Taken together, they have a 3% prevalence in the general population and are the main cause of cardiac morbidity and mortality also among young, being a predisposing factor for sudden cardiac death. The clinical heterogeneity of cardiac diseases is associated also to complex molecular bases. Indeed, their molecular diagnosis has been greatly improved by the diffusion of next generation sequencing-based strategies allowing the simultaneous analysis of a high number of genes. In this context, we have designed and implemented in routine diagnostic setting a 207 genes panel for cardiac diseases analysis. From 2020 to May 2024 a total of 704 patients have been analyzed. For each patient genomic DNA was extracted from peripheral blood sample, used to obtain an enriched library (Agilent SureSelect technology) and sequenced using the MiSeq instrument (Illumina). Specific bioinformatic tools have been used for variants annotation and interpretation. Totally, 115 patients carried a pathogenic variant, most occurring in the MYBPC3, MYH7, OBSCN, DES, PKP2, TNNI3, LMNA, SCN5A, KCNQ1, and KCNH2 genes. Notably, pathogenic variants were found also in genes less common for cardiac diseases. Several not reported variants were also detected in different genes and some were classified as pathogenic/likely pathogenic variants based on the predicted effect at protein level. Finally, variants of unknow significance, whose pathogenicity cannot be defined based on current knowledge were also identified. Our data shows the reliability of large genes panel analysis for the molecular diagnosis of heterogeneous conditions like cardiac diseases. In addition to well-established pathogenic variants, novel and unknown significance variants were also detected for further investigations. Finally, the cascade genetic test at the family level allowed the identification of at risk carriers that have been admitted to proper cardiologic examinations and surveillance programs.

EP159

Reference values for serum Cystatin C in Very Preterm Infants at birth

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Background

Preterm infants suffer high rates of perinatal morbidity and mortality. With an estimated incidence of 12-18%, acute kidney injury (AKI) is among the main causes of mortality in the preterm population, especially in those of a lower gestational age. Serum cystatin C (CysC) is a promising biomarker of kidney function. To aid in the interpretation of serum CysC concentrations in preterm infants, we aimed to establish a reference interval (RVs) in this population.

Methods

The study subjects consisted of preterm infants born at Policlinico Modena Hospital (Italy) and admitted to the local Neonatal Intensive Care Unit (NICU). Patients with any anamnstic, clinical or laboratory evidence of renal and/or urinary tract disease were excluded.The correlation between CysC levels and gestational age was tested with Spearman coefficient of correlation and comparing CysC concentration in 2 groups of preterm infants (23+0-27+6 wks vs 28+0-30+0 wks) with Mann-Whitney U test.CysC was measured in serum samples withdrawn within 24 hours from delivery with an immunoturbidimetric method (PETIA) on a AU5800 analyzer (Beckman Coulter).RVs were estimated using the robust method suggested by CLSI 28-A3 after exlcuding outliers (Tukey). Statistical analysis and RV were calculated with MedCalc 18.2.1.

Results

A total of 82 preterm infants (47 male and 35 female) were included. Median Cys-C concentration was 1.61 mg/dL (min-max: 0.84-2.50; interquartile range: 1.46-1.83). No correlation was found between CysC and gestational age (Spearman's coefficient 0.06, 95%CI: -0.15;0.28, P=0.54); additionally, no difference was found in CysC concentrations between preterm infants born before or after 28 weeks of gestational age (medians 1.61 vs 1.69 mg/L, P=0.88).After the exclusion of a single outlier result, we obtained the following RVs: lower limit 1.10 mg/L (90%CI: 1.02-1.19), upper limit 2.17 mg/L (90%CI: 2.08-2.26).

Conclusion

We report a RVs for CysC in preterm infants. Since creatinine concentrations in neonates at birth are influenced by maternal concentrations, it is important to have accurate interpretive criteria for CysC in a pediatric population at high risk of renal complications.

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EP160

CONFRONTO TRA METODI: IL DOSAGGIO DEL RAME SIERICO COLORIMETRIA VS ASSORBIMENTO ATOMICO (GOLD STANDARD)

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INTRODUZIONE: Il rame è un oligoelemnto fondamentale per il corretto funzionamento di numerosi enzimi cellulari che sfruttano la sua facile e rapida conversione da Cu + (ione rameoso) a Cu2+ (ione rameico). Al di fuori dell'intervallo di concentrazione fisiologico (70 – 155 g/dl) si configurano gravi situazioni patologiche, quali il Morbo di Wilson (eccesso di Cu) e la Sindrome di Menkes (carenza di Cu). Il Gold-Standard per il dosaggio del Cu-Si è la Spettrofotometria in Assorbimento Atomico. In questo contributo si presentano i risultati del confronto di una Metodica Colorimetrica con il Gold Standard.

MATERIALI E METODI: Abbiamo analizzato 104 campioni e confrontato i valori ottenuti dai dosaggi effettuati con la metodica di riferimento, con i valori ottenuti dai dosaggi effettuati con la Metodica Colorimetrica - Sentinel Diagnostic applicata su piattaforma Siemens-Dimension. I risultati sono stati analizzati utilizzando metodi statistici di regressione Passing-Bablock e Bland-Altman. Sono state effettuate prove di Ripetibilità e Riproducibilità del metodo.

RISULTATI: Dall'analisi condotta sui dati ottenuti con le due metodiche abbiamo ottenuto una retta di regressione con intercetta 14,96 e pendenza 0,99. Il coefficiente di correlazione di Pearson è 0,94 e il coefficiente di determinazione R2 è 0,89. Dal confronto effettuato con l'analisi di Bland-Altman abbiamo ottenuto un BIAS di 14,3 e un intervallo di confidenza al 95% con limite superiore di +36,9 e un limite inferiore di -8,3.

CONCLUSIONI: Il confronto ha dimostrato una ottima performance del metodo colorimetrico rispetto al Gold Standard. L'elevata affidabilità ed efficacia del test applicata su piattaforma ad alta automazione, rende fruibile il test in tutti i laboratori, consente una riduzione dei tempi di analisi e refertazione, oltre ad un'ottimizzazione delle risorse economiche e umane.

EP161

Diagnosis of a rare form of Tyrosinemia: Integration of NGS molecular analysis with mRNA analysis.

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Tyrosinemias are a group of autosomal recessive disorders due to the alteration of the tyrosine degradation pathway. These disorders may occur in an acute form showing hepatic and renal failure, while the subacute and chronic forms cause central and peripheral neuropathy. Diagnosis is based on the detection of altered tyrosinemias biomarkers levels integrated with the molecular analysis, to identify the specific enzymatic defect. Newborn screening (NBS) provides an early detection of tyrosinemias biomarkers, i.e. succinyl acetone (SA) and tyrosine. We report the case of a newborn showing increased levels of SA and tyrosine on NBS, and SA traces in the urine. Kidney and liver function biomarkers were normal. To perform a definitive diagnosis, patient underwent genetic test by NGS multigenic panel, including 5 genes associated with tyrosinemias. The molecular analysis showed no pathogenetic variants in FAH gene and the presence, in heterozygosis, of the c.68-12G>A and c.464_471delinsCTGGG (p. Val155_Asp157delinsAlaGly) variants in GSTZ1 gene, coding the maleylacetoacetate isomerase enzyme. Both variants were not reported in the HGMD mutational database and have been classified as variants of uncertain significance (VUS), according to the ACMG criteria. Family segregation allowed to determine the configuration in trans of the variants. In-silico analysis highlighted the possible formation of a cryptic splicing acceptor site in c.68-10. mRNA analysis confirmed the bioinformatics prediction and revealed a 10 bp retention, with shifting of the reading frame and the creation of a premature stop codon (p. Ala22Valfster28). Based on these findings, both variants were re-classified as pathogenic.This study demonstrates how an integrated extensive genetic analysis enabled the definitive diagnosis of maleylacetoacetate isomerase deficiency and optimized patient clinical management. Moreover, the mRNA analysis allowed the re-evaluation of the two identified variants, pointing out the importance of a molecular biology laboratory, that can perform functional studies of VUSs to definitively attribute pathogenicity.

EP162

A novel homozygous deletion in the Carbonic Anhydrase 5 (CA5A) gene associated with a severe form of hyperammonemia.

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Hyperammonemia is a metabolic condition, potentially lethal for patients of all ages, characterized by raised levels of ammonia. Hyperammonemias can be distinguished into primary, due to urea cycle deficiency, and secondary, that could indirectly interfere with urea cycle function. Secondary hyperammonemias can be caused by acquired non-genetic factors and hereditary factors as other congenital errors of metabolism: dibasic amino acid transport deficiency, organic aciduria, defects in fatty acid oxidation. Genetic test allows to establish an accurate diagnosis as it identifies the specific enzymatic alteration. A newborn showing hyperammonemia came to our attention, after experiencing respiratory distress with rapid development of intestinal necrosis, following hypotensive shock. The Newborn Screening (NBS) highlighted the presence of hypocitrullinemia. Due to severe hyperammonemia, genetic test was performed promptly. The NGS gene panel consists of 76 genes including both genes associated with urea cycle defects and genes involved in secondary hyperammonemias. The molecular analysis did not reveal any potentially pathology-associated point mutations in the analysed genes. Subsequently we performed visual inspection of the NGS reads, comparing the patient reads against three different healthy controls. This analysis revealed the absence of reads at exon 1 locus of the CA5A gene, suggesting the presence of a homozygous deletion including the entire exon 1. The deletion was confirmed by duplex PCR. It is a novel mutation in the CA5A gene, coding VA carbonic anhydrase: definition of the genomic coordinates, comprising the deletion breakpoint, is in progress.The genetic test enabled a definitive diagnosis of encephalopathy due to VA carbonic anhydrase deficiency and led to define an accurate medical treatment.This study proved how the integration of bioinformatics with biochemical-clinical data allowed the identification of a large deletion, despite the well-known NGS limitation to identify large macro-rearrangements.

EP163

RNA m6A modification in obese children affected by nonalcoholic fatty liver disease

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Objectives: Non-alcoholic fatty liver disease (NAFLD) encompasses a broad spectrum of pathologies ranging from pure fatty liver to steatohepatitis with fibrosis and even cirrhosis. It is the most common form of chronic liver disease in the paediatric population. The molecular mechanisms leading to the progression of NAFLD remain unclear, but recent studies have shown that epigenetic modification of the mRNA N6-methyladenosine (m6A) contributes to the progression of this disease. M6A refers to the methylation of the sixth N atom of adenine in RNA molecules. The aim of this study is to investigate the changes in the level of RNA m6A methylation in NAFLD obese and non-NAFLD obese children.Methods: The study population included 31 overweight/obese (BMI ≥ 85th and 95th percentiles for sex and age, respectively) children and adolescents with NAFLD and 28 overweight/obese children and adolescents without NAFLD (11.8±2.4 vs. 10.6±2.6 years) participated in the study. Liver disease was defined using abdominal ultrasonography. Fresh blood samples were collected from all subjects in 3 mL EDTA evacuated blood tubes. Total RNA was extracted via TRIzol (Invitrogen, CA, USA), and RNA quality was assessed using NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA). The m6A modification level of total RNA was investigated using EpiQuik m6A RNA Methylation Quantification Kit (Epigentek Group Inc., Farmingdale, NY, USA) according to the manufacturer's instructions. Differences were assessed with Mann-Whitney test, and diagnostic performance was calculated with Receiver Operating Characteristics (ROC) curve analysis. The level of statistical significance was set at $p<0.05$.Results: We found that m6a% was significantly lower in overweight/obese NAFLD patients than in those without NAFLD (mean 0.001±0.0002% vs. 0.017±0.013%, $p<0.0001$). The area under the ROC curve (AUC) for m6a% was 0.83 (95% confidence interval: 0.71-0.97, $p<0.0001$).Conclusions: Our preliminary results suggest that RNA m6a% may influence the pathologic mechanism of NAFLD. Further studies are therefore required to validate these findings.

EP164

Role of serum subclasses Immunoglobulin G (IgG) and serum free Light Chains (fLCs) Kappa and Lambda in the progression of liver fibrosis

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Background and aims

Chronic liver diseases (CLDs) are characterized by chronic inflammation of the liver that causes formation of fibrotic tissue and in more severe cases to cirrhosis, finally leading to hepatocellular carcinoma (1-2). Hepatic fibrosis results from several triggers such as chronic infection, excessive alcohol consumption (alcoholic liver disease-ALD), non-alcoholic fatty liver disease (NAFLD) and autoimmune liver diseases (3). B cells play a critical role in CLDs causing polyclonal synthesis of immunoglobulins (Ig), mainly IgG, that may modulate monocyte activity towards a pro-inflammatory and profibrotic phenotype. Its outcome may be influenced by IgG subclass distribution since each IgG isotype displays different biological properties (4). Moreover, serum free light chains (fLC), reflecting the immune function of B-cells, is strongly associated with inflammation and disease activity (5). We investigated serum IgG subclasses and fLCs distibution in patients with different CLDs conditions. In particular, we studied patients with cirrhosis compared to steatosis-NASH and healthy controls (HCs).

Methods

IgG1–4 subclasses and fLC Kappa and Lambda were evaluated in 50 patients with CLDs and 30 HCs by turbidimetry. (Binding Site – Optilyte).

Results

We observed high IgG1 and IgG3 subclasses levels in cirrhosis patients compared to controls [(p* value < 0,013, 0,035)] and steatosis-NASH patients [(p*value<0,023, p*value<0,038). Other IgG subclasses don't show significant differences between patients analyzed. fLC Kappa and Lambda show a significant increase in cirrhosis patients compared to controls [(fLC Kappa p* value < 0,001),(fLC Lambda p* value < 0,004)] and steatosis-NASH patients [(fLC Kappa p* value < 0,03),(fLC Lambda p* value < 0,04)].

Conclusion

Our data highlight an increase in IgG1, IgG3 subclasses and fLCs in advanced stage of CLDs. This could be related with a higher inflammatory state in severe patients. These biomarkers are directly proportional to the staging of the disease.

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EP165

Analisi del liquido ascitico in pazienti epatopatici con sospetta peritonite batterica spontanea

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INTRODUZIONE

La peritonite batterica spontanea (PBS) è un'infezione batterica del liquido ascitico in assenza di cause intra-addominali primitive o di altre infezioni principali.

Lo scopo dello studio è stato quello di confermare la diagnosi di PBS nei pazienti epatopatici afferenti all'Unità di Epatologia dell'Ospedale Evangelico Betania.

MATERIALI E METODI

320 pazienti ricoverati nel reparto di Epatologia, con sospetto clinico di PBS, sono stati sottoposti a paracentesi con prelievo di liquido ascitico ed inviati tempestivamente in laboratorio.

Del liquido aspirato, una quota è stata rapidamente inoculata nei flaconi per aerobi ed anaerobi (BD-BACTEC FX40) e messi in incubazione a 37°C per 10 gg,

mentre l'altra quota è stata messa in provette (Vacutainer), effettuato l'esame chimico fisico con dosaggio di albumina, LDH, glucosio (COBAS-PRO-Roche)

e l'esame Polimorfonucleati (PMN) con contaglobuli automatico (DxH900 Beckman Coulter).

RISULTATI

Dei 320 pazienti, 296 hanno confermato attraverso gli esami clinici e microbiologici la diagnosi di PBS,

mentre gli altri 24 avevano altro tipo di ascite.

In accordo con i criteri adottati, 43 avevano una classica PBS con PMN > 250 cellule mm3 ed esame colturale positivo,

mentre gli altri 253 pazienti avevano PMN > 250 cellule mm3 ed esame colturale negativo (ascite neutrocitica).

Dei 43 pazienti con esame colturale positivo, il 62,79% dei germi isolati erano Gram negativi. Inoltre, dai risultati dell'esame

chimico-fisico, è stato dimostrato che i pazienti con PBS,

rispetto a quelli con altro tipo di ascite, avevano un aumento di LDH, enzima sempre presente in casi di infiammazione, e una diminuzione di glucosio, substrato utilizzato dai batteri nel loro metabolismo.

CONCLUSIONI

La diagnostica di laboratorio gioca un ruolo fondamentale per la gestione tempestiva e appropriata dei pazienti con PBS.

È chiaro che la conta dei PMN nel liquido ascitico rappresenta il "gold standard" per la diagnosi.

EP166

EVALUATING BIOMARKERS FOR ACCURATE STAGING OF LIVER FIBROSIS: A NON-INVASIVE APPROACH

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Background and aims

Chronic liver diseases (CLD) represent a global health problem (1). It is known that chronic liver inflammation leads to development of fibrotic tissue and in more severe cases to cirrhosis, finally leading to the development of hepatocellular carcinoma (2). Novel circulating markers for CLD staging are in high demand. Extracellular matrix (ECM) components offer considerable promise in this area(3). Given the key role of ECM components in liver fibrosis we have focused our attention on hyaluronic acid (HA), laminin (LN), collagen type III N-peptide (PIIIP N-P), type IV collagen (C-IV), cholylglycine (CG) and Golgi protein-73 (GP73) (4). The introduction of these biomarkers could be a promise field in the early diagnosis and staging of liver fibrosis(5).

This case-control study evaluates the diagnostic potential of ECM-related markers in HCV-positive patients with different degrees of fibrosis.

Methods

96 patients were grouped into mild-to-moderate (F1-F2, METAVIR score, n=50) and advanced fibrosis (F3-F4, n=47) groups. Inclusion criteria were detectable HCV RNA and absence of other liver diseases/co-infections. Levels of HA, LN, PIIIP N-P, C-IV, CG, and GP73 were measured with MAGLUMI 800 CLIA.

Results

Circulating ECM markers were significantly increased ($p<0.001$) in the pathological group (F3-F4) compared to controls (F1-F2), namely CG (0.8 to 2.3 μg/mL), C-IV (15 to 55 ng/mL), HA (65 to 85 ng/mL), PIIIP N-P (267 to 462 ng/mL), and GP73 (15 to 27 ng/mL). C-IV demonstrated the highest classification performance (ROC-AUC=0.88, 95%CI:0.81-0.95). Multivariate regression, including also age and gender, indicated an AUC of 0.85 (95% CI: 0.78-0.92) for C-IV alone, and of 0.92 (95% CI: 0.87-0.97) when combined with PIIIP N-P.

Conclusions

This study underscores the potential of ECM-related biomarkers in non-invasively staging CLD, suggesting their integration in diagnostics could reduce invasive biopsies.

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EP167

Red Blood Cell Distribution Width-to-Platelet Ratio (RPR): A Non-Invasive Marker for Predicting Decompensation in Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) Related Chronic Liver Disease.

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BACKGROUND-AIM

First Decompensation Event (DE) is looked upon as a watershed in Chronic Liver Disease (CLD) related to MASLD. Both acute (AD) and non-acute (NAD) DE significantly worsens severity and prognosis of the disease. Despite the high reliability of Clinically Significant Portal Hypertension (CSPH) in predicting DE, its usage is very restricted due to invasiveness and costs. Hence, novel non-invasive predictive tools are needed. RPR has proven to indicate hepatic fibrosis in MASLD, while no data related to MASLD-Compensated Advanced CLD (MASLD-cACLD) prediction is currently available.

METHODS

In this study, 40 controls and 150 MASLD-cACLD patients were enrolled and followed semiannually for 3 years. Baseline assessments included biochemical and clinical parameters, Liver Stiffness Measurement (LSM), Child-Pugh (CP) score, Model for End-stage Liver Disease (MELD) score, AST to Platelet Ratio (APRI), Fibrosis-4 (FIB-4) score, Albumin-Bilirubin (ALBI) score, ALBI-FIB4, and RPR. Decompensation events (DEs) were recorded, and CSPH was assessed according to guidelines.

RESULTS

Out of 150 MASLD-cACLD patients, 43 (28.6%) progressed to decompensated ACLD (dACLD) within a median of 28.9 months (29 NAD; 14 AD). Baseline RPR values were significantly higher in cACLD patients compared to controls, and the values of MELD, CP, APRI, FIB-4, ALBI, ALBI-FIB4, and LSM were significantly elevated in progressed dACLD patients compared to those who remained stable (all $p < 0.0001$, except for FIB-4 [$p = 0.007$] and ALBI [$p = 0.011$]). ROC analysis identified RPR values > 0.472 and > 0.894 as the best cut-offs in predicting first DE within 3 years and AD-related mortality, more than other biological markers such as APRI, FIB-4, ALBI, ALBI-FIB4, MELD, CP, and LSM (all $p < 0.0001$). Overall, RPR (adjusted Hazard Ratio [aHR]: 1.91; 95% CI: 1.72-1.98; $p = 0.02$) and baseline CSPH (aHR: 1.84; 95% CI: 1.72-1.91; $p = 0.04$) were significantly associated with DEs. Patients with both baseline CSPH and RPR > 0.472 had a higher risk of DE (HR: 3.10; $p = 0.0023$).

CONCLUSIONS

These findings propose RPR as a valid non-invasive tool for predicting the timing and modalities of DE in MASLD-cACLD patients.

EP168

The utility of GP73 in patients with NAFLD

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Background: The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide with unclear etiology and pathogenesis. Golgi protein 73 (GP73) is a type II Golgi membrane protein upregulated in livers from patients with a variety of liver diseases such as viral and non-viral hepatitis, cirrhosis and hepatocellular carcinoma. We evaluate the utility of GP73 in the early diagnosis of non-alcoholic fatty liver disease (NAFLD).Methods: Fifty-one patients with NAFLD were allocated in groups according to their status of fibrosis (significative or advance), and there were 15 healthy controls. Serum GP73 was determined with a chemiluminescence immunoassays (Maglumi X8 -Snibe Co. Ltd). The sensitivity (SE), specificity (SP), and the area under the receiver operating characteristic curve (AUROC) were calculated.Results: The serum GP73 concentration was significantly higher in patients with NAFLD than controls (mean 27.5±9.02 ng/ml and 19.6±29.7 ng/ml, respectively), and in patients with advance fibrosis than significative fibrosis (mean 25±8 ng/ml and 32±8 ng/ml, $p>0.05$, respectively). The area under ROC curve is 0.84 (95%CI 0.7-0.9). Using a cut off of 45 ng/ml, as indicated by the company, the SE was 50% (95%CI 1.26% to 98.74%) and SP was 23% (95%CI 13.53% to 35.19%). Our preliminary results suggest an optimal cut off 12.6 ng/ml with SE and SP of 98% and 63%, respectively.Conclusion: Serum GP73 may be an effective and reliable marker for the diagnosis of advanced fibrosis and may be useful for management of NAFLD patients, but the evidences are still limited. Considering that the concentration of GP73 is consistent with the stage of liver fibrosis, GP73 may be an indicative marker for advance fibrosis. Further studies to assess the diagnostic accuracy of GP73 for NAFLD patients are needed, and the combination with other markers should be evaluated.

EP169

Bioelements (Mg, Fe, Zn, and Cu) content in liver biopsies from healthy donors and patients with cirrhosis and hepatocarcinoma

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Bioelements such as magnesium, iron, zinc, and copper play an essential role in key metabolic processes in the liver. The occurrence of certain pathologies can lead to a dysregulation of their homeostasis. This study aims to determine the content of these elements in liver biopsies obtained from healthy donors and from patients with cirrhosis and hepatocarcinoma.The levels of magnesium, iron, zinc, and copper were measured using atomic absorption spectrometry in biopsies obtained at the time of liver transplantation in 36 patients with cirrhosis (CIRs) and hepatocarcinoma (HCCs) and in biopsies obtained from 18 deceased donors with healthy livers (CTRLs). The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Sapienza University–Policlinico Umberto I (Ref. No. 1129/14.12.06).In liver biopsies from healthy donors, the levels of magnesium (159±15.8 µg/g wet wt), iron (124±115 µg/g wet wt), zinc (73±20.4 µg/g wet wt) and copper (6.99±3.73 µg/g wet wt) were within the range of those reported in the literature. In a previous paper we investigated the role of magnesium in the liver of cirrhotic patients [1] and the results of the present study confirm that different liver pathologies as cirrhosis and HCC can influence hepatocyte bioelement metabolism, producing a significant decrease in magnesium and zinc content accompanied by an increase in copper levels. No significant changes were observed in iron content. Hepatocellular magnesium and zinc depletion may be related to inflammation, while copper elevation may lead to increased oxidative stress, suggesting a critical role for these elements in the interplay between oxidative stress and inflammation in liver diseases.[1] Parisse, S.; Gianoncelli, A.; Isani, G.; Gambaro, F.L.; Andreani, G.; Malucelli, E.; Aquilanti, G.; Carlomagno, I.; Carletti, R.; Mischitelli, M.; et al. Severity of Hepatocyte Damage and Prognosis in Cirrhotic Patients Correlate with Hepatocyte Magnesium Depletion. Nutrients 2023, 15, 2626. https://doi.org/10.3390/nu15112626

EP170

Variants in NOTCH2 gene and clinical signs of Alagille syndrome

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Introduction: Alagille syndrome (ALGS) is a rare autosomal dominant genetic disorder caused by pathogenic variants in two genes: Jagged Canonical Notch Ligand 1 (JAG1 about 98-99% of cases) and Notch Receptor 2 (NOTCH2 about 1-2% of cases). It is characterized by multiorgan clinical signs and with incomplete penetrance and variable expression. The prevalence is approximately 1:30.000-1:70,000. We aim to report rare NOTCH2 variants identified in patients with different liver diseases to increase the knowledge about of NOTCH2 variants.
Methods: We performed genetic analysis of 230 patients with cholestasis and hepatopathies by Next Generation Sequencing (NGS) using a panel of 59 genes associated with liver diseases. Rare variants were confirmed by Sanger sequencing. Pathogenicity assessment was performed by ACMG guidelines. Bioinformatic predictions were also performed.
Results: Eleven variants in NOTCH2 gene were identified in 10 unrelated patients. Two variants were classified as pathogenic variants (1 nonsense variant and 1 frameshift variant), the remaining 8 missense variants and 1 synonymous variant were classified as variant of uncertain significance (USV). Three patients did not meet the criteria to suspect ALGS and carried variants predicted not pathogenic. Whereas 7 patients had symptoms consistent with clinical suspicion of ALGS: 2 with null variants, 2 with variants predicted to impact protein function by bioinformatics, 1 with a synonymous variant together with variants in glycosylation-related genes (COG5 and ALG1), and 2 carried variants predicted as benign.
Conclusion: Our results increased the knowledge about NOTCH2 variants and the related phenotype, allowing to improve genetic diagnosis of ALGS.

EP171

Golgi protein 73 and its diagnostic value in patient with HCC-HCV correlated: a case report

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Golgi protein 73 (GP73) is a type II Golgi membrane protein mainly expressed by bile duct epithelial cells in the normal liver, and it is poorly expressed in hepatocytes. In patients with acute or chronic liver diseases and especially in patients with hepatocellular carcinoma (HCC), the expression of GP73 is significantly up-regulated in hepatocytes. Similarly, serum levels of alpha fetoprotein (AFP) are high in more than 70% of HCC patients, and it is widely used for diagnosis of HCC. Although the mechanisms leading to elevated GP73 levels are unknown, its potential role in the diagnosis of HCC are of great interest.Here, we describe a case of a 55-year-old man, with cirrhosis due to hepatitis C (HCV) infection and HCC, who was referred to our institute in 2023, with an indication to perform orthotopic liver transplantation (LT). Before the LT, patients had high levels of AFP, transaminases, platelets count and gamma GT, supporting the state of chronic hepatopathy. After LT, the serum levels of these biomarkers were in the normal ranges, and patients had received a pharmacological therapy with tacrolimus. We determined the serum levels of GP73 after the LT using a chemiluminescent immunoassay (manufacturer's range <45 ng/mL), and we observed a significantly high GP73 levels in patient's serum (123 ng/ml). The current literature reports discordant opinions about the kinetics of GP73 before and after surgery in HCC patients who have received curative resection, some authors suggest that its levels decrease after surgery, others suggest that they remain elevated, as in our case report.At present, the biological function of GP73 is not fully understood. Further investigations are needed to assess the diagnostic value of GP73, and determine if its serum levels can be used to monitor patients with liver disease and predict patient's outcomes, or develop a new therapeutic target for HCC.

EP172

Relationship between lower PSA screening opportunity during the COVID-19 pandemic and increased mortality for prostate cancer in Italy

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Background: There is confirmed evidence that several cancer screening programs in Italy were disrupted during the coronavirus disease 2019(COVID-19) pandemic, including prostate specific antigen (PSA) testing, the demand for which had drastically decreased, especially in the early phase of the pandemic. We therefore planned this study to investigate whether the reduced PSA screening opportunities may have led to higher early mortality from prostate cancer.Methods: We performed a digital search of the official database of the Italian National Institute of Statistics (ISTAT) to identify the total number of deaths registered for prostate cancer in the first two years of the COVID-19 pandemic (2020-2021; pandemic period) compared with the previous two years (2018-2019; pre-pandemic period). The search was carried out according to the following variables Area (entire country), age (5-year classes), sex (male), cause of death according to the European Short List (prostate cancer) and period (from year 2018 to 2021). Results: The total number of deaths due to prostate cancer increased by approximately 3.0% during the COVID-19 pandemic compared to the previous two years (15856 vs. 15399). A particularly high mortality rate was observed in the older age groups, with a 7.5% increase in mortality for this type of cancer in people aged 80 years or older. The largest increase in deaths from prostate cancer during the pandemic period was observed in the very elderly (i.e. those aged 95 years and older; +19.5%).Conclusions: The COVID-19 pandemic has significantly impacted healthcare in Italy and prostate cancer screening, especially in the elderly, was no exception. The increased mortality observed during the COVID-19 pandemic could therefore be due to the postponement or cancellation of PSA testing, which has led to a delayed diagnosis of a considerable number of curable cancers.

EP173

Involvement of nicotinamide N-methyltransferase enzyme in radioresistance of head and neck squamous cell carcinoma cell.

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Head and neck squamous cell carcinoma (HNSCC) arise from the mucosal epithelium of the oral cavity, pharynx and larynx. It represents the sixth most common neoplasm worldwide, being responsible for approximately 2% of all cancer-related deaths. Despite progress in diagnosis and therapy, 5-year survival rate is less than 50%, due to diagnostic delay and resistance to traditional chemo- and radiotherapy. Therefore, the discovery of new molecules able to modulate cancer cell response to conventional therapies and that could be used as novel therapeutic target is crucial.Nicotinamide N-methyltransferase (NNMT) enzyme was upregulated in many solid tumors, including oral squamous cell carcinoma, among HNSCCs. NNMT was found to positively influence tumor cell proliferation, migration and invasiveness, as well as resistance to chemotherapy. Enzyme levels were also markedly increased in association with cancer stem cells (CSCs). Given the role played by CSCs in tumor aggressiveness and resistance to radio- and chemotherapy, these results strongly highlight the leading function exerted by NNMT in cancer cell metabolism.In this study, distinct parental (PA) and radio-resistant (RR) cell subpopulations were selected from the commercial FaDu cell line, obtained from a hypopharyngeal tumor of a HNSCC patient. Subsequent Real-Time PCR analyses were performed on both cell populations, in order to evaluate the expression level of stem cell markers and NNMT. Further phenotypic characterization was achieved by subjecting FaDu-PA and FaDu-RR cell clones to colony formation assay.Results obtained showed that FaDu-RR cells exhibited significantly higher expression levels of stem cell markers OCT4 and NANOG compared with FaDu-PA. Moreover, radio-resistant cells demonstrated a markedly enhanced clonogenic ability with respect to that of parental counterpart. Interestingly, NNMT expression was found to be increased in FaDu-RR versus FaDu-PA cells.Results obtained, although preliminary, seem to suggest that the enzyme could serve as a promising target for effective anticancer therapy. Further ongoing analyses will speculate and clarify the potential contribution of NNMT to the molecular and cellular mechanisms promoting resistance of HNSCC cell to radiation treatment.

EP174

FTIR Imaging as a new tool for investigating Oral Tongue Squamous Cell Carcinoma

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Oral Tongue Squamous Cell Carcinoma (OTSCC) is the most common malignancy of the oral cavity, with ca. 40% of all oral cancers. It derives from the stratified squamous epithelium of oral mucosa, causing the invasion of the connective tissue. Due to the high density of nerve and muscle bundles and the complexity of the lymphovascular system, it shows high aggressiveness and locoregional recurrences. Pathological staging, mainly based on morphological features (primary tumor size, invasion of adjacent tissues, metastasis in regional lymph nodes and other organs), is not always sufficient, due to the presence of patients with the same OTSCC staging diagnosis, who show a poor outcome to therapeutic protocols.This study aims to investigate a new analytical approach, based on Fourier Transform InfraRed Imaging (FTIRI) spectroscopy coupled with multivariate and univariate analyses, to improve the prognostic and predictive potential of tumor staging through the identification of new reliable spectral markers and deepen knowledge on tumor growth mechanisms verifying the involvement of the peritumoral and extratumoral regions. N. 29 Formalin-Fixed Paraffin-Embedded (FFPE) biopsies (N. 26 with histological diagnosis of Stages I – IV OTSCC, and N. 3 without OTSCC, taken as control samples) were analyzed. A different macromolecular composition was found in the tumor mass in relation with the stage, providing reliable spectral markers which improved the morphological staging characterization. Moreover, similar spectral features between the tumor mass and the region very close to it were observed mainly in advanced stages (III and IV) respect to early ones (I and II), letting hypothesize the presence of a major tumor involvement in the peritumoral region.

EP175

Longitudinal detection of somatic mutations in oral cancer patients' saliva

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Background: Oral squamous cell carcinoma (OSCC) is a relatively common malignancy worldwide with poor prognosis and high mortality and recurrence rates after treatment. It is, therefore, crucial to be able to detect recurrence or minimal residual disease following radical treatment early so that salvage surgery can be performed while the disease is still resectable. In this scenario, aim of this study is to create a liquid biopsy-based analytical pipeline able to detect somatic tumor mutations in a cohort of 17 oral carcinoma-affected patients undergoing follow-up for minimal residual disease detection.
Methods: The first saliva sample was collected before surgery while the rest were collected during the subsequent visits according to the follow-up schedule. Salivary DNA (sDNA) samples were extracted and a 52-gene panel was used for somatic variants detection.
Results: Overall, 41.2% of samples collected before surgery bore a deleterious variant (n=7/17). Moreover, 29.2% of samples harboured a deleterious somatic variant (n=21/72). The most frequently mutated genes were TP53 (80%), FBXW7 (8%), PDGFRA (4%) and PTEN (4%). Finally, three patients experienced a loco-regional relapse by clinical evaluations, anticipated in 67% of cases by the molecular one (n=2/3).
Conclusion: Taken together, our data indicate that sDNA could aid in the monitoring of patients' follow-up as low-frequency somatic mutations could be assessed from the saliva of oral cancer patients. Prospectively, these results suggest that salivary-based liquid biopsy might pave the way for personalized molecular therapies based on mutational data of OSCC patients.

EP176

APPROCCIO PROGNOSTICO – PREDITTIVO AL GLIOBLASTOMA: LA BIOLOGIA MOLECOLARE NELLO STUDIO DEI GENI IDH, IDH2 E MGMT

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I principali markers molecolari utilizzati nell'analisi e nello studio dei glioblastomi sono l'ipermetilazione del promotore di MGMT, la delezione del braccio corto del cromosoma 1 e del braccio lungo del cromosoma 19 (co-delezione completa 1p/19q) e la mutazione dei geni che codificano per l'isocitrato deidrogenasi 1 e 2 (IDH1 e IDH2). Lo stato mutazionale dei geni IDH1 e IDH2 è un'informazione utile per la stratificazione prognostica, in quanto le mutazioni sono presenti nei glioblastomi secondari (di basso grado) mentre non sono state osservate nei gliomi primari.Tra le varianti patogenetiche dei geni IDH1/2 conosciute in letteratura quelle clinicamente più significative in quanto rappresentano bersagli per la terapia mirata sono quelle che determinano i cambiamenti amminoacidici a livello dei codoni G105 e R132 del gene IDH1 e R140 e R172 del gene IDH2.La sovraespressione di MGMT nel tessuto tumorale conferisce un effetto protettivo contro la morte cellulare indotta dalla chemioterapia alchilante. Esiste una forte correlazione tra l'espressione della proteina e la resistenza della terapia: le cellule tumorali esprimenti MGMT risultano 4-10 volte più resistenti alla bis-cloroetilnitrosourea (BCNU), al temozolomide (TMZ) e ad altri composti. Il silenziamento epigenetico di MGMT, mediante metilazione delle isole CpG del suo promotore, è un importante fattore prognostico nei pazienti con glioblastoma multiforme (GBM), gliomi anaplastici o di basso grado. Pazienti affetti da GBM che presentano ipermetilazione di MGMT possiedono un chiaro vantaggio dal trattamento con TMZ in aggiunta alla radioterapia standard infatti l'ipermetilazione del promotore di MGMT rende le cellule tumorali più sensibili alle terapie alchilantiSono stati comparati i risultati dell'analisi dello stato mutazionale dei geni IDH1/2 ed il grado di metilazione del promotore del gene MGMT di pazienti affetti da glioblastoma ottenuti con due metodiche diverse: il Pirosequenziamento e la. La concordanza tra le due metodiche è risultata elevata per entrambe le analisi; i dati ottenuti sono congruenti e riproducibili e il risultato delle due metodiche sovrapponibile. L'utilizzo della Real-Time PCR costituisce un vantaggio in termini di accorciamento del TAT e di riduzione del tempo dedicato alla produzione del dato da parte del personale tecnico.Nonostante esistano difficoltà nel definire un valore di cut-off univoco per la determinazione dello stato di metilazione di MGMT, le grandi metanalisi suggeriscono che è meglio definire metilato il promotore di MGMT quando la percentuale di metilazione del campione è superiore al 9%.Parte del lavoro è stato dedicato alla verifica del valore di cut off utile a discriminare i campioni metilati da quelli non metilati quando viene utilizzata la metodica Real-Time PCR di valutazione qualitativa della metilazione del promotore del gene MGMT.

EP177

NAD+/SIRT1 pathway as a novel biomarker to counteract the progression of Actinic Keratosis

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Introduction & Objectives: Actinic keratosis (AK) is a precursor to invasive squamous cell carcinoma, primarily caused by ultraviolet radiation exposure. Early diagnosis and treatment of AK are crucial to prevent its progression. Among the preventive strategies sunscreen use and oral nicotinamide (NAM) intake are the most relevant. NAM administration reduces AK progression, mitigates inflammation, and repairs UVR-induced DNA damage. Moreover, NAM is a precursor of nicotinamide adenine dinucleotide (NAD+), an activator of sirtuin (SIRT)1, a protein with anti-inflammatory, antioxidant and anti-cancer properties. Although controversial data exist on the role of SIRT1 in AK, no data are related to the systemic modulation of this protein in AK. Thus, this study aims to understand the potential role of SIRT1 and NAD+ as possible biomarkers in counteracting AK progression following NAM administration.
Materials & Methods: In this clinical trial, 30 patients with AKs and a history of non-melanoma skin cancer were treated with NAM (1 g/day) over a 24-months period. Hematological, biochemical, and skin condition assessments were conducted every 6 months. Blood samples were collected for serum and peripheral blood mononuclear cells (PBMCs) extraction and for the evaluation of different targets.
Results: Our results reported a significant variation in some biochemical parameters. In particular, we observed a decreased in basophils, monocytes, total cholesterol and blood glucose levels, suggesting reduced inflammation and increased physiological activity following NAM administration. Interestingly, NAM treatment significantly enhanced NAD+ sera levels, leading to a substantial increase in nuclear SIRT1 activity in PBMCs, in association to a significant reduction in the number of AK lesions.
Conclusion: NAM administration significantly reduced AK progression, supporting NAM's role as a chemopreventive agent in AK management and highlighting its implications for skin cancer prevention and treatment. Although preliminary, our results innovatively, showed as the reduction in AK progression was concomitant with a significant increase in NAD+ serum levels and in systemic nuclear SIRT1 activity, suggesting that these molecules could be considered novel potential biomarkers for monitoring the progression of AK and other skin cancers.

EP178

Ruolo del HE4 come biomarcatore nella diagnostica delle patologie annessiali del cavo pelvico. Confronto tra i risultati di laboratorio e la clinica.

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Introduzione: il dosaggio del biomarcatore HE4 è stato introdotto nella diagnostica di routine del nostro laboratorio da alcuni anni, dopo condivisione della scelta con i clinici della UO di Ostetricia e Ginecologia. Il dosaggio su siero dell'HE4 avviene contemporaneamente e sullo stesso campione del Ca125 i cui risultati vengono combinati nell' algoritmo chiamato R.O.M.A (Risk of Ovarian Malignancy Algorithm) che tiene conto anche dello stato di menopausa. Il test è prevalentemente rivolto alle donne in regime di ricovero o ambulatoriale, in età fertile e menopausa, su richiesta dei medici ginecologi. Sono oggetto della nostra osservazione i campioni processati nel 2022 e 2023, rispettivamente 894 e 960. Obiettivi: dal confronto dei risultati ottenuti nel corso del 2022 e 2023 e dalla diagnosi e trattamento riportata in cartella clinica si intende valutare la sensibilità e specificità del test rispetto all'efficacia clinica nella diagnosi e cura della paziente. Metodologia: questi risultati vanno riportati ai due diversi metodi e tecnologie utilizzati negli anni di riferimento. Nel 2022 i test sono stati dosati sullo strumento Ci2000 (ABBOTT) con metodica in Chemioluminescenza. Nel 2023, per aspetti organizzativi, i test sono stati dosati nello strumento COBAS e 411 (ROCHE) con metodica in elettrochemioluminescenza. Il sistema gestionale ABBOTT elaborava in automatico il calcolo dell'algoritmo R.O.M.A., mentre il sistema gestionale Cobas richiede una elaborazione di calcolo non automatizzata. Risultati attesi: Correlazione dei risultati di laboratorio ottenuti e la clinica. Verifica della sensibilità e specificità del test rispetto ai risultati ottenuti. Valutazione della correlazione dell'HE4-Ca 125 rispetto ad altre patologie annessiali di origine epitelioide. Conclusioni: lo studio dei numerosi casi osservati deve rafforzare la validità del test ai fini diagnostici e di monitoraggio secondo il riscontro con i dati clinici che vanno a validare la sensibilità e specificità del test.

EP179

DNA methylation status affects cell free DNA release in patients-derived colorectal cancer models

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Liquid biopsy based on the isolation and analysis of circulating cell-free DNA (cfDNA) is aimed at identification of specific genomic alterations, and could greatly improve cancer diagnosis and prognosis. Indeed, tumor cells release cfDNA into the bloodstream through tumor cell necrosis or apoptosis, and this analyte has proven to be highly informative at different timepoints of the disease. However, the mechanisms underlying cfDNA release have not been fully characterized. Furthermore, for unknown reasons cfDNA remains undetectable in a small fraction of advanced stage cancer patients. DNA methylation and chromatin status may affect the intrinsic propensity of tumor cells to release cfDNA. In order to verify the correlation between differentially methylated regions and cfDNA release a panel of colorectal cancer CRC cells and of patient-derived xenografts were evaluated by means of EPIC methylation microarrays. We found that low cfDNA release by CRC cell lines correlated with the presence of a high CpG Island Methylator Phenotype (CIMP). Furthermore, by methylome profiling a different signature of methylated regions between high and low cfDNA releasing CRC cells was found. This signature was applied to a dataset of 490 CRC patient-derived xenograft and CIMP-high tumors were enriched in samples predicted as low cfDNA releasers (* $P < 0.005$). Finally, when applied to an independent set of 174 CRC cell lines and patient derived organoids, the same signature successfully predicted their intrinsic propensity to release cfDNA in the supernatant. As further demonstration of the DNA methylation and cfDNA release association, we genetically ablated DNA methyl transferases in CRC cells. Whole genome sequencing (WGS) and ATAC-Seq analysis were used to investigate the nuclear DNA and the supernatant DNA of CRC cells in which DNA methyl transferases had been genetically inactivated (DNMT1/DNMT3B knockout, named HCT116 DKO). We found that decreased methylation led to higher nucleosome accessibility of selected genomic regions, and to higher cfDNA fragments production. Together, our results confirm a positive correlation between methylation loss and increased cfDNA levels and give more insights for the prediction of the cancer cells intrinsic ability in cfDNA releasing.

EP180

Testosterone levels after vitamin D supplementation at two levels of intensity in women diagnosed with breast cancer

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Background:
Sex hormones are known to modulate breast cancer (BC) risk and recurrence and vitamin D may modulate circulating androgens and estrogens. However their interactions are still debated. We analyzed serum levels of 25-hydroxyvitamin D (25(OH)D) and testosterone (T) after two-year treatment with oral vitamin D3 in women previously diagnosed with BC participating in a multicentric randomized controlled trial conducted in Italy of the effect of lifestyle modifications on BC recurrence (DEDiCa).
Methods:
Eligible women with primary histologically-confirmed BC (stages I-III) were randomized within 12 months of diagnosis to follow either one of two lifestyle treatments for 33 months: Mediterranean diet with low glycemic index carbohydrates and daily brisk walking + oral vitamin D3 to reach blood levels of 60 ng/ml (group A) or standard advice to follow a Mediterranean diet and avoidance of sedentary behaviour + oral vitamin D3 to reach 30 ng/ml (group B). Serum 25(OH)D and T levels were evaluated at baseline and yearly thereafter. Mean values were compared within and between treatments in the study population (n=253) and in a subgroup without hormone-suppressive therapy (n=46). Wilcoxon rank-sum test or chi-square and Fisher-exact tests were performed as appropriate. Analytes variation was evaluated using a likelihood ratio test on a mixed-model repeated-measures, and results were adjusted with linear multivariable models.
Results:
No differences in baseline characteristics were reported between randomization groups. Significant increases in serum 25(OH)D concentrations were observed in group A and B after two years of study treatment (26±14 to 54±11 ng/mL, 26±13 to 31±8 ng/mL, respectively, $p<0.001$). No significant changes were found in T levels within or between study groups (0.169±0.145 to 0.195±0.170 ng/mL, 0.191±0.163 to 0.205±0.141ng/mL, respectively, $p=0.930$). No significant differences were observed in the subgroup without hormone suppressive therapy. In multivariate analysis, serum 25(OH)D variations did not account for serum T variations.
Conclusion:
This study shows that increasing serum vitamin D levels up to 60 ng/ml following oral treatment did not affect serum testosterone concentrations.

EP181

Real-time PCR quality expression by using different amplification platform from formalin-fixed paraffin-embedded tissues (FFPE)

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Background: The survival rates of non-small cell lung carcinoma (NSCLC) patients have notably increased due to advancements in identifying therapeutic targets. According to international guidelines, targeted therapies are recommended only when specific molecular targets have been identified. KRAS (Kirsten Rat Sarcoma Virus) is the oncogene that is most frequently mutated in NSCLC, and the most clinically relevant variation is KRAS G12C. In recent years, small inhibitory molecules have been discovered to bind selectively to Cysteine 12 of KRAS G12C, blocking the mutated protein in its inactive state. The aim of this study was to evaluate the analytical performance of two different amplification platforms. Methods: We utilized 40 formalin-fixed paraffin-embedded (FFPE) tissue samples to compare KRAS mutational status with two main PCR-based platforms the IdyllaTM platform (Biocartis, Mechelen, Belgium) and EasyPGX® qPCR instrument 96 (Diatech Pharmacogenetics, Jesi, Italy). The analysis on Idylla™ technology was performed with 4um FFPE tissue sections, while we extracted DNA from human NSCLC tissues with QIAamp DNA FFPE Tissue kit (Qiagen GtmbH, Germany) for qPCR with EasyPGX®. Results: Twenty-one samples had the mutation, while the remaining 19 were identified as wild type. The results obtained with both platforms were highly comparable and correlated. Conclusions: These results suggest no qualitative differences between the two platforms, confirming their validity. Idylla requires less operator time and fewer preparation procedures, even if the sample material could not be reprocessed. EasyPGX requires instead pre-extraction of DNA and longer processing times, but enables the utilization of extracted material for further analyses. Considering these aspects is essential to choose the most appropriate method to include in each laboratory's routine.

EP182

Comparison between a new PSA assay with the well-established Beckman Coulter immunoassay: a preliminary report

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Background

Prostate Specific Antigen (PSA) stands as a critical biomarker for prostate cancer patient clinical management.Reproducibility of serum PSA measurement is essential in the application of this analyte. In this study we sought to compare three different immunoassays (CLIA), Beckman Coulter Access® Hybritech® (PSA-B) (reference method), Immulite® 2000 PSA (PSA-I) and the recent developed AtellicaTM IM PSA assay (PSA-A).

Methods

We selected serum samples from our routine workload at University Federico II Hospital between April and May 2024 from 106 men with a median age of 65 years (interquartile range= 56-73). Total PSA was determined by using three different assays included PSA-B, PSA-A, PSA-I. The determinations were made on Beckman Coulter Access 2 immunoassay (Beckman Coulter®), AtellicaTM IM (Siemens) and Immulite ® 2000 analyzers (Siemens) respectively.

Results

A significant strong correlation between PSA-B and PSA-I assays was found for samples in the overall population (Spearman r= 0.99, p value <0.0001). In addition, we analyzed the correlation between PSA-B and PSA-I stratifying values according to different range of clinical interest; PSA-I displayed a good correlation with PSA-B for values below 2 ng/mL (Spearman r= 0.98, p value <0.0001), for values between 2 ng/mL and 10 ng/mL (Spearman r= 0.97, p value <0.0001) and for values higher that 10 ng/mL (Spearman r= 0.98, p value <0.0001). A significant positive correlation was found between PSA-B and PSA-A in the overall population (Spearman r= 0.95, p value <0.0001). In addition, a significant association between PSA-A and PSA-B were found stratifying values below 2 ng/mL (Spearman r= 0.81, p value <0.0001), from 2 ng/mL to 10 ng/mL (Spearman r= 0.93, p value <0.0001). and higher than 10 ng/mL (Spearman r= 0.96, p value <0.0001). The correlation between PSA-B and PSA-I (Spearman r= 0.99) was significantly higher compared to the correlation between PSA-B and PSA-A (Spearman r= 0.95) in the overall population (p <0.0001).

Conclusion

Although both PSA-I and PSA-A demonstrated a significant positive correlation with PSA-B, the reference method for PSA measurement, PSA-I displayed a significant better correlation with PSA-B compared to PSA-A.

EP183

Clinical validation in multiple sclerosis of the LUMIPULSE immunoassay for plasma neurofilament light chain

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INTRODUCTION

Neurofilament light (NfL) is an intermediate filament protein found specifically in the neuronal cytoskeleton. It can be released into the extracellular space through axonal degradation, and it has been shown that varying NfL levels are an indicator of neuroaxonal damage, irrespective of the cause, and can therefore be associated with a variety of neurological diseases such as multiple sclerosis (MS). A number of ultra-sensitive assays have been developed to quantify plasma NfL (pNfL), with potential associations with clinical features.

OBJECTIVE

To evaluate the LUMIPULSE immunoassay for pNFL in MS as a marker of clinical features.

METHODS

We recruited consecutive people with diagnosis of MS based on 2017 revision of McDonald criteria. Blood samples were collected and were centrifuged within 3 h after draw at 1100 rpm x 10 min, aliquoted into polypropylene tubes and stored at -80°C. Concentrations of plasma neurofilament light chain (pNFL) were determined by an assay system based on CLEIA technology consisting of two-step immunoassay method on the LUMIPULSE G System. In this preliminary analysis of our ongoing study, we have used linear regression models to estimate associations between pNFL and, separately, each clinical variable.

RESULTS

We included 131 people with MS (age 48.6±12.0 years, females 67.1%, disease duration 16.3±14.2 years, EDSS 3.5 (0-8.0)), and excluded 3 due to recent delivery (n=1), pneumonia (n=1), and concomitant inflammatory bowel disease (n=1). Mean pNFL was 15.5±5 pg/mL. We found significant associations between pNFL and presence of clinical or radiological disease activity (5.3%) (Coeff=49.35; 95% CI=31.57, 67.12; p<0.01), and presence of cardiovascular risk factors (30.5%) (Coeff=11.74, 95% CI=2.32, 21.16; p=0.01).

CONCLUSIONS

In MS pNfL is significantly associated with clinical or radiological disease activity (as a consequence of acute inflammatory neuroaxonal damage) and to concomitant cardiovascular risk factors (causing long-term neuro-axonal damage and clinical progression). Further largescale population studies are necessary to clinically validate pNfL as a promising biomarker for disease activity, progression, prognosis and monitoring effectiveness duration of therapy.

EP184

Identification of Porphyromonas Somerae in the Urine of Bladder Cancer Patients by the Use of ddPCR

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Background: In a recent study on bladder cancer (BK) patients we profiled a bacterial signature in first-morning urine samples. In particular, we demonstrated the possibility of using the first-morning urine samples (FM-U) as an advantageous specimen for urobiome profiling, suggesting Porphyromonas Somerae species as a possible biomarker for BK.Aim: The aim of this ongoing study is to confirm the presence of the P. Somerae species in subjects affected by bladder cancer by using droplet digital Polymerase Chain Reaction (ddPCR).Materials and methods: Bacterial DNA was extracted from FM-U samples of male patients with bladder cancer (BK; n=37), prostate cancer patients (PK; n=41), and non-oncological volunteer male subjects (HC; n=24). The quantification of positive and negative droplets from ddPCR was performed by QuantaSoftTM software. Comparative analyses between groups were performed by using the Kruskal-Wallis H test and Mann–Whitney U test, adding the False Discovery Rate (FDR) correction. In addition, a correlation analysis including 64 previously sequenced samples was made by using Z-score normalization and Spearman's rank coefficient. Results: Preliminary data showed a statistically significant difference in P. Somerae positive droplets count in both comprehensive (H test, p-value=0.0033) and pairwise (BK vs PK, U test q-value=0.0014; BK vs CO, U test q-value=0.008) analyses. Furthermore, the analysis of 64 samples tested with both ddPCR and NGS revealed a significant (p-value = 0.0002) moderate correlation (Spearman's r=0.44) between the number of droplets count and the number of reads associated with the P. Somerae. Conclusions: Data obtained with quantification of P. Somerae positive droplets demonstrated the increased abundance of this bacterial species, confirming urobiome sequencing data of our previous work. These preliminary results indicate that ddPCR may be a useful and less expensive alternative in detection of bacterial urobiome species associated with bladder cancer. However, validation of these findings requires an expanded patient cohort.

EP185

Oral intake of lactolycopene reduces oxidative stress induced by epidermal growth factor receptor (EGFR) inhibitors

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Epidermal growth factor receptor (EGFR) inhibitors are a class of drugs used to treat several common malignancies, including breast, colon, lung, and pancreatic cancer. EGFR inhibition leads to the production of reactive oxygen metabolites, which may interfere with cytotoxic effects of antineoplastic agents reducing the rate of cell proliferation and contributing to side effects of treatment. EGFR drugs, such as panitumumab, are associated with characteristic skin toxicities. Lycopene and its derivate β-carotene have an extreme antioxidant activity and, due to their hydrophobic structure, accumulate specifically in the skin (1) Lycopene dietary intake and the assessment of its blood bioavailability may be particularly relevant in the evaluation of lycopene effectiveness in reducing skin toxicity induced by panitumumab. Panitumumab treated subjects (n =28) were randomly assigned to ingest 20 mg lycopene/d for 10 weeks as lactolycopene or placebo. Plasma concentrations of lycopene, β-carotene and malondialdehyde (MDA), as index of oxidative stress, were determined using reverse-phase high-performance liquid chromatography (HPLC) with UV and fluorescence detection, respectively (ß-Carotene and MDA in serum/ plasma HPLC Kit, Chromsystems, Germany). In the placebo group, mean plasma lycopene concentration was of 420±66 ng/ml, β-carotene 245±48.5 ng/ml and MDA 0.61±0.05 µM/ml. In the lactolycopene group, mean plasma concentrations of lycopene and β-carotene were significantly higher (664±88.8 ng/ml (p=0.0016) and 321±44.6 ng/ml (p=0.018), respectively)), while the MDA concentration was significantly lower (0.55±0.07 µM/ml (p=0.02)). A reduction in skin toxicity was also observed. Our results show that oral intake of lactolycopene, by increasing plasma concentrations of lycopene and B-carotene, reduces oxidative stress induced by EGFR inhibitors and protects the skin.

1) The Anti-Cancer Activity of Lycopene: A Systematic Review of Human and Animal Studies Kapa#a, M. Szlendak, E. Motacka Nutrients, 2022 Dec 3;14(23):5152.

EP186

Urinary steroidome investigation by dilute & shoot LC-MS analysis: a potential novel tool for characterizing adrenal tumors

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The analysis of 24h urine steroidome has been widely used in the context of adrenal tumors in the last decades. Indeed, the performance of liquid chromatography coupled to mass spectrometry (LC-MS) have provided useful tools to investigate wide panels of unconjugated steroids in specific clinical settings. However, the LC-MS capability of measuring intact phase II metabolites in urine could represent a potential novel tool to be employed for adrenal tumors characterization.

A “dilute and shoot” LC-MS method was developed for measuring more than 30 steroidal compounds from just 10µL of 24h urine sample. The list of measured compounds includes free steroids (e.g., Cortisol, Cortisone) as well a wide panel of intact glucuro- and sulpho-conjugated phase II metabolites. The developed method was validated in accordance with ISO 17025 requirements for quantitative methods and it was tested on more than 100 real 24h urine samples already analyzed in routine for urinary free cortisol and cortisone by LC-MS.The optimized analytical method allowed to efficiently separate target analytes, including challenging isobaric isomers and guaranteed a sufficient sensitivity for detecting steroid hormones and metabolites down to 1 ng/mL. The performed validation protocol, including the evaluation of selectivity, quantitative performance and carry-over gave satisfactory results. The application to real 24h urine samples allowed assessing method’s robustness in the context of clinical biochemistry laboratory’s routine, opening the way to its use in selected clinical studies.The presented analytical platform proved to be suitable for free steroid hormones and phase II metabolites measurement in 10µL of 24h urine samples. This simple and accurate method could represent an innovative and useful tool to further characterize adrenal tumors. The use of a very limited sample volume represents an advantage for its application to urine samples already collected during previously performed clinical studies within the European Network for the Study of Adrenal Tumours (ENSAT).

EP187

Analysis of metabolic pathways by high-resolution mass spectrometry: a tool for studying neuroblastoma complexity to improve tumor diagnosis and monitoring

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Introduction: Neuroblastoma (NB) is a complex pediatric tumor with heterogeneous molecular features, which poses major challenges in tumor diagnosis and monitoring. Metabolic profiling techniques may represent a useful resource to unravel the intricate biochemistry of NB in order to increase diagnostic accuracy and improve tumor monitoring strategies. Through an untargeted metabolomics study, we have previously shown that in NB different metabolic pathways such as catecholamines, amino acids, methionine and polyamines are differentially altered depending on tumor biology. The aim of this work is to define an extended panel of metabolites belonging to these pathways using high-resolution mass spectrometry (HRMS) as a tool to further investigate NB metabolism. Methods: Using the HRMS system coupled to liquid chromatography (Vanquish duo- Orbitrap Exploris 120, Thermofisher Scientific) we set up the analytical conditions to identify in the urine of patients with NB a very broad panel of metabolites from 20 mL of urine only. From the results obtained in our previous metabolomics work, we quantitatively validated the molecules that were significant by adding the semi-quantitative profile of all metabolites of the L-DOPA metabolic pathway present in patients' urine. Results: We identified 24 molecules of the L-DOPA pathway, including conjugated forms (sulfates and glucuronidates) in addition to the 8 metabolites routinely analyzed in the NB biochemistry centralization protocol at the Gaslini Institute. To these results we added the quantification of some amino acids, especially the metabolic pathway of methionine, cysteine and polyamines. Conclusions: Biochemical analysis by HRMS allows a very accurate definition of a panel of metabolites of various metabolic pathways from a very low volume of urine. This work lays an important foundation for in-depth studies of NB metabolism in order to develop new diagnostic and tumor monitoring strategies during therapy and in relapses.

EP188

Il DIRA test: l'esperienza del Settore di Protidologia della ASL BT

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Il Mieloma Multiplo (MM) è una neoplasia ematologica caratterizzata dalla proliferazione clonale di plasmacellule o linfociti B secernenti nel siero e/o nelle urine una Componente Monoclonale (CM). L'introduzione degli anticorpi monoclonali (mAb) anti-CD38 nel trattamento del MM ha migliorato l'outcome dei pazienti. Il Daratumumab è un mAb umanizzato isotipo IgG1κ, prodotto mediante tecnologia del DNA ricombinante, che negli schemi terapeutici è utilizzato in associazione a immunomodulatori (IMiDs) e Inibitori del Proteosoma. Questo farmaco, in base alla concentrazione e alla frequenza della terapia, può raggiungere livelli di concentrazione sierica tali da renderlo rilevabile su elettroforesi sieroproteica (SPE) e su immunofissazione sierica (sIFE). Il Daratumumab Interference Reflex Assay (DIRA) test consente di comprendere se la IgGκ tipizzata in sIFE corrisponda al farmaco somministrato o sia una CM. Il test è stato introdotto nella diagnostica specialistica del Settore di Protidologia della ASL BT e viene eseguito utilizzando il kit Hydrashift Daratumumab (Sebia) su strumentazione Hydrasys II della stessa Ditta.Nella nostra routine, se nei pazienti in follow-up elettroforetico appare un picco non quantizzabile (<2 g/L) che migra in posizione catodica nella zona gamma, tipico dell'interferenza da Daratumumab, si contatta il clinico per l'anamnesi farmacologica e si effettua isotipizzazione; se questa rivela una IgGk, si esegue il DIRA Test.In caso di esito positivo, il referto è accompagnato da un commento che informa della presenza di un picco in zona gamma causato dall'interferenza del farmaco. Quando la SPE evidenzia ipogammaglobulinemia, ma non il picco in gamma e la sIFE rileva una IgGk, acquisite le informazioni sulla farmacoterapia, si esegue il DIRA Test riflesso: se positivo, l'interferenza farmacologica è segnalata nel referto. Dal 2022 al 2024 abbiamo valutato 35 pazienti, 30 dei quali sono risultati positivi al test. L'86% dei pazienti ha ripetuto il test durante il follow-up clinico.Il DIRA test rappresenta un'importante implementazione a supporto dell'attività clinica, in quanto la valutazione dell'interferenza farmacologica è di fondamentale importanza nel follow-up del MM, per la diagnosi differenziale di eventuale recidiva di malattia.

EP189

Cystic Fluid LDL-Cholesterol and Lymphocytes: Worrisome Biomarkers of Intraductal Papillary Mucinous Neoplasms (IPMN)

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Introduction: Pancreatic Cystic Neoplasms (PCNs) pose a risk of malignant transformation, notably in the prevalent IPMN type. Current biomarkers like CEA, amylase and glucose are not capable of distinguishing mucinous from non-mucinous PCN. Our study delves into metabolic indices, lymphocyte subsets, and mesenchymal stem cells (MSCs) to differentiate high and low-risk IPMN, offering potential insights for clinical decision-making when concerning features arise.Methods: Following ethical approval and participants' consent, 26 patients (11 males, mean age 69.5±9 years) subjected to EUS-FNA were consecutively enrolled. Blood, serum, and cystic fluid samples were collected for comprehensive analyses. Glucose, CEA, total, HDL and LDL cholesterol and total proteins were determined in serum and cystic fluid. Immunophenotyping in both peripheral blood and cystic fluid samples was investigated by flow cytometry to detect lymphocyte subsets and MSCs.Results: Final diagnosis comprised mostly IPMN (25/26), and patients were categorized as low or high risk of malignancy based on imaging worrisome features, cystic fluid CEA (over 45 ug/L), and elevated serum CA19-9 (above 26 Ku/L). Demographic analysis revealed no gender disparity, but high-risk were significantly older than low-risk patients (p=0.003). 69% of patients had anaemia. MSCs were not detected in cystic fluids, while in this fluid lymphocytes exhibited a significant difference between high and low-risk patients (p=0.005), being notably scarce in high-risk patients. In blood, non-MHC restricted cytotoxic T cells were significantly higher (p=0.019), and MSCs tended to be lower in high-risk patients. Biochemical analyses revealed lower cystic fluid total proteins and LDL-cholesterol in high-risk patients (p=0.005, 0.031). ROC curves indicated comparable discriminant abilities of cystic fluid lymphocytes (AUC=0.868), total proteins (AUC=0.859), and LDL cholesterol (AUC=0.795) together with glucose (AUC=0.625), and CEA (AUC=0.878).Conclusions: Premalignant cells in high-risk IPMN exhibit enhanced energy uptakes through metabolic reprogramming, emphasizing the co-assessment of metabolic resources involving glucose, LDL-cholesterol, and total proteins along with lymphocytes as biomarkers of malignancy.

EP190

Ruolo del dosaggio sierico degli anticorpi anti-p53 nei pazienti con tumore del polmone

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INTRODUZIONE

L'oncosoppressore p53 svolge un ruolo determinante per garantire la stabilità genomica, pertanto la perdita di funzionalità di p53 rappresenta spesso un prerequisito della tumorigenesi. La nota correlazione tra anticorpi anti-p53 (s-p53-Abs) e cancro suggerisce il potenziale uso di tali anticorpi come biomarcatori nel monitoraggio della malattia. Tuttavia i dati in letteratura sull'utilizzo di s-p53-Abs sono contrastanti, verosimilmente a causa delle differenze nei metodi utilizzati per le determinazioni quantitative. Tali anticorpi venivano rilevati mediante tecniche di ELISA non standardizzate, mentre più di recente è stato introdotto un test quantitativo immunologico automatizzato in elettrochemiluminescenza altamente specifico e rapido. L'obiettivo di questo studio è determinare il possibile ruolo degli s-p53-Abs nel monitoraggio dei pazienti con tumore del polmone.

METODI

In questo studio sono stati arruolati 49 pazienti afferenti alla Chirurgia Toracica dell'Istituto Nazionale dei Tumori IRCCS "Fondazione G. Pascale". Gli s-p53-Abs sono stati dosati mediante test immunologico Elecsys Anti-p53 (Roche) su campioni di siero e plasma prelevati prima dell'intervento (T0) e su campioni di siero a tre mesi dall'intervento (T1).

RISULTATI

Gli s-p53-Abs sono stati rilevati al T0 in 6 pazienti (12,24%), con valore mediano di 0,83 mg/mL (IQR 0,44 – 2,54 mg/mL). I test di linearità, effettuati eseguendo 20 ripetizioni di un campione di siero con alti livelli di s-p53-Abs (6,74 mg/mL), hanno mostrato un Coefficiente di Variabilità del 7,4%. Il Test di Pearson ha evidenziato un'ottima correlazione tra i valori di s-p53-Abs ottenuti su siero e plasma (p<0,001). Nel siero dei due pazienti con s-p53-Abs elevati al T0 di cui erano anche disponibili campioni al T1, si è avuta una riduzione rispettivamente del 93,3% (da 0,46 mg/mL a 0,03 mg/mL) e dell'82,6% (da 6,74 mg/mL a 1,17 mg/mL).

CONCLUSIONI

Il test Elecsys Anti-p53 ha dimostrato un'ottima linearità e un'ottima correlazione tra siero e plasma. L'importante riduzione dei livelli di s-p53-Abs dopo l'intervento chirurgico sembra indicare un loro possibile ruolo prognostico nei pazienti con tumore del polmone. La conferma di questo dato richiede ulteriori studi.

EP191

EPIGENETICS IN CHRONIC MYELOID LEUKEMIA: THE ROLE OF POLYCOMB GENES

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Introduction: Chronic myeloid leukemia (CML) is characterized by the BCR::ABL1 fusion protein that converts myeloid precursors into leukemic stem cells (LSC) but also induces epigenetic reprogramming. Polycomb Repressive Complexes, which include EZH2 and BMI1, are a group of epigenetic regulators that can be dysregulated in CML LSC.

Aim: The aim of this study was to evaluate the expression of BMI1 and EZH2 in 24 patients at diagnosis and after 3, 6, and 12 months of therapy with TKIs. We also evaluated expression levels of these 2 genes in 21 patients at the Treatment-Free Remission (TFR). Then, we tested potential correlations between the Polycomb genes and BCR::ABL1 transcript levels, with the purpose of finding their eventual predictive role on molecular response.

Methods: A novel digital droplet PCR (ddPCR) method capable of simultaneously analyzing three genes (BMI1, EZH2, and GAPDH - with the last chosen as the reference gene) has been set by using the Droplet Generator and Droplet Reader instruments and the QuantaSoftTM Pro Software (BioRad, Italy). This study has been performed in the context of the project Bando Salute 2018 “StemCMLCure”(PI Prof. Bocchia).

Results: Overall, we observed: 1) a significant correlation between expression levels of BMI1 and EZH2 at all timepoints; 2) a lower BMI1 expression at diagnosis vs later timepoints (Pearson's= 0.814-0.486, $p<0.05$); 3) a correlation (even if not statistically significant – probably for the small number of cases) between higher BMI1 expression at 3 months of therapy with imatinib and a lower probability of achieving deep MR after 12 month; 4) a decreased BMI1 expression at the molecular relapse vs the moment of TKI interruption in the 6 patients who failed TFR ($p=0.005$).

Conclusions: The correlation between BMI1 and EZH2 levels, already observed by our group in aggressive lymphoma, might be explained by the fact that EZH2, by deregulating the mir-200c, sustains the BMI1 expression. The lower BMI1 expression at diagnosis and at the TFR failure could be considered as an indirect marker of stemness and resistance to TKIs (high BMI1 levels have been measured also in healthy donors). Moreover, BMI1 independence from BCR::ABL1 makes its expression as a new possible independent marker of disease behavior.

EP192

Assessment of DNA extraction methods from formalin-fixed paraffin-embedded tissues (FFPE) and their implications

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Background: the identification of therapeutic targets has significantly enhanced the survival rate of non-small cell lung cancer (NSCLC) patients. According to international guidelines, targeted therapies can only be administered if specific molecular targets have been identified. Extracting DNA from formalin-fixed paraffin-embedded tissues (FFPE) for molecular analysis is challenging due to fragmentation and cross-linking. The objective of this study was to compare the efficiency of DNA extraction using three different techniques, to evaluate their suitability for routine applications. Methods: We used 82 FFPE tissue samples to compare the manual extraction technique with the QIAsymphony (Qiagen GtmbH, Germany) and QIAcube (Qiagen GmbH, Germany). The total amount of DNA was quantified spectrophotometrically by measuring the absorbance at 260 nm. DNA purity was assessed by determining the A260/A280 ratio (reference interval: 1.8-2.0) and A260/A230 ratio (reference interval: 2.0-2.2) using the Spectrophotometer Nanodrop2000 (Thermo Scientific, Rockford, IL, USA). Results: The analysis revealed an average concentration of nucleic acids of 160.15 ng/µl for manual extraction; 80.7 ng/µl for the QIAcube method and 37.9 ng/µl for the QIAsymphony. Manual extraction yielded the highest DNA concentration ($p<0.0001$). All methods provided DNA within an A260/A280 ratio close to the optimal range, but the A230/A280 ratio analysis indicates that QIAsymphony retained more organic contaminants compared to QIAcube ($p<0.01$) and manual extraction ($p<0.0001$). Conclusion: Our results suggest that manual DNA extraction resulted in the highest number of samples meeting the purity criteria. Although manual extraction demonstrated the highest efficacy, it is not suitable for modern laboratory workflows due to its time-consuming nature. Automation, while time-saving, often compromises DNA yield and quality. These findings underscore the trade-offs between automation and extraction efficiency, emphasizing the importance of selecting the appropriate technique according to the required DNA quality.

EP193

Inherited breast and ovarian cancers beyond BRCA analysis: a case study

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Hereditary Breast and Ovarian Cancer (HBOC) syndrome accounts for about 15% of all breast cancer (BC) cases and is mainly related to BRCA1/2 pathogenic variants. Nevertheless, they explain just a fraction of all HBOC cases suggesting the existence of other genetic risk factors. In this scenario, we implemented a second-level molecular test for the simultaneous analysis of 33 genes in BRCA negative patients with a clinical suspicion of HBOC. Here, we report the case of a 67-year-old woman who came to our attention because she was affected by BC and had a notable family history of breast, prostate, colon, stomach and bladder cancers. BC was diagnosed at age of 58 years and surgically treated by unilateral mastectomy. Histological examination allowed to diagnose an invasive breast carcinoma of no special type (NST) G2 pT2 N0, expressing estrogen receptors (ER:95%, PgR:25% and c-erbB:3+). Thus, after adjuvant therapy, hormone therapy was done for five years up to October 2022 and the patient is currently in follow up. Finally, an atypical nevus was removed last April. Based on personal and family history, genetic test for BRCA evaluation was carried out revealing no pathogenic variants. Therefore, the genetic analysis was extended to our HBOC genes panel showing a pathogenic DNA variant in ATM gene, known to be a pivotal checkpoint kinase in cell cycle and a regulator of a wide variety of downstream proteins. The above-mentioned variant, namely c.7271T>G, p.(Val2424Gly), has been associated with a deleterious impact on protein structure and functions, resulting in a significantly decreased ATM kinase activity able to confer a risk to comparable to BRCA1/2 pathogenic variants. Based on this result, familial genetic test was carried out. Patient's brother and 2 nephews were analyzed, one of the latter (a healthy 29-year-old man) was found to carry the ATM variant. This case report emphasizes the importance to expand the genetic analysis to genes other than BRCA and to enlarge the molecular analysis to affected and not affected family members in order to adopt preventive measures and targeted treatments able to positively impact patients' outcomes.

EP194

Determinazione e supplementazione della vitamina D: un problema ancora aperto

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La nota AIFA 96 per la "prescrizione di farmaci a base di vitamina D" ha aperto un dibattito su molti aspetti della determinazione di questo misurando, sui livelli decisionali che dovrebbero essere raccomandati ed adottati dai laboratori clinici e sull'appropriatezza della richiesta e determinazione della vitamina stessa. Dal punto di vista analitico, è importante sottolineare come il programma di standardizzazione avviato dal National Institute of Health (NIH) nel 2010 abbia dimostrato l'esigenza non solo di ricalibrare i metodi commerciali in uso, ma soprattutto di rivedere i livelli decisionali sulla base dell'evidenza che i dati dei maggiori studi clinici disponibili sono affetti da un grado significativo di inaccuratezza (bias). Ad oggi, tuttavia, i livelli raccomandati da vari organismi e società scientifiche, e che quindi i laboratori clinici dovrebbero riportare nel referto sono: a)<30 nmol/L stato carenziale; b) 30-50 nmol/L insufficienza, c) 50-125 nmol/L adeguatezza,d) 75-125 nmol/L valori ottimali in pazienti affetti da osteoporosi o con condizioni cliniche a rischio di ipovitaminosi D e d)> 250 nmol/L eccesso. Inoltre, la determinazione della Vitamina D appare appropriata e raccomandata: a) In tutti i casi di sospetto clinico di osteomalacia (conferma dell'ipotesi diagnostica e/o diagnosi differenziale) e va ripetuta a distanza di 3-6 mesi; b) In tutti i casi di sospetto iperparatiroidismo, sia primario che secondario, e nei pazienti diagnosticati per questa condizione morbosa; c) Nei pazienti con insufficienza renale cronica per la valutazione ed il monitoraggio delle malattie renali croniche con disturbi del metabolismo minerale e osseo (CKD-MBD) e d)Nei soggetti di età pediatrica con fattori di rischio per deficit, con ritardi nella crescita e nel caso di lunghi periodi di ospedalizzazione/istituzionalizzazione. Infine, viste le acquisizioni sul complesso meccanismo della regolazione del metabolismo osseo, si ritiene essenziale la determinazione in alcuni casi non solo del vitamero 25(OH)D, ma anche della forma attiva diidrossilata, come pure del Paratormone ed in alcune specifiche situazioni cliniche dell'FGF23.

EP195

Immunofissazione e immunosottrazione: metodiche a confronto

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Le discrasie plasmacellulari sono un gruppo di malattie eterogenee dal punto di vista clinico e biochimico, caratterizzate dall'anomala proliferazione di un clone plasmacellulare e dalla presenza nel siero e/o nelle urine di una immunoglobulina monoclonale (CM), strutturalmente ed elettroforeticamente omogenea.L'esame di elezione per la rilevazione della CM è l'elettroforesi sieroproteica (sEF) in tecnica capillare (CZE). L'immunotipizzazione può essere eseguita con immunofissazione su gel di agarosio (IFE) o con immunosottrazione in tecnica capillare (ISE).La IFE è una tecnica elettroforetica zonale che utilizza antisieri monospecifici verso le catene pesanti e verso le catene leggere delle immunoglobuline. La reazione tra antisiero e campione diluito determina un immunoprecipitato che, dopo migrazione e colorazione, consente l'isotipizzazione della CM. La ISE prevede il trattamento preliminare del campione con gli antisieri monospecifici verso le catene pesanti e leggere delle immunoglobuline, che causa l'immunoprecipitazione della CM. Il confronto tra il tracciato elettroforetico del sovranatante privo di CM e quello del siero non trattato consente la isotipizzazione della CM: sEF del sovranatante è caratterizzata dalla scomparsa del picco monoclonale corrispondente agli antisieri reattivi.Le CM possono migrare diversamente a seconda della metodica utilizzata per la loro isotipizzazione. Riportiamo il caso di un paziente di 73 anni con due CM che sia in ISE che CZE migrano in zona Beta2 e in Beta2-Gamma (CM + Proteine Beta2: 14.8 g/l) e in Beta2-Gamma (16.1 g/ l), mentre su gel di agarosio "slittano" in zona gamma. I nostri protocolli prevedono la isotipizzazione della CM con la IFE in quanto l'esame è il gold standard. Tuttavia in caso di difficoltà interpretative, conseguenti alla differente migrazione della CM rispetto al tracciato elettroforetico, si procede all'esecuzione della ISE al fine di confermare l'esatta zona di migrazione della CM.

EP196

Programma di screening per il riconoscimento precoce del deficit genetico di alpha1-antitripsina (AATD) tramite elettroforesi delle sieroproteine (SPE): analisi preliminare di uno studio prospettico monocentrico

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Il AATD è una condizione miss-diagnosticata associata a circa il 60% di rischio di sviluppare una malattia polmonare ostruttiva. I programmi di screening raccomandati da diverse Società scientifiche raramente vengono implementati in pratica clinica, disattendendo molto spesso la possibilità di offrire una diagnosi precoce. Anche il recente documento SIBIOC 2024 sull'armonizzazione del referto SPE raccomanda l'inserimento di un commento interpretativo in caso di riduzione della frazione α1globulinica. In questo studio presentiamo i risultati preliminari di un programma di screening basato sulla SPE per il riconoscimento precoce del AATD.

MATERIALI E METODI: è stato analizzato un campione di pazienti con età tra i 18 e i 70 anni per cui era disponibile almeno un'EFP capillare effettuata presso il Laboratorio analisi del PO di Pescara nel periodo da gennaio 2022 a marzo 2024. I pazienti con frazione α1≤2,9% sono stati selezionati per la determinazione dell'AAT tramite metodo turbidimetrico e, in caso di livelli sierici inferiori a 0,9 g/L, per la ricerca delle mutazioni correlate al AATD, effettuata presso il Laboratorio dedicato del Policlinico San Matteo di Pavia. Il test genetico è stato offerto anche ai familiari volontari di primo grado. I portatori di una variante PI*M sono entrati nei programmi di prevenzione presso l'ambulatorio pneumologico di riferimento.

RISULTATI: 5736 SPE sono risultate con α1≤2,9%, di cui il 5% (287) con α1≤2,3%. La maggior parte erano esami richiesti per i donatori di sangue e per i pazienti dei reparti di pneumologia e malattie infettive. Al momento l'esito del test genetico è stato ottenuto per 34 pazienti: 6 PI*MS (deficit lieve), 16 PI*MZ (deficit intermedio), 12 varianti rare associate a un deficit severo (eterozigosi per *M1Q0amersfort, *Mwhitstable, *MMwurzburg, *MMprocida, *MQ0clayton, *MZ e omozigosi per PI*ZZ e PI*SS). Altri 35 pazienti devono ancora essere contattati per l'esecuzione dell'esame genetico.

CONCLUSIONI: I dati ottenuti finora risultano in linea con quelli di prevalenza nazionale e l'SPE si conferma un test di primo livello ideale per lo screening del AATD. Tuttavia, la raccolta dati è ancora in corso e risulterebbe utile estendere l'arruolamento anche ai casi di sdoppiamento della frazione α-1globulinica.

EP197

Evaluation of the quantitative turbidimetric Bence Jones protein (BJP) assay compared to urine immunofixation: A step forward with the quantitative evaluation of BJP.

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Background-Aim

The (BJP) refers to monoclonal free immunoglobulin light chains in urine (u-FLC), kappa (K) and lambda (λ). The determination and dosage of BJP is clinically useful in the diagnosis and follow-up of patients affected by neoplastic B lymphocyte pathologies, such as Multiple Myeloma (MM), Monoclonal Gammopathy (MG), Monoclonal Gammopathy of Undetermined Significance (MGUS), Amyloidosis (AL), and related disorders. Guidelines recommend the use of urinary immunofixation (gold standard) to detect BJP while its quantification by densitometric calculation of the BJP band. The study aims to evaluate whether the turbidimetric dosage of BJP in the 24 hours urine samples can correlate with the standard method based on densitometric analysis.

Methods

A total of 256 serum and urine samples were collected at "San Filippo Neri" Hospital in Rome, from routine samples. Patients were enrolled with the request for determination of BJP with a densitometric method. The residual samples were transported to the University Hospital "Policlinico Tor Vergata" to measure the u-FLC K and λ with a quantitative turbidimetric method.

Results

The 256 samples resulted: 50 BJP negative, 206 BJP positive (of these last 146 BJP K positive, and 60 BJP λ positive samples). For the 146 patients with positive K, we find a u-FLC median value of 95.86 mg/L and in the 60 patients with positive λ we have a u-FLC median value of 17.43 mg/L. For the 50 negative samples, we found u-FLC median K and λ values of 7.18 mg/L and 1.0 mg/L respectively. We carried out a correlation between densitometric and turbidimetric quantification showing good levels of correlation for both BJP K and λ. Furthermore, of the 146 samples positive for BJP K, 33 samples were undetectable by the densitometric technique. On the contrary, the turbidimetric method still provided a positive value for the chain involved.

Conclusions

This study highlights a method to improve laboratory practices in the management of plasma cell diseases. The u-FLC turbidimetric determination can be used in conjunction with densitometry to support the clinician in the patient management, but it needs better harmonization and standardization before being used in common clinical practice.

EP198

A rare hemoglobin variant (Hb Austin) reported for the first time by capillary electrophoresis

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Glycated hemoglobin (HbA1c) is a biomarker that reflects the average level of blood glucose over the past 2–3 months. HbA1c measure is a validated test to monitor blood glucose control in people with diabetes. Various assay methods are used to measure HbA1c, and many factors may interfere with its measurement according to assay method used. We report here the case of an unusually undetectable HbA1c value caused by the interference of a rare hemoglobin variant (Hb Austin), never reported before in capillary electrophoresis (CE). A 59 year-old female patient came to our laboratory to carry out a series of tests for suspected diabetes mellitus (DM). HbA1c test performed by CE did not provide any results due to an interference of a hemoglobin variant migrating close to the HbA0 on the electrophoretic trace. To confirm the hemoglobin variant the sample was analyzed in capillary electrophoresis with the Hemoglobin (E) mode. The electrophoretic trace did not show abnormal peaks, but the presence of a variant that co-migrated with HbA0 could not be excluded. Hence, the sample was sent to a reference laboratory for further investigations, where both HPLC (High Performance Liquid Chromatography) and genotyping were performed. While detecting the presence of a variant, the HPLC analysis was not affected by it and provided a numerical value for glycated hemoglobin. The molecular analysis made possible to typify the variant which turned out to be a very rare variant, never reported before in CE, called Hb Austin. Although it's clinically silent, the present results show how Hb Austin variant can affect the results of HbA1c analysis through CE, but not that obtained from the HPLC method. Hence the importance of being able to integrate various analytical methods to determine HbA1c since there are also hemoglobin variants that interfere with HbA1c dosage in HPLC and not in CE. Furthermore efforts have to be made in the research of alternative HbA1c methods free of interferences.

EP199

THE IMPORTANCE OF LABORATORY IN THE DIAGNOSTIC PROCESS FOR A PATIENT WITH SUDDEN SEVERE BONE PAIN

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Light-chain multiple myeloma (LCMM) is a less frequent type of multiple myeloma (~15% of all cases), with a more aggressive course and poorer prognosis. A distinctive feature of LCMM is the absence of complete clonal immunoglobulins secretion by malignant plasma cells, with no M-spike visible in serum protein electrophoresis (SPE) which can lead to miss the diagnosis. We describe the clinical case of a patient with l LCMM to highlight how quick laboratory diagnosis means establishing early treatment and improving prognosis.A 77-years-old woman was admitted to Emergency Department for sudden severe back pain. Her serum sample was submitted to Clinical Pathology Unit (S.Eugenio Hospital, Rome) and biochemical parameters were measured on Abbott Alinity ci system (photometry, potentiometry, chemiluminescence). Since one diagnostic option was the presence of a monoclonal component, SPE was performed on Capillarys 3 TERA(SEBIA). The laboratory professionals decided to carry on further studies: s-IFE and urine Immunofixation (u-IFE) were both performed in agarose gel using Hydragel IF/BJ (HR) (SEBIA). The quantification of serum free light chains (sFLCs) k and l was determined on Siemens BN ProSpec nephelometer. Laboratory examinations evidenced creatinine in normal range (0,73 mg/dL), mild hypocalcemia (7,2 mg/dL), anemia (11.1 g/dL). SPE revealed hypogammaglobulinemia (2,9 g/L) with flattening of g zone and no M-spike visible. S-IFE performed with standard antisera (IgG, IgA, IgM, k and l) showed a monoclonal band only in antisera to l in a1 region without corresponding heavy chain band; a subsequent s-IFE with antisera to IgD, IgE, free k and free l confirmed it. FLCs l were 34600 mg/L; u-IFE revealed a Bence Jones proteinuria l. According to laboratory findings, bone marrow biopsy and clinical data, the diagnosis was l LCMM.LCMM is an uncommon type of MM difficult to recognize, since despite there are excessive serum clonal FLCs, SPE has no M-spike. So, when there is clinical suspicion, the expertise of the laboratory professional is pivotal in defining a procedure of “personalized medicine”, carried out on the basis of the available guidelines, which can allow a rapid diagnosis and early initiation of treatment, essential for improving LCMM patients’ outcomes.

EP200

Pentraxin-3 as a promising biomarker in different inflammatory conditions

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Pentraxin 3 (PTX3) is overexpressed in several pathological conditions by exerting a pivotal role both as a regulator and indicator of inflammatory response. Based on this evidence, in the first part of this analysis we conducted a case–control study to compare the PTX3 serum levels and several immune-inflammatory mediators of 80 healthcare workers who were subdivided into subjects who were previously infected with SARS-CoV-2 (n = 40) and individuals who were never infected (n = 40). Using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA), PTX3 and various immune-inflammatory protein levels were assessed in serum samples, while also considering possible variables (e.g., gender-related differences). We have shown elevated levels of PTX3 and other inflammatory proteins in previously infected COVID-19-positive subjects ($p < 0.001$). Moreover, the obtained data also indicate a degree of severity influenced by gender, as shown by the subgroup analysis, in which PTX3 expression was more pronounced in previously COVID-19-positive males ($p < 0.001$) than in females ($p < 0.05$) compared to the respective controls. In addition, our data further validate, through a direct comparison of previously COVID-19-positive subjects, greater pro-inflammatory levels in males than in females.

As well, literature data have shown that PTX3 could have an impact on follicle growth and development, influencing women fertility. Thus, as second aim of this investigation we probed the role of PTX3 in the context of blastocyst and implantation outcomes. PTX3 protein levels and other cytokines were assessed in the follicular fluid of 23 subjects, under the age of 40 years, undergoing in vitro fertilization cycles, including females without achieved implantation (n=11) and those with implantation (n=12). From our data, PTX3 emerged as a strong predictor, more than TNFα and IL-1β, of implantation failure and related inflammatory follicular state.

Overall, our results may support the validity of PTX3 as a reliable biomarker in inflammatory responses in both pathological contexts, thus, its modulation could constitute an effective therapeutic strategy for improving the recovery from COVID-19 and a valuable target to improve artificial reproductive treatments outcomes.

EP201

Alzheimer's disease: preliminar data on blood-based biomarkers

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βAlzheimer's disease (AD), a primary cause of dementia globally, is traditionally diagnosed with cerebrospinal fluid (CSF) tests and positron emission tomography (PET). These methods are invasive and expensive, with limited accessibility. Recent advancements in sensitive immunoassays have identified potential blood-based biomarkers, such as Aβ42/Aβ40 ratios and phosphorylated tau (p-tau) species. In this preliminar work we evaluated the clinical utility and reliability of these biomarkers on a cohort of 60 patients with cognitive impairment followed by Clinical Neurology, University Hospital of Trieste.We measured plasma and CSF concentrations of pTau181, Aβ42, Aβ40 through an immunoenzymatic assay (Lumipulse G System). Statistical analysis was performed by Analyse-it (Microsoft Excel). Patients were classified on the base of their liquoral profile in four groupes: A+T+, A+T-, A-T+ and A-T- (A refers to β-amyloid deposition, T to pathologic Tau). The four groups were analyzed comparing the results of plasma pTau181, Aβ42, Aβ40 and Aβ42/Aβ40 ratios with that of CSF. Plasma pTau181 was also evaluated in relation to CSF Aβ42/Aβ40 ratio. Plasma pTau181 showed very good performances in the distinction of the different patient subsets (A+ vs A-, A+ T+ vs A- T-, either $p<0.0001$), while discrete performances of Aβ42/Aβ40 ratio were obtained, due, perhaps, to the small patients cohort. No statistical significance was found comparing plasma and CSF pTau181 or Aβ42/Aβ40 ratio in the A+T-, A-T+ groups.

ROC curves for the different subset of patients were calculated. In the case of plasma pTau a cut-off value of 1.5 was found able to discriminate between A- T- and A+ T+ patient groups.These results show as plasma pTau181 and to a lesser extent Aβ42/Aβ40 ratio are promising and not invasive blood-biomarkers in AD diagnosis, follow-up, prognosis and treatment response. Much more effort should be do on developing more robust assays in order to achieve as high performance as core CSF and PET and in the research of new biomarkers able to early identify patients with probable AD.

EP202

The cryoglobulinemia-type trend over the years in the Province of Modena

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INTRODUCTION. Cryoglobulins (GCs) are proteins characterised by the reversible phenomenon of precipitation at 4°C and dissolution at 37°C. By keeping the serum of cryoglobulinemic patients at 4°C for 7 days, we can note the appearance of a measurable cryoprecipitate (CPT). Cryoglobulinemia is referred to CPT consisting of immunoglobulin (Ig) and according to the Brouet classification, simple cryoglobulinemia consists of a single monoclonal Ig (type I), mixed cryoglobulinemia is produced by two circulating Ig: a monoclonal one and a polyclonal one (type II), or both polyclonal (type III).
OBJECTIVE. To assess the cryoglobulinemia-type trend over time, from 2015 to 2023, in the province of Modena.

METHODS. Data were extracted from the Laboratory System (LIS) and processed with Office Excel (Microsoft 2019).

RESULTS: The obtained data indicate the number of positive CGs by year: type 1, 2, 3 and total respectively. 2015: 25, 73, 86, 184; 2016: 20, 77, 95, 192; 2017: 10, 46, 89, 145; 2018: 10, 63,68,141; 2019: 6,35,62,103; 2020: 4,37, 41, 82; 2021: 6, 4, 67, 117; 2022: 1, 41, 99, 141; 2023: 3, 37, 82, 122. Those data were related to patients who came to the laboratory for the first time.

CONCLUSIONS: From the obtained data we can assume:
1) A decrease, almost to the disappearance, of the type 1 CGs, typical of haematological patients. Those are usually cryoglobulins that do not produce symptoms, and for this reason, are not always researched.
2) A substantial reduction of type 2 GCs, often associated with HCV infection, has been almost eradicated thanks to new direct antiviral action drugs and prevention campaigns. As a result, the elimination of HCV-related cryoglobulinemia was observed in 50% of treated cases, thus could explain the decrease.
3) A decrease in type 3 CGs up to 2020, then an increase until 2022 and a further decrease in 2023. CGs type 3 could be due to the evolution of CGs type 2 which lose monoclonality as attenuated by therapies, but could also be associated with autoimmune diseases increased after the COVID-19 pandemic.

EP203

Valutazione dell'Efficacia dell'Immunosottrazione nella Rilevazione delle Catene Leggere Libere nelle Gammopatie Monoclonali.

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Nella diagnosi e nel monitoraggio delle discrasie plasmacellulari, è fondamentale classificare la componente monoclonale (CM) identificando le classi di immunoglobuline coinvolte e l'eventuale presenza di catene leggere libere (CLL), kappa (κ) o lambda (λ). L'approccio standard prevede l'elettroforesi delle proteine sieriche per l'identificazione della CM e il successivo approfondimento per la sua caratterizzazione tramite l'immunofissazione sierica (IFIX). Un'alternativa maggiormente automatizzata potrebbe essere rappresentata dall'immunosottrazione (IT). Presso l'Ospedale San Raffaele di Milano, è stata realizzata una valutazione preliminare al fine di definire la capacità dell'IT di rilevare la presenza di CLL rispetto all' IFIX. A questo scopo, sono stati inclusi nella valutazione 85 pazienti noti per gammopatie monoclonali, per i quali sono state eseguite IFIX (G26 - Interlab Sebia) e successiva IT (Capillary 3 TERA - Sebia). L'IT è stata eseguita mediante elettroforesi capillare, trattando i campioni con antisieri specifici per le catene pesanti (IgG, IgA, IgM) e leggere (κ e λ). La caratterizzazione della CM è stata effettuata osservando la riduzione o la scomparsa di un picco nell'elettroferogramma del campione trattato rispetto a quello non trattato. In 66 pazienti, IFIX ha rilevato la presenza di CM caratterizzate da catene leggere legate e assenza di CLL, confermata all'IT. Nei restanti 19 pazienti, IFIX ha evidenziato anche la presenza di CLL (14 λ libere e 5 κ libere), non identificabili all' elettroferogramma ottenuto con IT. Entrambe le metodiche sono in grado di rilevare la presenza di catene leggere legate alle classi di immunoglobuline G – A – M, ma nel 100% dei pazienti in cui sono presenti anche CLL, la metodica IT non è in grado di evidenziare queste ultime. Pertanto, è evidente come nella classificazione della componente monoclonale delle discrasie plasmacellulari, l'IFIX resti la metodica di riferimento, in quanto in grado di identificare la presenza sia di catene leggere legate che libere. L'IT pur presentando vantaggi in termini di automazione ed essendo efficace nel rilevare le catene leggere legate, non presenta la stessa accuratezza dell'IFIX nella rilevazione delle catene leggere libere.

EP204

Biomarker-based renal response and progression criteria in AA amyloidosis: results from the Pavia-Heidelberg study

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In AA amyloidosis the kidney is involved in almost 100% of cases. The aim of treatment is attaining low levels of serum amyloid A (SAA), which is associated with prolonged survival. However, differently from systemic immunoglobulin (AL) amyloidosis, renal response and progression criteria have not been validated so far. Here we propose new renal response and progression criteria for AA amyloidosis. The study was conducted in the prospectively maintained database of newly diagnosed patients with AA amyloidosis of the Pavia (n=233) and Heidelberg (n=303) Amyloidosis Centers. Patients with a second evaluation were included in the study. Cut-offs of variations in 24h-proteinuria and estimated glomerular filtration rate (eGFR) (at 12 months) from baseline best predicting end-stage renal failure (ESRD) at 24 months were identified by ROC analysis. Impact of SAA normalization (i.e. <6.4 mg/L; at 12 months) on ESRD was also evaluated. Renal response and progression criteria were tested in the Pavia cohort. The Heidelberg series served as validation cohort. One hundred forty-seven patients from Pavia and 156 from Heidelberg were included in the study. Kidney involvement was present in almost 95% of patients in both cohorts. Median follow-up was 7.2 months and 7.0 months in the Pavia and Heidelberg groups, respectively. Progression to ESRD was observed in 54 (41%) patients in the Pavia cohort and 44 (34%) in the Heidelberg group. Renal response was defined as reduction of 24h-proteinuria >40% from baseline, without a worsening of eGFR >20%, and best predicted progression to ESRD in the testing (at 5 years: 5% vs. 34%, P=0.003) and validation cohort (at 5 years: 11% vs. 30%, P=0.009). A reduction of eGFR >20% from baseline predicted shorter time to ESRD both cohorts (at 5 years in the testing cohort: 14% vs. 44%, P<0.001; at 5 years in the validation cohort: 19% vs. 34%, P=0.003). Finally, SAA normalization resulted in prolonged time to ESRD in the Pavia (at 5 years 7% vs. 36%; P<0.001) and Heidelberg cohort (at 5 years 4% vs. 30%; P=0.045). We identified and validated renal response and progression criteria in AA amyloidosis, which can be used for the refinement of patient management and the design of new clinical trials.

EP205

Una forma non comune di cristalli di acido urico nelle urine

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Caso clinico. Nel gennaio 2024, è stata ricevuta dal nostro Laboratorio una richiesta di esame chimico-morfologico delle urine in una donna di 83 anni con occlusione intestinale alta in quadro di carcinoma localmente avanzato. Il campione è stato analizzato su un sistema modulare Sysmex UN (Sysmex Corp, Kobe, Giappone). L'esame chimico delle urine riportava solo emoglobina 0,03 mg/dL, e normalità per altri parametri. Per le regole impostate nel sistema è scattato l'esame microscopico su UD-10, che ci ha permesso di individuare numerosi cristalli di forma atipica a pH acido (pH 5,0). È stato quindi valutato il sedimento anche in microscopia in contrasto di fase a 400x.

Background. L'esame del sedimento urinario viene largamente utilizzato nei laboratori clinici, e la presenza di cristalluria è utile per diagnosticare malattie ereditarie litogeniche, identificare cristalli di farmaci, valutare disturbi metabolici associati alla formazione di calcoli e del rischio di recidiva della calcolosi. L'eliminazione del cristallo di acido urico nell'urina è conseguente al catabolismo degli acidi nucleici endogeni o a causa dell'introduzione eccessiva di alimenti con basi puriniche. Nelle urine è possibile trovare diversi tipi di cristalli acido urico, che a pH acido appaiono in una varietà di forme, tra cui rombica, prismatica e ovale con estremità appuntite, cunei, rosette e piastre irregolari.

Discussione. Dopo revisione della letteratura, è stato possibile trovato solo un altro caso simile, descritto da Baroni et al. (doi: 10.1016/j.cca.2018.01.018), nel quale è descritto un caso di cristalli atipici simili ad aghi e matite, parzialmente simili a quelli riportati da altri che li ritenevano dovuti ad acido urico. Gli autori hanno eseguita l'indagine spettroscopica infrarossa, che ne ha confermato la loro come strutture di acido urico. Vista l'alta variabilità di tipologie di cristalli che hanno come costituente principale l'acido urico, è interessante notare e riportare varianti più rare, per rendere più facile il riconoscimento di queste forme da parte di altri professionisti.

EP206

Studio Metabolico per rischio nefrolitisiaco: Un approccio Integrato tra Patologia Clinica e Nefrologia

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La nefrolitiasi, o calcolosi renale, è una condizione diffusa. Il test metabolico per il rischio nefrolitisiaco è essenziale per valutare i fattori di rischio metabolici e pianificare strategie terapeutiche personalizzate. Il laboratorio di Patologia Clinica, in collaborazione con il reparto di Nefrologia e Dialisi, ha intrapreso un progetto per ottimizzare la gestione dei pazienti con nefrolitiasi. Ai pazienti è stato fornito un kit per la raccolta delle urine delle 24 ore, con istruzioni dettagliate. Il campione urinario delle 24 ore è stato raccolto in due contenitori, uno con acido cloridrico e uno con clorexidina, suddividendo ogni minzione equamente. È stato richiesto anche un campione delle urine della mattina, in cui si sono misurati creatinina, calcio e proteine. Nelle urine delle 24 ore sono stati dosati calcio, ossalato, magnesio, citrato, fosforo, sodio (urine acidificate), potassio, cloro, ammonio, acido urico, creatinina, proteine, urea e pH (urine in clorexidina). I parametri urinari sono stati confrontati con valori soglia standard. Il referto, include la valutazione della saturazione dell'acido urico e degli indici di rischio secondo le formule di Tiselius, insieme all'analisi dell'assunzione di proteine, sale e fosforo. Un commento finale fornisce ulteriori raccomandazioni cliniche. Dall'analisi dei dati raccolti su 20 pazienti con calcolosi renale nota già in follow up, sono emersi i seguenti risultati: Ipercalciuria di origine metabolica (12 pz, 60%); pH urinario aumentato (15 pz, 75%); Ipocitraturia (7 pz, 35%); Iperossaluria (3 pz, 15%); Indici di rischio elevati per la formazione di calcoli di fosfato di calcio (8 pz, 40%); Rischio elevato per calcoli di brushite (3 pz, 15%), per calcoli di acido urico (2 pz, 10%), calcoli di ossalato di calcio (3 pz, 15%). Il test metabolico ha permesso di individuare e correggere i fattori di rischio specifici, non noti, potenzialmente riducendo il tasso di recidiva dei calcoli renali. Questo approccio integrato e personalizzato rappresenta un significativo avanzamento nel trattamento della nefrolitiasi, offrendo una possibilità terapeutica con strategie basate su evidenze metaboliche specifiche. Sono necessari ulteriori studi su un campione più ampio per confermare questi risultati preliminari.

EP207

Il Valore Diagnostico dell'Indice Sistemico di Infiammazione e del Rapporto Neutrofili-Linfociti nelle Malattie della Prostata

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Scopo: Questo studio ha l'obiettivo di valutare i valori diagnostici dell'indice sistemico di infiammazione (SII) e del rapporto neutrofili-linfociti (NLR) in pazienti con iperplasia prostatica benigna (BPH), lesioni precancerose (PL) e cancro alla prostata (PC).Metodi: Tra settembre 2018 e aprile 2024, un totale di 514 soggetti sono stati arruolati per la diagnosi iniziale presso la Clinica Urologica dell'Ospedale Universitario (AOU) di Sassari, Italia. Questo studio trasversale ha incluso 166 pazienti con BPH, 59 con PL e 289 con PC localizzato. I marker dell'emocromo completo, tra cui NLR, rapporto piastrine-linfociti (PLR), rapporto linfociti-monociti (LMR), volume medio piastrinico (MPV), piastrinocrito (PCT), cellule grandi non colorate (LUC), SIRI, AISI e ampiezza di distribuzione dei globuli rossi (RDW) sono stati esaminati e analizzati. L'analisi della curva ROC è stata eseguita per valutare la capacità discriminante dei marker infiammatori e la loro combinazione con PSA per il PC. Un modello di regressione logistica binaria è stato utilizzato per valutare l'associazione tra i marker infiammatori significativi e il PC.Risultati: I nostri risultati rivelano differenze significative nei neutrofili e negli indici compositi correlati, come NLR, SIRI e AISI, suggerendo un potenziale ruolo di questi marker nella diagnosi del cancro alla prostata. L'analisi logistica ha indicato che un conteggio più elevato di neutrofili e indici compositi aumentati erano associati a una maggiore probabilità di progressione del cancro alla prostata. Non sono state osservate differenze significative nei pazienti con lesioni precancerose.Conclusione: Questo studio evidenzia il valore diagnostico di SII e NLR nel differenziare il cancro alla prostata dall'iperplasia prostatica benigna e nell'identificare la progressione del cancro alla prostata. Le differenze significative nei conteggi dei neutrofili e negli indici correlati tra i pazienti sottolineano il potenziale ruolo di questi marker infiammatori nella pratica clinica.

EP208

Extended Next Generation Sequencing-based genes panel analysis as molecular test supporting inherited nephropathies diagnosis

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Nephropathies are a group of diseases with a progressively increasing incidence worldwide and a variable, often multifactorial etiology. More than 200 different kidney diseases have been described that taken together have a prevalence of 6-8 cases per 10.000 people, and may occur both in childhood and adulthood as well. In recent years, the role of genetic factors in nephropathies onset has been emphasized since several variants in an increasing number of genes have been identified. Moreover, Inherited kidney diseases (IKDs) are among the leading causes of early-onset chronic kidney disease and are responsible for at least 10–15% of cases of kidney replacement therapy. Their correct identification is crucial to improve patients' clinical management supporting a more correct prognostic evaluation and therapeutic choices. To investigate IKDs' etiology and improve their molecular analysis, an extended NGS custom genes panel has been developed including a high number of genes possibly explaining such medical conditions. The in-house designed genes panel consists of 205 genes known to be associated with different IKDs, including glomerular, tubular and interstitial kidney diseases, and congenital anomalies of the kidney and urinary tract. Since December 2023 until today, 32 patients, whose DNAs were obtained from peripheral blood samples, have been analyzed through the preparation of Agilent enriched libraries and Illumina MiSeq system sequencing; then reads were analyzed using the web-based tool Seqr. Thus far pathogenic variants have been detected in 5 unrelated patients in 4 different genes, each involved in several molecular pathways: among these, CYP24A1, related to infantile hypercalcemia, CEP290 that is mutated in Bardet-Biedl and Meckel syndromes, KCNJ1 notoriously linked to Bartter syndrome, SLC3A1 impaired in cystinuria cases. Moreover, a huge number of variants of uncertain significance were found, making difficult their correct interpretation since no definitive data regarding their pathogenicity are currently available. Our results show that the use of extended genetic test could be significant to better understand the molecular bases of rare IKDs and to improve medical care for both patients and at-risk families as a step towards precision nephrology.

EP209

Screening delle batteriurie con analizzatore automatico UAS 800 (SIEMENS)

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Introduzione: Il gold standard diagnostico delle infezioni alle vie urinarie è l'urinocoltura, un'analisi complessa che determina un alto numero di campioni negativi ed un elevato carico di lavoro. Per questo, lo scopo dello studio è stato quello di valutare la capacità di screening delle batteriurie dell'analizzatore automatico UAS 800 (SIEMENS) al fine di sottoporre ad esame colturale solo i campioni positivi.
Materiali e metodi: In questo studio sono stati analizzati 350 campioni di urina mitto intermedio o da catetere con richiesta di esame colturale raccolti tra Febbraio e Maggio 2024. Questi sono stati sottoposti ad analisi chimico fisico e del sedimento urinario con piattaforma NOVUS-UAS 800. L'urinocoltura è stata poi eseguita su terreno differenziale cromogeno CHROMIDCPS incubato a 37°C per 48h. I campioni considerati positivi allo screening avevano un numero di leucociti superiori a 17.82 particelle/µl e/o un numero di batteri superiore a 44.88 elementi/µl.
Risultati: Dei 350 campioni analizzati, 276 (78,9%) sono risultati negativi e 74 (21,1%) positivi. Il microrganismo maggiormente isolato è stato Escherichia coli (71.6%). Dal punto di vista della soglia di positività batterica, la sensibilità e specificità sono risultate 97.3% e 23.1%. Il valore predittivo positivo (VPP) e negativo (VPN) 25.3% e 96.9%. Per quanto riguarda le soglie di positività relative ai leucociti, la sensibilità e la specificità sono risultate 80.3% e 81.1%, mentre VPP e VPN rispettivamente 52.3% e 94.1%. Considerando entrambi i parametri, la sensibilità e la specificità sono risultate 100% e 22.1 % mentre VPP e VPN 25.6% e 100%.
Conclusioni: La combinazione tra i valori di sensibilità, di VPN e l'assenza di falsi negativi (considerando leucociti e batteri insieme) ci hanno permesso di stabilire che il sistema UAS 800 può essere utilizzato come metodo di screening delle batteriurie riducendo così il numero di campioni da sottoporre ad urinocoltura, i costi e le tempistiche di refertazione. L'interpretazione dell'operatore resta comunque necessaria alla risoluzione di particolari casistiche quali la presenza di alcuni elementi (sali e cristalli amorfi) classificati erroneamente come batteri o eventuali leucociti dalle forme anomale e quindi non conteggiati.

EP210

The close relationship between podocyte and suPAR in diabetic nephropathy

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Early identification of developing diabetic nephropathy (DN) is crucial. DN manifests with impaired glomerular filtration rate (eGFR), microalbuminuria and progresses to end-stage renal disease. Soluble urokinase receptor (suPAR) has been associated with podocytopathy and its high levels were increased in Diabetes Mellitus. Podocytes are constituents of the glomerular filtration barrier and suPAR has a role in regulating interactions with extracellular matrix proteins. This study investigated whether high levels of suPAR may predict podocytopathy when albuminuria and eGFR decrease not yet appeared. suPAR was measured in 142 diabetic patients (67F/75M; median age 67). Urine Albumin/Creatinine Ratio (uACR) and eGFR were recorded and associated with suPAR levels distribution based on the risk category of CKD in the KDIGO. After a median time of 105 days, 43 patients returned for a second blood collection. The KDIGO identified 80 patients as low risk, 47 as moderate risk, and 13 as high risk of CKD. Mean plasma suPAR levels are 4.613±3.331 ng/mL and showed a significant (P=0.0001) inverse and positive correlation with eGFR and HbA1c respectively. suPAR significantly increased from low to high risk; in particular, the increase is higher between the first two KDIGO category (4.176 vs 6.285 ng/mL) compared to the highest (7.557 vs 7.427 ng/mL). We stratified the 80 patients at low risk considering suPAR quartiles and 52 of them had suPAR in the third and fourth quartiles. Only 5 patients out of 52 had HbA1c levels above the therapeutic target of 53 mmol/mol. Among the 43 patients with a second evaluation timing, 16 had worsening in eGFR or uACR or both and 13 of them had suPAR in the fourth quartile at enrollment. Logistic regression analysis, corrected for age and gender, showed that high suPAR is significantly associated with the worsening (OR: 1.4 [95% CI 1.04-1.88]). suPAR shown to be a better candidate to stratify low-risk KDIGO patients with good glycemic compensation but, that according to suPAR, may need a more careful follow-up. suPAR displayed a significant link with the worsening of renal function and microalbuminuria regardless of gender or age; it could became a predictor for DN and a key strategy for prevent worse outcome in these patients.

EP211

Preliminary evaluation of an innovative diagnostic algorithm software (DAS) for differentiating urinary protein profiles in CKD

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The presence of proteinuria above physiological limits is a known independent risk factor for chronic kidney disease (CDK) [1]. The evaluation of the excreted urine by High Resolution Electrophoresis (HR) distinguishes among the three main pathological proteinuria: glomerular, tubular, and overflow. Specifically, glomerular and tubular proteinuria, are represented by the presence in the urine of macro (>67 kDa) and micro proteins (<67 kDa) respectively. Overflow proteinuria is marked by an excessive amount of low molecular weight proteins, constituted mainly by poly or monoclonal free light chains. Urinary marker proteins measurement may be crucial for distinguishing proteinuria patterns. Thus, the evaluation of proteinuria of 84 spot urines samples from patients with different hematological diseases and/or CDK, was performed through HR by three independent operators and alongside by the diagnostic algorithm software (DAS). This is a unique 'proteinuria pattern definition database', based on total proteins urinary (TPU), creatininuria and specific proteins quantification. All measurements were performed by Optilite turbidimeter (Binding Site) except for TPU and creatinine by Roche Cobas while concordance between methods by Cohen's Kappa (κ) statistical coefficient.

Of the 84 samples analyzed, HR and DAS showed correspondence for most proteinuria types. Firstly, HR recognized 23 physiological proteinuria compared to 25 by DAS, with a good concordance (κ=0.60). Secondly, the overflow parameter was detected in 16 samples by both methods, while glomerular in 33 samples by HR and 27 by DAS. Interestingly, we obtained similar results for mixed proteinuria (15 by HR and 11 by DAS) and tubular proteinuria with 4 recognized by HR and 5 by the algorithm. Preliminary data showed also a moderate concordance (κ=0,567) between two methods in Bence Jones' detection, with 35 identified by HR and 39 by DAS. Finally, for most samples, our analysis relies on a combination of HR and DAS. Furthermore, DAS proved fundamental to classify ambiguous cases of tubular proteinuria, thanks to α-1 and β-2 macroglobulines and Retinol Binding Protein measurements. These preliminary results showed the algorithm's post-analytical support to better distinguish between different proteinuria.

[1]Enrico Valvo:"Proteinuria: pathophysiology, diagnostic and therapeutic approach" Italian Journal of Medicine 2009;3(2):116-122

EP212

Determinazione degli Ossalati Urinari: Implicazioni Diagnostiche e Sfide di Standardizzazione

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La quantificazione degli ossalati urinari rappresenta una sfida analitica cruciale per la diagnosi e il trattamento delle nefropatie ossaliche, in particolare dell'iperossaluria primaria di tipo 1 (PH1). La PH1 è una rara malattia autosomica recessiva causata da mutazioni nel gene AGXT, che compromette l'attività dell'enzima alanina-glyoxalato amino transferasi, con conseguente accumulo di ossalato. L'acido ossalico in elevate concentrazioni forma cristalli di ossalato di calcio nei tubuli renali che precipitano causando calcolosi renale, nefrocalcinosi e insufficienza renale negli adulti e nei bambini. Questo studio ha lo scopo di valutare se l'iperacidificazione delle urine (pH<2) possa preservare l'ossalato garantendone la solubilità, evitando una sottostima della sua concentrazione, e successivamente confrontare la capacità del metodo enzimatico di rilevare con precisione un eccesso di produzione urinaria di acido ossalico, comparandolo con il metodo HPLC.13 campioni di urina delle 24h sono stati prima aliquotati (10mL) ed in seguito iperacidificati con 100 μL di acido cloridrico 40% 6N, osservadoli al m.o. e analizzati con metodo enzimatico pre e post acidificazione. Altri 30 campioni sono stati aliquotati con la medesima operatività ed analizzati utilizzando due distinti metodi analitici: enzimatico e HPLC con rilevatore UV. Abbiamo così potuto osservare come l'iperacidificazione nel 62% dei campioni analizzati abbia determinato un aumento della concentrazione di acido ossalico misurata con metodo enzimatico, probabilmente a causa di una ri-sospensione degli ossalati precipitati. Il confronto delle due metodiche analitiche utilizzate mostra una sottostima del 30% della concentrazione di ossalati misurata con il metodo enzimatico rispetto all'HPLC che evidenzia la necessità di standardizzare le metodiche studiate.La quantificazione degli ossalati urinari, insieme alla composizione chimica dei calcoli e alla diagnostica molecolare, dovrebbero essere presenti nel portfolio di un laboratorio per un corretto inquadramento diagnostico e terapeutico dei pazienti con sospetto di nefropatie ossaliche.

EP213

ALGORITMO PER LA DIAGNOSI DELLA SINDROME DI LYNCH: UTILITA' DELLE INDAGINI SOMATICHE PRELIMINARI

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Circa il 5% dei tumori del colon-retto (CRC) è ascrivibile alla Sindrome di Lynch. L'identificazione dei soggetti affetti da questa forma di predisposizione allo sviluppo di tumori ricopre una notevole importanza clinica poiché permette una significativa riduzione della mortalità ma, nonostante la sempre più ampia diffusione delle tecniche di sequenziamento NGS, uno screening di massa attuato mediante sequenziamento dei geni causativi non è applicabile a causa dei costi elevati e della complessità dell'analisi.L'algoritmo diagnostico proposto dalle più recenti linee guida è stato adattato e modulato nel contesto della realtà del territorio ai fini di razionalizzare ed ottimizzare le risorse in essere presso la SS di Genetica e Biologia Molecolare dell'AO S. Croce e Carle di Cuneo. Grazie all'anamnesi personale e famigliare raccolta in sede di consulenza genetica, è stato possibile individuare i soggetti ad alto rischio di essere affetti da S. di Lynch. L'analisi genetica è stata direttamente proposta ai pazienti rientranti nei Criteri di Amsterdam II, mentre a quelli che soddisfano i Criteri di Bethesda rivisitati sono state in prima istanza proposte analisi molecolari su tessuto tumorale quali la valutazione dell'instabilità dei microsatelliti (MSI), dell'inattivazione somatica del gene MLH da metilazione del promotore (per i ca. endometriali) e la ricerca della mutazione V600E nel gene BRAF (per i CRC). La ricerca delle varianti germinali nei geni del mismatch repair (MMR) non è stata effettuata per i tumori che non presentavano MSI o anomalie nell'espressione genica delle proteine MMR. Riguardo i CRC con mancata espressione di MLH è stata dapprima ricercata la mutazione V600E di BRAF: in caso di sua assenza è stata ricercata l'inattivazione somatica di MLH.Ai pazienti il cui DNA tumorale presentava un profilo suggestivo di S. di Lynch è stata proposta la ricerca delle varianti germinali dei geni MMR eseguita presso il Laboratorio di riferimento Regionale.Lo studio molecolare della MSI costituisce un dato utile anche per i pazienti non affetti da S. di Lynch; la MSI è infatti associata a miglior prognosi per via della buona risposta alla terapia con inibitori del checkpoint immunitario: per questo la valutazione MSI viene anche richiesta dall'Oncologo a fini prognostico/predittivi di risposta ai farmaci mirati e, in caso di presenza di MSI, il dato viene commentato inserendo nel referto il suggerimento a rivolgersi alla consulenza genetica oncologica.I risultati ottenuti presso la SS di Genetica e Biologia Molecolare confermano i dati della letteratura scientifica circa l'inadeguatezza dei criteri di Amsterdam II e di Bethesda rivisitati se utilizzati singolarmente, data l'insufficiente inclusività dei primi e la scarsa selettività dei secondi evidenziando però la loro utilità clinica, se utilizzati insieme e soprattutto in sinergia con le analisi molecolari su tessuto tumorale.

EP214

AN EPIGENETIC AMPLIFICATORY APPROACH FOR CYSTIC FIBROSIS THERAPY

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Cystic Fibrosis (CF) is an autosomal recessive disease caused by pathogenic variants in the CFTR gene. CF has a high complexity at cellular level, as demonstrated by the recent finding of the FOXI1-expressing ionocyte, which has been added to the already complex cellular context of lung. We setup a patient-specific cellular system of nasal epithelial stem cells (called CF-CRC-AESC), inducible to respiratory differentiation, to test gene expression and epigenetic modulation. The targetable amount of CFTR mRNA may be variable and it may be usefully enhanced by amplificatory and ionocyte-inducing experimental therapies. CFTR and FOXI1 mRNA expression in nasal brushing samples and CF-CRC-AESC cultures from CF patients, carriers and wild-type individuals revealed a great interindividual variability, mostly unrelated to the CFTR genotype as well as to the FOXI1 SNPs profile. This variability can affect the therapeutic response. Moreover, the expression pattern of CFTR in nasal brushing is correctly reproduced in differentiated CF-CRC-AESC, highlighting a possible epigenetic control of CFTR and FOXI1 gene transcription, as well as of respiratory epithelium differentiation. Focusing on the therapeutic aspect, we tested an epigenetic amplificatory strategy by using the hypomethylating drug 3-deazaadenosine (3-DZA) aimed to the enhancement of the mRNA expression of CFTR (the disease marker) and FOXI1 (a respiratory epithelium differentiation marker) genes in CF-CRC-AESC. The 3-DZA enhanced both the CFTR and FOXI1 expression, in differentiated CF-CRC-AESC with wild-type and F508del/F508del genotypes. The mRNA levels of both the CFTR and FOXI1 genes seem crucial for the enhancement of precision targeted therapy. The 3-DZA could be exploited as an amplifying drug, since it showed to be able to amplify the gene expression of both CFTR and FOXI1, with possible increase of protein production. The increased amount of CFTR protein, even if hypofunctional due to pathogenic variants, could provide an increased substrate for CFTR modulatory drugs. These findings provide new insights into the role of DNA methylation in the genotype-phenotype relationship in CF, as well as into epigenetics as a new CF therapeutic strategy.

EP215

De novo factor VIII gene intron 22 inversion in a male inherited from the mother as probable gonadal mosaiscism

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INTRODUCTION: Haemophilia A (HA) is a blood clotting disorder caused by a genetic deficiency in clotting Factor VIII (F8), thereby resulting in significant susceptibility to bleeding. This condition occurs almost exclusively in males born to carrier mothers due to X-linked recessive inheritance. Three classes of pathogenic variants in the F8 gene are responsible for HA: inversions of intron 1 or intron 22, deletions/ duplications and point changes. Nevertheless, rare, isolated cases emerge from de novo mutations.MATERIALS AND METHODS: DNA was extracted from patients and amplified by PCR using specific primers pairs #anking exon-intron junctions of the twenty-six exons and the promoter of the F8 gene. PCR products were analyzed by direct sequencing of amplified regions. The inversion of the intron 22 was performed by using Long Distance - PCR with two appropriate primer pairs. To search for macrodeletions/duplications in the gene coding for Factor VIII Multiplex ligation-dependent Probe Amplification (MLPA) was performed.RESULTS: From 2021 to today, 38 patients have been analyzed at the Ceinge Biotecnologie Avanzate in Naples. 33 males were affected by type A haemophilia, highlighting, in hemizygosis, the presence of a point mutation in 23 patients, an intron 22 inversion in 9 patients and a deletion of exon 26 of the F8 gene in only one of these. Furthermore, five females were negative. Among the nine patients carrying the intron 22 inversion, a 1-year-old child came to our attention due to suspicion of severe HA having F8 values lower than 0.1%. This mutation seems to have arisen de novo, being absent in the expected mother as an obligate carrier. CONCLUSIONS: De novo intron 22 inversion is described in few cases and it has been thought that it may be a case of gonadal mosaicism in the mother. Germinal mosaicism in hemophilia appears to be rare as reported to date3.Molecular investigations to detect gonadal mosaicism involve the observation of germ cells. So it is necessary to deepen the examinations in the mother of the proband, sampling from buccal brushings or hair radical bulbs. Unfortunately, as it is impossible to confirm that causal variants arise at conception or very early in zygotes, guidelines recommend and propose prenatal diagnosis in subsequent pregnancy.

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EP216

L'impatto del favismo in una coorte di pazienti con errori congeniti dell'immunità: quali scelte terapeutiche?

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INTRODUZIONE: La profilassi antibiotica a lungo termine è una pratica consolidata nella gestione di alcuni errori congeniti dell'immunità (Inborn Errors of Immunity, IEI) di cui modifica la storia naturale garantendo una riduzione dell'incidenza degli episodi infettivi. Il deficit di glucosio-6-fosfato deidrogenasi (G6PD-D) è il più comune deficit enzimatico eritrocitario, X-linked e può limitare la scelta della molecola da adottare in quanto antibiotici ad elevato potere ossidante possono indurre una crisi emolitica acuta.MATERIALI E METODI: Sono stati analizzati 120 pazienti per sospetto IEI, sottoposti a work-up immunologico completo ed indagine genetica mediante Next generation sequencing. Nei pazienti risultati portatori di varianti di classe I è stato effettuato dosaggio enzimatico.RISULTATI: Abbiamo identificato la presenza di una variante patogenetica di G6PD in 4 pazienti (3% della casistica) tra cui una donna che non ha impattato sulle scelte terapeutiche. Gli altri 3 pazienti presentavano difetto di Myd88, neutropenia congenita e sindrome Iper-IgM da difetto di CD40L. Il difetto di funzione è stato confermato mediante dosaggio enzimatico. Nel paziente con difetto di Myd88 e nel paziente con difetto di CD40L i riusltati della genetica hanno indotto la modifica della scelta della terapia, sostituendo il cotrimossazolo con l'amoxicillina. Nel paziente con neutropenia congenita è stata instaurata una terapia appropriata, evitando l'uso di molecole a rischio, in caso di insorgenza di infezioni.CONCLUSIONI: La profilassi e la terapia antibiotica hanno un ruolo ineludibile nella gestione di molte IEI per ridurre il rischio di infezioni batteriche gravi, talvolta potenzialmente mortali. La scelta dell'agente antibatterico può risultare problematica in caso di deficit di G6PD in cui alcuni farmaci sono preclusi in quanto riconosciuti come sostanze ad elevato potere ossidante. Considerata l'incidenza del difetto nella popolazione generale la coesistenza di questo difetto con le IEI non è infrequente, dato confermato dall'analisi della nostra coorte. E' pertanto opportuno praticare screening genetico per escludere tale condizione nei pazienti con IEI che necessitino di terapia antibiotica profilattica a lungo termine o a dosaggio terapeutico per il trattamento di infezioni.

EP217

GENOTIPIZZAZIONE DELLE IPERFENILALANINEMIE IN CAMPANIA DAL 2021 AL 2023

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INTRODUZIONE: Le iperfenilalaninemie (HPA), l'errore congenito più comune del metabolismo degli aminoacidi, sono principalmente causate da mutazioni nel gene della fenilalanina idrossilasi (PAH). METODI: Sul DNA estratto da sangue periferico di neonati risultati positivi allo screening neonatale per HPA dal 2021 al 2023, seguiti presso il centro di riferimento regionale di Napoli, sono state amplificate le regioni comprendenti i tredici esoni e le regioni introniche fiancheggianti del gene PAH e sottoposte a sequenziamento automatico con metodo di Sanger. L'analisi dei dati è stata effettuata mediante software CodonCode Aligner. RISULTATI: Sono stati analizzati 113 pazienti italiani con HPA (18 PKU, 18 HPA II, 72 HPA III) e sono state riscontrate mutazioni in 108 pazienti (detection rate 95%). Sono state identificate un totale di 50 diverse varianti genetiche, confermando l'elevata eterogeneità del gene PAH. Le mutazioni più frequenti sono: c.782G>A p.(Arg261Gln), c.1208C>T p.(Ala403Val) e c.1066-11G>A. Inoltre, è stata identificata una nuova mutazione (c.983C>T), de novo in una famiglia. Le mutazioni sono localizzate nel gene PAH come segue: gli esoni 6 e 7 presentano il 46% delle mutazioni totali; l'esone 11 contiene il 12%; l'esone 2 contiene il 10%; gli esoni 3, 4, 5, 8, 9, 10 e 12 ne contengono meno del 10%. Infine non è stata identificata nessuna mutazione nell'esone 1. Considerando i diversi fenotipi, la mutazione c.1066-1G>A è quella più frequente (28%) nei pazienti con PKU, seguita dalla mutazione c.782G>A (16%). Le mutazioni più frequenti nei pazienti HPA II sono c.782G>A (12%), c.143T>C (10%), c.1066-11G>A (8%). Infine, nei pazienti HPA III, le mutazioni più frequenti sono c.1208C>T (44%) e c.898G>T (24%). La mutazione c.638T>C è presente solo nei pazienti PKU, la mutazione c.1223G>A nei pazienti HPA II e le mutazioni c.898G>T e c.734T>C negli HP AIII. Infine, le mutazioni c.782G>A e c.1066-11G>A sono associate a tutti e tre i fenotipi. CONCLUSIONE: L'analisi molecolare dell'HPA risulta fondamentale per la corretta caratterizzazione dei pazienti analizzati, inoltre potrebbe confermare e/o predire il fenotipo biochimico, contribuendo così alla programmazione di un'approccio dieto-terapeutico appropriato. Infine, la previsione del fenotipo biochimico per un dato genotipo consente un adeguato counseling genetico, la diagnosi prenatale e la programmazione del follow-up e dell'outcome a lungo termine dei pazienti.

EP218

DISTROFIA MUSCOLARE DI DUCHENNE: I PROGRESSI TERAPEUTICI RICHIEDONO UNA DIAGNOSI MOLECOLARE PRECOCE

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La distrofia muscolare di Duchenne (DMD) è una miopatia progressiva letale a trasmissione X-linked recessiva. Essa colpisce prevalentemente i maschi (1/3.500-9.300) ed è causata da mutazioni del gene DMD (Xp21.2), che codifica per la proteina distrofina. Le alterazioni genetiche più frequenti (60-70% dei casi) sono delezioni/duplicazioni (CNV) che comprendono uno o più esoni, seguite da piccole mutazioni nonsenso/missense/frameshift (SNV). Il fenotipo più severo (DMD) è generalmente correlato a variazioni che alterano la cornice di lettura (out-of-frame) del gene DMD o che introducono stop codon prematuri; le varianti in-frame sono più spesso associate al fenotipo Becker, meno grave. Non c'è una terapia efficace per la cura della DMD, ma sono in uso diverse strategie terapeutiche mirate a correggere specifiche alterazioni.Dal 2018 al 2024, abbiamo eseguito il test molecolare di DMD-BMD in 204 soggetti (64 femmine, 140 maschi), riferiti per segni clinici o familiarità. La ricerca di CNV e SNV è stata eseguita, rispettivamente, mediante MLPA e next generation sequencing (NGS) di oltre 200 geni associati a distrofie muscolari. Tra i pazienti con valori di CPK sierica 1000-40000 U/L, ma senza altri segni evidenti di malattia, 15 maschi e 5 femmine di età compresa tra 1 e 40 anni sono risultati positivi al test. Di questi, 12 maschi e 3 femmine presentano delezioni/duplicazioni di 2 o più esoni, sia in-frame che out-of-frame, che correlano con l'esordio e la gravità del fenotipo. Negli altri 5 pazienti, l'NGS ha identificato 4 nuove mutazioni nonsenso e una mutazione di splicing.Lo sviluppo e l'ottimizzazione di nuove terapie sta progressivamente portando ad un aumento delle aspettative di vita dei pazienti. Recentemente, la Food and Drug Administration ha approvato la prima terapia genica per la DMD, indicata per il trattamento di pazienti con diagnosi genetica, deambulanti e di età compresa tra 4 e 6 anni. Estendendo questi requisiti ai pazienti individuati dalla nostra analisi, 6 potrebbero beneficiare della terapia genica. Questi sviluppi, in concomitanza al trial clinico europeo attualmente in corso, evidenziano la necessità di effettuare uno screening precoce, anche molecolare, per individuare tempestivamente i pazienti eleggibili per la terapia.

EP219

Tre pazienti di etnia Rom portatori della stessa delezione del gene MYD88 provenienti da uno stesso Centro: effetto fondatore?

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RAZIONALE DELLO STUDIO: Il deficit di MyD88 è una rara immunodeficienza caratterizzata da suscettibilità a infezioni piogeniche invasive favorite da una risposta infiammatoria inadeguata, con mancato incremento indici di flogosi. Ciò può ritardare l'identificazione di infezioni potenzialmente letali con mortalità massima in epoca infantile. La metà dei pazienti segnalati è di etnia Rom, un gruppo itinerante a rischio di mortalità infantile derivante dall'accesso limitato ai servizi sanitari e dalle condizioni abitative spesso non adeguate. Immunodeficienze congenite dovrebbero essere sempre sospettate in questo gruppo poiché esso rappresenta un isolato genetico. MATERIALI E METODI: Abbiamo analizzato tre pazienti affetti da deficit di MyD88, appartenenti a tre famiglie di etnia Rom, non imparentate, portatori della stessa delezione (E66Del) di cui ipotizziamo un effetto fondatore, sulla base anche della revisione della letteratura. L'indagine genetica è stata condotta mediante l'analisi di un pannello NGS targeted per geni associati a immunodeficienze congenite. RISULTATII : Tre pazienti hanno presentato infezioni piogeniche ricorrenti dal primo anno di vita. Il work-up ha mostrato IperIgE e l'indagine molecolare in NGS la delezione E66Del del gene MYD88, descritta nel 66% dei pazienti della letteratura dello stesso gruppo etnico. E' stato avviato follow-up e terapia profilattica. Tutti sono andati incontro a un drammatico miglioramento clinico. Tuttavia, P1 e P2 hanno interrotto la profilassi e il follow-up e hanno successivamente presentato infezioni severe (encefalite pneumococcica e meningite a eziologia non nota, rispettivamente), fatale nel caso di P1. CONCLUSIONI: L'analisi genetica, sui tre pazienti di etnia Rom, ha mostrato in tutti la stessa delezione nel gene MYD88, già segnalata nel 66% dei pazienti affetti della stessa etnia, suggestivo di effetto fondatore. Questa diagnosi può essere sottovalutata a causa della discrepanza tra la gravità delle infezioni e i reperti laboratoristici. In assenza di strumenti di screening precoce, è necessario mantenere un elevato livello di allerta nei pazienti di etnia Rom con insorgenza precoce di infezioni. Data la frequenza della p.E66del, il sequenziamento diretto potrebbe essere utilizzato per uno screening rapido in questi pazienti con fenotipo suggestivo. Infine, si osservano tipicamente livelli aumentati di IgE per cui tale immunodeficit dovrebbe essere incluso nella diagnosi differenziale delle sindromi da iper-IgE.

EP220

Method comparison for expanding analyte profiling in drug of abuse screening on urinary matrix

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Introduction: In today's clinical setting of emergency departements, there is a growing need to implement rapid and reliable urine screening methodologies for drugs of abuse.
Methods: sixty urine samples from emergency department patients (65% males) with the request of toxicological analyses for clinical and not medical forensic purposes. Two different screening approaches were compared, providing semi-quantitative data: AU 5800© (Beckman Coulter, USA), based on an enzyme immunoassay technique, used routinely in the laboratory and including seven urinary drug kits (amphetamine, cocaine, barbiturates, benzodiazepines, cannabinoids, opioids,methadone), that has been implemented with 3 additional kits (fentanyl, oxycodone, and ketamine), and MULTISTAT EV4455© (Randox, UK), a multiparametric method based on a POCT-like approach performing chemiluminescence competitive immunoassays to detect up to 29 analytes.
Results: sample positivity rates obtained with the two instruments were comparable, despite small differences due to the different assay techonologies: for example AU found 1.6% positivity for oxycodone, while Multistat reported 5% positivity, Multistat found 33.3% positivity for fentanyl compared to 26.5 % found by AU. By adopting the expanded screening panel new drug positivities were observed: ketamine (17 with AU and 15 with Multistat), oxycodone (1 with AU and 3 with Multistat), fentanyl (16 with AU and 20 with Multistat). The data show excellent agreement, with Cohen's K values ranging from modest (0.50 for oxycodone) to very high (0.96 for cocaine).
Conclusions: both analyzers can be used as a screening method for accurate and timely diagnosis in the emergency department setting. The choice of the optimal instrument for drug of abuse screening should be based on clinical aspects related to the patient population referred to the laboratory and organizational aspects inherent to the workflow and sample pathway. In the face of an approach based on multiparametric clinical chemistry analytical platform with established use in laboratories, it will be interesting to evaluate the possible role of a dedicated instrument with a wider panel of analytes that could provide complementary results in selected patient populations.

EP221

ANALYTICAL PERFORMANCES VERIFICATION OF SIX IMMUNOMETRIC METHODS FOR URINE DRUG SCREENING IMMUNOASSAYS

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Background: Drug misuse represents a social plague which has led an increase of the toxicological test requests and the need to obtain quick and accurate results. Urine immunoassay screening method is the most common approach to satisfy these demands, but there are several limitations. Aim: The study aims to evaluate, according to CLSI EP15-A2 and CLSI EP12-A2 guidelines, the method verification and diagnostic accuracy of six semi-quantitative enzyme immunoassay methods (Emit® II Plus) applied to Atellica® Solution, using urine specimens in order to verify urine drug screening in our practice. Methods: Method verification and diagnostic accuracy for Amphetamine, Ecstasy, Methadone, Cannabinoid, Cocaine and Opiate consisted of four parts: a) between-day precision evaluation (PWL) study obtained running three replicates once a day for five days of two quality control materials (BIO-RAD©); b) limit of blank (LoB) evaluated testing three replicates per run, for five runs using a negative sample; c) trueness obtained using a calibrator tested in triplicate for three days; d) diagnostic sensitivity (Se) and specificity (Sp) performed using a total of 170 urine specimens. To confirm all results an UPLC-MS/MS method was used. Results: For all assays, PWL was <5% (from 1,33% to 2,99% for low level and from 1,31% to 3,28% for high level). The highest LoB value was 1,27 µg/L, definitely below the cut-off used in our laboratory. Trueness showed a Bias% of -1.07% for Amphetamine (at 500 µg/L), -4,03% for Ecstasy (at 1000 µg/L), -0,22% for Cannabinoid (at 50 µg/L), +0,76% for Methadone (at 500 µg/L), -1,44% for Cocaine (at 300 µg/L) and +2,22% for Opiate (at 300 µg/L). For all tests, Se was 100% while Sp was 100% for Amphetamine, Cocaine and Opiate, except for Cannabinoid (99,3%) due to the presence of 2 false positive (FP), Methadone (98,6%) linked to 1 FP and Ecstasy (99,4%) with 1 FP probably due to the presence of Quetiapine (5107 µg/L) which might belong to structurally unrelated compounds of MDMA capable to produce a positive result, as indicated by cross-reactivities manufacturer's document. Discussions: Verification and diagnostic accuracy results meet the requirements adopted by our laboratory, ensuring the reliability of the analytical data.

EP222

Metabolic Profiling of Elexacaftor/Tezacaftor/Ivacaftor (ETI) by liquid chromatography-high resolution mass spectrometry (LC-HRMS)

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Cystic fibrosis transmembrane conductance regulator (CFTR) modulators are drugs designed to restore CFTR protein function in cystic fibrosis (CF) patients. The combination of elexacaftor (ELX), tezacaftor (TEZ), and ivacaftor (IVA) (ETI) is approved for patients aged six and older with at least one F508del mutation in the CFTR gene and it is prescribed at a standard dose for adults and adjusted by weight for children. Treatment response variability has been observed, but its causes are poorly studied. A definitive correlation between pharmacokinetic (PK) parameters and ETI efficacy is not yet established. ETI is metabolized in the liver by cytochrome P450 (CYP450) enzymes, particularly CYP3A4 and CYP3A5. This CYP450-dependent metabolism can lead to altered ETI exposure due to drug-drug interactions, pharmacogenetic variants affecting enzyme activity, or liver impairment.
In this study the semi-quantitative profiling of ETI was conducted on 53 anonymized plasma samples from CF patients treated with ETI according to the summary of product characteristics. Analyses were performed by liquid chromatography-high resolution mass spectrometry (LC-HRMS) with a UHPLC Vanquish Transcend Duo system coupled to a Orbitrap Exploris 120 Mass Spectrometer (Thermo Fisher Scientific, Milan, Italy). Ionization was achieved with an ESI source in both positive and negative modes using C18 and HILIC chromatographic columns. Data were acquired in MS Full Scan mode with a resolution of 120000 (mass range 100–1000 m/z). Raw data files were processed with Compound Discover software, simulating Phase I and Phase II metabolic reactions. Ionic currents corresponding to m/z values were extracted and filtered based on peak chromatographic quality. Putative metabolites were compared to pure ETI standards, selecting those with a mass spectrum fragmentation pattern match score above 80%. This procedure allowed the identification of ELX, TEZ, IVA and the metabolites: ELX-M23, TEZ-M1, TEZ-M2, TEZ-M5, IVA-M1, IVA-M6. Significant variability in the drug-metabolite ratio among patients was observed, suggesting a need for further investigation into the role of metabolism in inter-individual variability of clinical response to treatment.

EP223

Cannabis intoxication in two children: case report and pharmacokinetics

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Introduction: The accidental exposure of children to edible cannabis products is a growing concern. Symptoms typically appear between one and a half to three and a half hours after ingestion, with common signs including lethargy, ataxia, hypotonia, mydriasis, tachycardia, and hypoventilation. The nonspecific nature of these symptoms in infants can lead to delayed diagnosis and inappropriate treatments. Toxic screening is essential for accurate diagnosis and treatment. We present the pharmacokinetic (PK) profile of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) following the unintentional ingestion of a marijuana edible in two children (aged 1 and 2 years). Methods: The PK study was based on a sparse sampling strategy at random time-points to better fit in the clinical pediatric setting. Since the exact time of ingestion was unknown, in both clinical cases, the time of the collection of the first sample was considered as time zero. Subsequent samples were collected 16, 27, and 126 hours later for case 1, and 4, 30, and 82 hours later for case 2. THC and CBD measurement in plasma was performed by a validated liquid chromatography-tandem mass spectrometry method. The 8-point calibration curve ranged from 0.2 to 300 ng/ml for both THC and CBD. PK parameters have been computed utilizing a non-compartmental analysis (NCA) approach from the available concentration–time data. THC plasma concentrations were used to calculate the constant elimination rate (kel), estimated by log-linear regression analysis of the terminal phase of the plasma concentration vs the time curve. The half-life (t1/2) was calculated as t1/2 = ln2/kel. Results: CBD was undetectable in one case while, in the second it was detectable only in the first sample with a concentration of 1.11 µg/L. For THC, the PK parameters in the two cases were as follows: the kel was 0.02 h#¹ and 0.04 h#¹, the t1/2 was 42 h and 16 h, respectively. Conclusion: Given the highly variable and difficult to predict PK parameters in the two cases presented we can conclude that monitoring drug levels over time is a useful tool for the management of intoxications in children.

EP224

Quidel "Triage TOX Drug Screen" THC Screening Assay Evaluation: comparison of Rapid Drug Test and LC-MS/MS Measurement Method

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Background: the diagnostic activity in Romagna (a geographical area that includes more than one million inhabitants) is organized with an HUB laboratory and seven spoke laboratories (LRR) located within the main hospitals. THC assay in LRRs is measured on site using Triage TOX Drug Screen urine immunoassay (Quidel). This test is easy to use and available 24 hours a day,7 days a week, butthe main disadvantage of immunoassays is the lack of analytical specificity due to cross-reactivity with drug metabolite sand other analytes. For this reason, samples testing positive for THC with Triage TOX Drug Screen were after tested with a second immunochemical rapid test SureStep (Abbott). Samples with discordant results (positive on Quidel and negative on Abbott) were further verified at the HUB laboratory using the liquid chromatography technique coupled to mass spectrometry (LC-MS/MS), reference method accredited according to the International Standard ISO 15189.
Methods: the Triage TOX Drug Screen test has a THC-COOH cut off of 50 ng/ml. The SureStep test has a THC-COOH cut off of 50 ng/ml.LC-MS/MS for THC-COOH assay, has a cut off of 15 ng/ml.
Results: in 2 years (2022/2023), 7780 urinary screening analyzes carried out in patients belonging to the various emergency rooms of the Romagna AUSL were reported. Considering the positive tests, only 13 samples (0.17% of the total number of samples analysed) had discrepant results between Triage (positive result) and SureStep (negative result).Mass spectrometry analyses put in evidence, in 4 of 13 samples tested, the presence of traces of THC-COOH (inactive metabolite indicating THC intake) above the cut off of 15 ng/ml but still lower than the cut off of the immunochemical kits of 50 ng/ml.
Conclusion: in this work we demonstrated that the Quidel Triage TOX Drug Screen THC assay showed acceptable analytical correlation performance compared to LC-MS/MS. These methods can be adopted in the clinical laboratory for fast THC drug monitoring of patients who require rapid treatment.

EP225

Operating Procedures for the determination of psychotropic drugs in biological matrices in Drug-facilitated crimes

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The term Drug-facilitated crime (DFC) generally refers to the violation of law under altered behavior and perceptions due to the effects of psychotropic substances, intentionally or unintentionally administered to adults, elderly, and children. In particular, drug-facilitated sexual assaults (DFSA) occur when a person is subjected to sexual acts while unconscious, incapable of understanding or reacting due to intentional or unintentional ingestion of alcohol, and/or psychotropic substances, also called rape drugs. Usually, these substances are pharmacologically potent, fast-acting central nervous system depressants with anesthetic effects provoking similar effects to those of alcohol acute intoxications. In Italy, the Decree of the President of the Council of Ministers (DPCM) of 24 November 2017 "National Guidelines for Health Authorities and Hospitals on first aid and social- health assistance for female victims of violence" is the tool to ensure an adequate and integrated intervention in the treatment of the physical and psychological consequences on women victim of violence. Many Regional Health Authorities have activated a "Pink Code" Protocol to clearly define common procedures for the admission and care of victims of psychological, physical, sexual or economic abuse belonging to fragile categories (women, minors, the elderly, non-EU citizens, homosexuals). In this context, a Regional Reference Laboratory for toxicological analysis in DFSA cases should be established to compose a capillary network on the national territory to achieve the same standards in all the regions and collect accurate information on the issue. Beside the conventional and unconventional matrices, the toxicology laboratory should be prepared for the storage and analysis of gastric content, due to the relevance of this matrix in the evaluation of DFSA. Furthermore, a list of substances has been proposed as minimum standards to be analyzed in the toxicology laboratory in DFSA cases.In conclusion, toxicology laboratories should support the medico-legal judgments by providing robust and reliable analytical results ensuring high quality standard on the entire national territory, in order to face the growing issues of DFSA.

EP226

PROPOSAL FOR THE IMPLEMENTATION OF A PROTOCOL FOR THE ASSESSMENT OF ALCOHOL ABUSE TO DRIVE IN LICENCE REGRANTING PROGRAM.

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Introduction: The condition resulting from alcohol dependence compromises the physical and mental requirements needed for the evaluation of fitness to drive. "The local medical commission must determine fitness to drive and, for this purpose, may utilize individual consultants or specialized medical institutes belonging to public structures, with the cost borne by the person being examined" (D.P.R. 495/92, art.330,c.6). For these assessments, a protocol has been established in the Liguria region (Resolution 321 of 19/12/2018) that includes not only the traditional quantification of Carbohydrate-Deficient Transferrin (CDT) in serum but also the measurement of ethyl glucuronide (ETG) in keratin matrix, excluding all traditional indirect markers (transaminases, MCV ecc…). Through this retrospective study, we will verify the validity of the two selected markers to identify individuals who abuse alcohol, with the aim of evaluating the best and most effective tool for monitoring the fitness to drive of individuals penalized under Article 186 of the Highway Code, while also seeking to further simplify the procedure to be adopted.Methods and materials: The retrospective study was conducted on 2976 individuals penalized under Article 186 of the Highway Code, examined by the local medical commissions of Liguria in 2023 (ASL5-Spezzino, ASL4-Chiavarese, ASL2-Savonese), using a protocol that included the quantification ETG in keratin matrix and CDT in serum. The analyses were performed at the regional reference toxicology laboratory for eastern Liguria, located at San Bartolomeo Hospital in Sarzana. The quantification of ETG in the keratin matrix was carried out using a liquid chromatograph (Agilent Infinity 1260) coupled with a tandem mass spectrometer (Agilent QQQ 6470). CDT, on the other hand, was evaluated using high-pressure liquid chromatography (Thermofisher Ultimate 3000) with a UV-Vis detector. Sampling of the keratin matrix was performed on a 3-6 cm segment of hair taken from the nuchal area; alternatively, chest hair was sampled (SOHT consensus 2019). For reporting, the cut-off values suggested by the ISS guidelines, forensic toxicology, and SOHT were considered. Results: From the observation of our data related to the local medical commissions in the Liguria region, it emerges that out of 2976 individuals monitored in 2023 for driving under the influence, to whom our protocol was applied, CDT was positive for 29 individuals (0.97%) and ETG was positive for 510 individuals (17%). Only 18 samples were positive for both biomarkers (0.60%), while 11 samples were positive only for CDT (0.37%). The most interesting finding was the large number of samples positive for ETG alone.
Conclusions: This study, which compares CDT in serum and ETG in keratin matrix, reinforces the fundamental role of ETG as an indicator of excessive alcohol consumption. Traditional indicators, such as CDT, characterized by low sensitivity, are not ideal for assessing fitness to drive. It is clear that even the sole determination of ETG values in the keratin matrix is an effective and sufficient tool for monitoring fitness to drive. If possible, it would be preferable to analyze two different markers with the same diagnostic

EP227

Long-term assessment of the safety profile of Epidyolex®: analysis of THC plasma levels

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Highly purified cannabidiol (CBD) (Epidyolex®) serves as an adjunctive therapy for drug-resistant seizures in Dravet Syndrome (DS), Lennox-Gastaut Syndrome (LGS), and Tuberous Sclerosis Complex (TSC). Despite lacking psychotropic effects, some studies put in evidence potential accumulation of delta-9-tetrahydrocannabinol (THC) in adipose and brain tissues following repeated administration. This study aimed to evaluate the long-term plasma levels of THC in patients undergoing Epidyolex® treatment. A cohort of children with pharmacoresistant epilepsy, receiving Epidyolex® as adjunctive therapy, underwent baseline blood sampling. Plasma levels of both CBD and THC were measured using a validated microsampling method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) with deuterated internal standards. The study included 11 patients with a mean age of 14 years affected by DS (7 patients, 64%), TSC (3 patients, 27%), and LGS in (1 patient, 9%). The median follow-up period observed was 1.3 years (range, 5-52 months). All patients received incremental doses of Epidyolex® and the mean dose at the last follow up was 12 mg/kg/day. At the last follow-up, the mean plasma concentration of CBD was 203.2 μg/L, while THC levels consistently remained below 0.2 μg/L despite the incremental doses of Epidyolex® administered. These results are consistent with existing clinical evidence indicating the absence of psychotropic and psychoactive effects, further confirming the absence of accumulation at therapeutic dosages.

EP228

Progress in HIV Care: Validating an LC-MS/MS method for Multi-Drug Monitoring in Antiretroviral Therapy

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The management of HIV has been revolutionized by combination therapy using different antiretroviral agents, transforming HIV from a lethal disease to a manageable chronic condition. Despite their efficacy, the adverse effects, toxicities, and adherence to medication regimens remain critical aspects for continuous assessment. In this context, we have developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous quantification of seven antiretroviral drugs: Bictegravir, Emtricitabine, Doravirine, Cabotegravir, Lenacapavir, Fostemsavir, and Tenofovir alafenamide. Given the prodrug nature of the last two drugs, their active metabolites, Temsavir and Tenofovir, were also included in our analysis. Our validation process adhered to the European Medicines Agency guidelines (EMA). Analytical standards for each drug were prepared from 99% pure powders of each molecule, an internal standard—Amprenavir— was utilized for all testing sessions. Transition peaks were identified for each analyte and the corresponding calibration curves were obtained. Precision, accuracy, and repeatability were assessed using three sets of intraday and interday calibration curves, along with quality controls at low, medium, and high concentration over three different testing sessions. Matrix effect, recovery, stability, and carryover were also evaluated. Clinical evaluation on plasma sample from patients undergoing treatment was also obtained. All analytes' calibration curves demonstrated excellent linearity with R^2 values greater than 0.99. The precision, accuracy, and repeatability of the measurements were consistent with the standards outlined in the EMA guidelines. The method showed minimal matrix effects (93-113%), satisfactory recoveries (83-110%), and robust stability after multiple freeze-thaw cycles. Noteworthy, Cabotegravir was the only drug demonstrating significant carryover (14%). Clinical evaluation of plasma samples from treated patients confirmed the method's accuracy in reflecting the pharmacokinetics of the antiretrovirals tested. This method holds great promise for enhancing the monitoring of antiretroviral drug concentrations, potentially leading to optimized patient follow-up and personalized treatment strategies in the clinical management of HIV.

EP229

COLLABORAZIONE TRA TECNICO SANITARIO DI LABORATORIO BIOMEDICO E INFERMIERE PER LA RACCOLTA A VALENZA MEDICO-LEGALE DELLA MATRICE CHERATINICA A SCOPO TOSSICOLOGICO PRESSO IL CENTRO PRELIEVI DELL'OSPEDALE PIO XI DI DESIO

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INTRODUZIONE

Il Tecnico Sanitario di Laboratorio Biomedico (TSLB) è l'operatore sanitario che applica le conoscenze biomediche e le tecniche laboratoristiche in ambito diagnostico per l'analisi dei campioni biologici, utilizzando strumentazioni e tecnologie all'avanguardia e garantendo la qualità dei risultati ottenuti.

MATERIALI E METODI

La raccolta della matrice cheratinica è svolta presso spazi adibiti al Centro Prelievi attenendosi alle indicazioni contenute nell'istruzione operativa aziendale, redatta in collaborazione tra il personale infermieristico e tecnico.

RISULTATI

Nell'anno 2023 la figura del TSLB ha partecipato alla raccolta dei campioni di matrice cheratinica a scopo medico-legale nella percentuale del 20% sul totale dei prelievi effettuati.

CONCLUSIONI

Il TSLB ha integrato le competenze dell'infermiere in merito alla corretta raccolta della matrice cheratinica in termini di quantità di materiale, spessore e numero di ciocche necessarie per l'analisi. L'infermiere, a sua volta, ha supportato il TSLB in merito alla relazione con il paziente durante l'esecuzione della raccolta del campione. Il lavoro sinergico di diverse figure professionali ha quindi migliorato la qualità del processo, dalla raccolta del campione all'analisi.

Le competenze del TSLB possono però essere integrate a quelle dell'Infermiere per

migliorare la fase pre-analitica e la qualità del servizio offerto. Lo scopo di questo lavoro era organizzare la raccolta a valenza medico-legale della matrice cheratinica per analisi tossicologiche, inserendo la figura del TSLB presso il Centro Prelievi.

EP230

Riorganizzazione dell'area preanalitica e identificazione del "Sample Manager" tramite applicazione del LEAN Management.

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Introduzione: Il metodo LEAN, applicato all'attività diagnostica, mira al miglioramento continuo tramite l'eliminazione di criticità, maggiormente presenti in fase preanalitica, causa di errori di laboratorio con gravi conseguenze cliniche e non.

Scopo: migliorare la performance diagnostica dell'U.O.C. Laboratorio analisi del P.O. Giovanni Paolo II – ASP Ragusa, con focus alla fase preanalitica, tramite applicazione del metodo LEAN.

Metodi: un team multidisciplinare del Laboratorio, coadiuvato da consulenti esterni, ha avviato un iter di improvement a seguito dell'identificazione di criticità e di sprechi tramite l'analisi dei flussi di lavoro (Value Stream Map); la mappatura (Spaghetti Chart) dei percorsi degli operatori di laboratorio e non; il conteggio dal 15 al 20/04/24 degli accessi in laboratorio; il conteggio delle non conformità (NC) registrate nei mesi di febbraio e di maggio 2024 a seguito di introduzione dell'apposito registro.

Risultati: La riorganizzazione della fase di accettazione dei campioni, risultata a maggior impatto negativo, ha previsto che il suo svolgimento non fosse più in area analitica bensì in un'area dedicata all'ingresso del Laboratorio. Ciò ha consentito di snellire i percorsi effettuati dal personale interno/esterno al laboratorio e di eliminare in area analitica, nel turno antimeridiano, 60 accessi al giorno da parte del personale di reparto e addetto al trasporto per la consegna o transito dei campioni.È stato inoltre introdotto il ruolo di "Sample Manager", ossia di un TSLB dedicato all'accettazione dei campioni e alla rilevazione e gestione delle NC in maniera sistematica e tracciabile.

Discussione: l'applicazione del metodo LEAN ha ottimizzato i flussi di lavoro e avviato l'attività di gestione tracciabile delle NC tramite apposito registro (101 NC a febbraio e 125 NC a maggio 2024).

Per valutare l'impatto positivo dei cambiamenti messi in essere, si è avviato un programma di survey, con pianificazione semestrale, che ha come obiettivo la misura della soddisfazione degli operatori.

L'attività di improvement svolta prevede ulteriori e futuri interventi da realizzare sulle attività meritevoli di intervento.

EP231

Cybersecurity in Italian laboratories: results of a national survey

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Background: Due to the increasing number of cyberattacks on healthcare organizations in Italy and abroad, cybersecurity is of paramount importance (1). We report here on the national results of a survey conducted by the Task Force Preparation of Labs for Emergencies (TFPLE) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) to assess the state of the art of cybersecutiry in laboratory medicine in Italian laboratories.
Methods: The survey, composed of a series of general questions on local organization, perceptions and experiences of cyberattacks, was delivered with an EFLM newsletter to the email addresses of all potential EFLM contacts. The responses from the Italian centers were transferred to an Excel file and graphically analyzed.
Results: A total of 133 responses were received to the survey, 27 of which from Italy. When asked about their familiarity with malware, cyberattacks and the strategies of cyberterrorists, 74.1%, 55.6% and 47.1% of respondents said they were familiar with them. Over 80% of respondents said they were likely to be the target of a cyberattack in the future, while over 50% said they had been the victim of a cyberattack in the past. Just over 20% of all respondents stated that hospital and laboratory servers are located in the cloud. Over 50% of respondents used a remote connection to the hospital information system (HIS), but multi-factor identification was only used in 41% of cases. Antivirus software and firewalls were running in over 80% of Italian facilities, and over 80% of respondents had received cybersecurity recommendations from their hospital. Finally, less than 20% of respondents stated that there was an emergency plan for the entire hospital and less than 15% for their laboratory.
Conclusions: The results of this survey show that great efforts need to be made to increase cybersecurity in Italy.
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EP232

Technological transition from GC-MS to LC-MS/MS for Serum Long Chain Fatty Acids determination: development of an improved omega screening test

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The three long chain fatty acids, arachidonic acid (AA, ω6), eicosapentaenoic acid (EPA, ω3) and docosahexaenoic acid (DHA, ω3) are introduced in the organism via diet. Serum determination of these fatty acids is crucial for the assessment of metabolic conditions, state of aging and the general functioning of the organism. The commonly named Omega Screening test evaluates the ratio between AA/EPA and AA/DHA concentrations, providing indications on the organism conditions and establish any interventions on the diet to reset the general state of health. Determination of these analytes in biological samples is mainly performed using Gas Chromatography – Mass Spectrometry (GC-MS) platforms. GC-MS fatty acids analysis requires some pre-analytic procedures, such as liquid-liquid extraction and chemical derivatization, that are time consuming and not automatable. Our aim was to improve Omega Screening test, with a transition from GC-MS to liquid chromatography tandem mass spectrometry (LC-MS/MS). For this purpose, a LC-MS/MS method was developed on a platform consisting in a ExionLC AD and a Qtrap 6500+ (ABSciex, Framingham, USA) equipped with electrospray ion source, operating negative mode. This method includes some pre-analytical procedure as triglycerides hydrolysis and protein precipitation, which requires less time than the previous ones and could be easily automatized. In order to obtain a faster method also the chromatographic separation was improved, passing by a run time of 32 minutes in GC to 6.5 minutes in LC. Analytes separation was performed on a HSS T3 column (Waters Corporation) using an isocratic elution composed by 15% of mobile phase A (H2O + 0.001% FA + 2 mM Ammonium Formate) and 85% of mobile phase B (MeOH + 0.001% FA + 2 mM Ammonium Formate), with a flow rate of 0.5 mL/min. Mass Spectrometry experiment was conducted in multiple reaction monitoring (MRM) mode.The current method was validated following guidelines for LC-MS/MS methods in diagnostic, and 50 real samples results were correlated with the ones obtained with the original method.The developed method for omega screening test was rapid, simple and accurate. Technological transition had a positive impact on laboratory routine in terms of time and consumption, fully resuming our aim.

EP233

Screening e conferma della infezione da HCV - valutazione di un test combinato per l'identificazione di HCV anticorpi e antigene

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80 campioni positivi con valori di HCV Ab (Anticorpi contro il virus dell'Epatite C) compresi tra 1 e 50 ICO, eseguiti con il test Elecsys Anti-HCV II su sistema Cobas® pro (ROCHE), sono stati confrontati con il test recomLINE HCV IGG (RIBA) (MICROGEN Gmbh) su sistema AP BLOT ELITE (das); 38 campioni (47,5%) hanno confermato la positività, individuando mediante curva ROC un cut-off pari a 16 ICO con specificità di 94,73% e indice di Youden di 70,92%: questi risultati trovano accordo in letteratura, dove è emerso un cut-off pari a 12,23 ICO (J Infect Dev Ctries 2016 Sep 30;10(9):1031-1034). Pertanto, utilizzando tale nuovo cut-off, si eviterebbero 44 test di conferma.Gli stessi 80 campioni sono stati quindi analizzati con il test Elecsys HCV Duo, sempre su sistema Cobas® pro. Il test Elecsys HCV Duo è costituito da 2 moduli di test (HCV Ag e anti-HCV), nei quali i peptidi e gli antigeni ricombinanti, che rappresentano le proteine core, NS3 e NS4, vengono utilizzati per rilevare gli anticorpi anti-HCV, mentre gli anticorpi monoclonali vengono utilizzati per rilevare l'antigene core dell'HCV.Con il modulo HCV Duo Ab, 63 campioni hanno confermato la positività nel confronto con Elecsys Anti-HCV II (cut-off = 9, specificità = 98,11%, indice di Youden = 98%); il test recomLINE HCV IGG (RIBA) ha confermato 37 campioni (58.7%), mentre 26 (41.3%) sono risultati negativi. 13 campioni risultavano positivi sia all'anticorpo (HCV Duo Ab) che all'antigene (HCV Duo Ag), 1 era positivo soltanto all'antigene.Tra i 37 campioni negativi, 1 è risultato positivo al test recomLINE HCV IGG (RIBA) di conferma, ma negativo al test HCV RNA.I risultati ottenuti hanno avvalorato l'ipotesi di una possibile sostituzione del metodo Elecsys Anti-HCV II con il test HCV Duo nell'indagine di primo livello in quanto dotato di una superiore specificità. HCV Duo rappresenta un valore aggiunto ai fini della diagnosi di malattia da HCV precoce, potendo essere impiegato come test di screening per prevenire la trasmissione dell'HCV ai destinatari di trasfusioni di sangue, emoderivati, cellule, tessuti e organi.

EP234

PEG 400 Ion Suppression in Busulfan Detection by High-Performance Liquid Chromatography—Tandem Mass Spectrometry

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Background: Busulfan (Bu), an alkylating agent commonly used in chemotherapy and transplantation, exhibits high intraindividual pharmacokinetic variability and possible time-dependent variations in clearance, which complicate therapeutic drug monitoring.Numerous analytical methods have been developed to reduce analysis time and facilitate timely decision-making regarding treatment changes; however, the validation procedures rarely involve analysis of potentially interfering excipients. Macrogol 400 (PEG400) should be considered as a possible interfering agent in the detection of plasma Bu levels, especially as an ionization suppressor.

Methods: Six intravenous formulations of Bu were compared with identify at least 1 common excipient (PEG 400). During the 176 therapeutic drug monitoring analyses of Bu, one of the PEG 400 specific mass-to-charge ratio transitions was determined using an instrumental method. After coelution with Bu and its internal standard (Bu-d8) was confirmed, all analyses were repeated usinga different experimental setup free of ion suppression induced by PEG. The concentration–time profile of PEG 400 was also analyzed.

Results: The area under the curve obtained from the 2 data sets was compared and analyzed using Lin concordance correlation coefficient and Bland–Altman plot analysis. The results from the 2 analytical methods were comparable: PEG 400 negatively affected the Bu-d8 coefficient of variation but not the Bu/Bu-d8 ratio.

EP235

Evaluation of the analytical and clinical performances of the Biolabo Kenza 450TX for immunoturbidimetric determination of HbA1c: a viable alternative to HPLC systems

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Background. Haemoglobin variant A1c (HbA1c) is traditionally determined using high-performance liquid chromatography (HPLC) systems. This study aimed to evaluate the analytical and clinical performances of the Biolabo Kenza 450TX (K450TX), an automatic clinical chemistry analyser by Biolabo SAS, Maizy (France), in the immunoturbidimetric determination of HbA1c, as a potential alternative to HPLC methods. The K450TX features a throughput of about 400 tests/hour, random access, up to 120 independent reagent positions and 98 positions for samples, calibrators and controls, along with a 'stat' function. The system supports various analytical calculations with simultaneous reading at nine wavelengths.Materials and Methods. Repeatability, bias, between-days and within-laboratory imprecision, and interferences were assessed following the CLSI guidelines. The performance of K450TX for measuring HbA1c of 195 leftover clinical samples, covering a range of medical decision levels, was evaluated in comparison to the reference Menarini/ARKRAY ADAMS A1c HA-8180V analyser (HA-8180V), a fifth-generation ion-exchange HPLC system. The study was approved by the Internal Review Board (protocol n.96268, 19/10/2020).Results. Results showed that between-days and within-laboratory imprecision, bias, and total analytical error were all below acceptable limits calculated on the biological variation and defined by the IFCC. Notably, there was a high degree of correlation between K450TX and HA-8180V. Passing-Bablok regression showed the perfect identity between the two methods with no proportional systematic error (slope 1; 95% CI: 0.99 to 1.00), and a constant systematic error of -1.00 mmol/mol (95% CI: -1.00 to -1.00). No interferences were observed within the limits specified by CLSI EP07-A2 with respect to manufacturers' claims. Statistical analysis was performed by using MedCalc version 18.2.1 and OriginPro version 8.5.1.Conclusions. The K450TX clinical chemistry analyser demonstrated satisfactory performance in the determination of HbA1c, offering a reliable alternative to traditional HPLC systems for routine diabetes management.

EP236

Validazione del dosaggio Beckman Access Hybritech PSA su plasma mediante protocollo CLSI EP35

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INTRODUZIONE

Il nostro laboratorio utilizza per il dosaggio del PSA totale la metodica Access Hybritech su strumentazione Beckman Coulter UniCel Dxl 800. La relativa IFU riporta come unica matrice utilizzabile il siero. In questo lavoro abbiamo testato il plasma come possibile matrice equivalente secondo il protocollo CLSI EP35 (2019).

MATERIALI E METODI

Per la determinazione di LoB e LoD abbiamo utilizzato il protocollo CLSI EP17-A2: due lotti di reagenti e un solo analizzatore per determinare, in 4 replicati, 4 campioni (calibratore 0, tampone, 2 campioni con bassissimo contenuto di analita) per 3 giorni successivi. Per la determinazione del LoQ abbiamo eseguito un profilo di imprecisione: due lotti di reagenti e un solo analizzatore per determinare 7 campioni (concentrazioni: 0,03, 0,1, 0,5, 3, 9, 12, 30 ng/ml) in 5 replicati per 5 giorni successivi. Lo studio di equivalenza è stato condotto utilizzando campioni di siero e plasma di 42 pazienti afferenti al punto prelievi dell'Ospedale di Urbino con concentrazioni di tPSA comprese fra 0,03 e 40 ng/ml. Previo consenso informato, i campioni sono stati anonimizzati e testati utilizzando un unico lotto reagenti su un unico analizzatore. Di ciascuna coppia siero/plasma sono stati effettuati due replicati in due corse diverse.

RISULTATI

Il valore di LoB e LoD è risultato pari a 0,0113 ng/ml con metodo non parametrico e 0,0126 ng/ml con metodo Currie; il valore di LoQ è risultato pari a 0,033 ng/ml (CV 10%). I risultati dello studio di equivalenza sono stati analizzati mediante regressione lineare e plot di Bland Altman. L'analisi mediante regressione lineare ha fornito i seguenti risultati: intercetta=0,08, pendenza=1,06, coefficiente di correlazione R=0,9973. L'analisi mediante metodo Bland Altman ha evidenziato un coefficiente di correlazione intraclasse (ICC) pari a 0,9974 per singola misura e 0,9987 per medie di misure. Il Bias è risultato pari a 7,56% e quindi inferiore al Bias desiderabile (10.6%) calcolato in base ai dati della variabilità biologica ricavati dal database EFLM.

CONCLUSIONI

Il nostro studio ha evidenziato una buona correlazione fra siero e plasma per il dosaggio Beckman Access Hybritech PSA. Lo studio di equivalenza fra matrici soddisfa le specifiche di qualità desiderabili.

EP237

Assessing the Performance of the CUBE 30 TOUCH ESR Analyzer Under Variable Clinical Conditions and Interfering Factors

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Background: The erythrocyte sedimentation rate (ESR) is a widely diagnostic test employed to reveal inflammatory conditions and influenced by all physiological and pathological conditions that can bias blood rheology. To confirm support for the application of ESR automatic analyzers in presence of interference factors in ERS diagnostic tests, it was necessary to lead investigations on interfering factors.Aim: The aim of this study was to evaluate the performance of the CUBE 30 TOUCH (DISSE, Siena, Italy) ESR analyzer in comparison with the gold standard, the Westergren method. Samples with the presence of lipaemia, hemolysis, and jaundice were examined, and it was defined the maximum time limits to ensure a reliable ESR measure.Methods: The study was conducted according to the requirements of the ICSH guidelines and was approved by the Siena Hospital Ethics Committee. Accuracy, intra-run and inter-run precision, and stability studies were performed. Moreover, hemolytic and lipemic samples were analyzed for interference study. Finally, fibrinogen sensitivity was assessed. Statistical analyses were performed.Results: Method comparison showed no statistical differences between the CUBE 30 TOUCH and Westergren method (Spearman Coefficient, R=0,95). In the intra-run precision, the CV% media obtained on samples with normal ESR level was 8,9%; with middle ESR level was 5,9% and with high ESR level the CV % was 4,3%. Inter-run precision test showed CV% of for single samples and overall samples in the range (12,3% for normal level and 4,8% for abnormal level). The samples stored at 4°C showed good stability up to 3 hours from collecting time. ESR samples showing hemolysis or jaundice showed good correlations with the gold standard method, Westergren tests was resulted more sensitive than CUBE 30 TOUCH to fibrinogen additions.Conclusions: The high comparability with Westergren method, both in normal and interfering samples, as well as the good precision, support the usefulness of CUBE 30 TOUCH in clinical routine laboratory.

EP238

Valutazione della stabilità del PSA totale in campioni di siero e plasma per l'accertamento dell'idoneità del donatore nel trapianto d'organo

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INTRODUZIONE

Le linee guida nazionali sul trapianto d'organo prevedono per la valutazione dell'idoneità del donatore il dosaggio del PSA totale per tutti i donatori maschi di età superiore ai 50 anni, possibilmente su campione antecedente il cateterismo vescicale. Quest'ultima indicazione comporta spesso la necessità di recuperare campioni refrigerati prelevati anche 72 ore prima. Il nostro laboratorio utilizza per il dosaggio di tPSA la metodica Beckman Coulter Access Hybritech PSA su UniCel Dxl 800 che prevede una stabilità massima di 24 ore a 2-8°C. Scopo di questo lavoro è stato quello di valutare la stabilità dei campioni di siero e di plasma anche a 48 e 72 ore.

MATERIALI E METODI

Lo studio è stato condotto utilizzando campioni di siero e plasma di quattro pazienti afferenti al punto prelievi dell'Ospedale di Urbino. I campioni, raccolti previo consenso informato e subito anonimizzati, avevano concentrazioni di tPSA pari a 2, 4, 8 e 30 ng/ml. Tutti i campioni sono stati testati ai tempi T0, T24, T48, T72 ore, in doppio per 5 volte ogni giorno. La stabilità è stata valutata calcolando la % di variazione secondo l'equazione:

Variazione % (Δ) = concentrazione al tempo T – concentrazione basale / concentrazione basale *100.

L'analisi statistica è stata eseguita mediante regressione lineare.

RISULTATI

Le variazioni percentuali per i quattro campioni (C1-4) di siero e plasma attraverso i tempi 24, 48 e 72 ore sono risultate comprese nei seguenti intervalli. C1 (2 ng/ml): Δ fra 9,2 e 12,8; C2 (4 ng/ml): Δ fra 7,5 e 17,5; C3 (8 ng/ml): Δ fra 3,9 e 12,5; C4 (30 ng/ml): Δ fra 8,7 e 9,3. L'analisi statistica non ha evidenziato differenze significative a nessuno dei tempi rispetto al valore del tempo zero, né per il siero, né per il plasma. Dalla regressione lineare è stata ricavata l'equazione che esprime la variazione della concentrazione in funzione del tempo. L'equazione per il siero è S=1,758-0.001T; l'equazione per il plasma è P=1,973+0,003T.

CONCLUSIONI

Il nostro studio non ha evidenziato variazioni significative nel dosaggio del PSA totale su campioni di siero e di plasma conservati a 2-8°C fino a 72 ore dal prelievo. L'eventuale ricorso a prelievi antecedenti il cateterismo vescicale per escludere falsi positivi non preclude la validità clinica del dato.

EP239

Evaluation of Stability and Accuracy Compared to the Westergren Method of ESR Samples Analyzed at VES-MATIC 5

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Introduction: The Erythrocytes Sedimentation Rate (ESR) measures the rate (mm/h) at which red blood cells form aggregates or rouleaux. The ESR is an estimator of severity of systemic inflammation because it depends on the acute-phase proteins circulating and by erythrocytes related factors (size, shape, number, surface charge). The stability evaluation of the sample is a crucial aspect of the preanalytical phase to guarantee the diagnostic quality of results. The purpose of this work is to assess both the stability at room temperature (RT) and at 4°C of EDTA blood samples for ESR analysis, and the accuracy compared to the Westergren reference method, using the VES-MATIC 5 DIESSE analyzer.
Materials and methods: Whole blood samples were randomly selected from daily routine of the San Donato Hospital in Arezzo, collected in EDTA tubes. The evaluation of stability at RT for 24 hours (4 h "T1", 6 h "T2", 8 h "T3", 10 h "T4", 24 h "T5") and at 4°C (24 h, 36 h and 48 h) was carried out using 635 total samples starting with T0 (within 2 h of venipuncture). For method comparison, 164 patients were enrolled and analyzed on VES-MATIC 5 and then diluted with sodium citrate for Westergren method, within 4 hours from collection.
Results: The decay of the samples at RT is statistically significant when compared between T0 and T1 ($p<0.05$), T2, T3, T4, T5 ($p<0.0001$) respectively. Regression showed a gradual decrease in correlation R=0.99 (T0 vs T1), R=0.97 (T0 vs T2), R=0.92 (T0 vs T3), R=0.87 (T0 vs T4) and R=0.40 (T0 vs T5). The stability of samples stored at 4°C and analyzed after 24h, 36h, 48h from each T0 measurement showed a worsening of regression comparing each to T0: R=0.99 (T0 vs 24h), R=0.97 (T0 vs 36h), and R=0.95 (T0 vs 48h) and an increase of Blant-Altman's bias. The method comparison between VES-MATIC 5 and Westergren manual method showed a correlation of R=0.96.
Conclusion: Samples began to decay at RT in a statistically significant way already 6 hours after collection, so the analysis of samples stored at RT more than 6 hours cannot be performed. When the samples were stored at 4°C the ESR can be measured until 36 hours after collection. Moreover, the VES-MATIC 5 accuracy performance compared to the Westergren reference manual method (R=0.96) is confirmed.

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Evaluation of ESR Reference Range by VES MATIC 5 and CUBE 30 touch respect to Westergren Method

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Introduction: Erythrocyte sedimentation rate (ESR) is a simple and worldwide diagnostic test employed to assess the inflammatory status with the widest application spread in chronic disorders as rheumatological and autoimmune diseases, in infections and tumors. The objective of this study is to generate reference intervals (RIs) for the automated analyzers, VES-MATIC 5 (VM5) and CUBE 30 touch (C30t) respect to gold standard method.
Materials: To establish IR, a total of 989 pre-selected healthy subjects from the San Donato hospital in Arezzo between October 2023 and May 2024 were enrolled and whole blood samples collected in K2EDTA tubes were used. They are divided according to sex. A subdivision was applied based on age for both groups. The study and the statistical analysis were conducted in respect to CLSI-C28-a3.
Results: The analysis dividing female (468) and male (521) pointed out significant differences between women and men, for all three analytical methods (VM5, C30t, Westergren). The ESR trend investigation based on age showed an ESR increase with age for both female from 7.8 mm/h to 10.6 mm/h and more evidently for male ranged from 2.8 mm/h to 9.2 mm/h. The reference ranges were calculated for each method, and for both sexes, considering the population based on the age, in three subgroups. The ESR mean with lower and upper limits (mm/h) of female ≥18 years but ≤49 years (n.171) were 8.01 (2.00-20.00) by Westergren, 7.11 (1.00-17.70; VM5) 8.78 (2.00-21.70; C30t). In female ≥50 years but ≤69 years (n.173) the ESR were 9.19 (2.00-22.33; Westergren), 8.05 (1.00-20.65; VM5) and 9.91 (2.00- 25.00; C30t). Female ≥70 years (n.124) had ESR mean of 9.81 (2.00-22.00; Westergren), 9.65 (1.13-22.63; VM5) 11.85 (3.00-25.88; C30t). The ESR mean (mm/h) of male ≥18 years but ≤49 years (n.197) were 3.96 (1.00-13.00; Westergren), 3.03 (1.00-11.00; VM5) 4.61 (1.00-14.05; C30t). In male ≥50 years but ≤ 69 years (n.200) the ESR were 4.83 (1.00-24.95; Westergren), 4.14 (1.00-20.98; VM5) and 5.55 (2.00-22.95; C30t). Male ≥70 years (n. 124) had ESR mean of 7.40 (1.00-26.50; Westergren), 7.07 (1.00-26.13; VM5) and 9.20 (2.00-32.13; C30t).
Conclusion: The study confirmed the ESR increasing trend with the age, with higher values in women. All three methods showed similar results.

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La frazione P3: un indizio prezioso per la corretta determinazione di HbA1c

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Nella determinazione della concentrazione di emoglobina glicata (HbA1c), i metodi basati su cromatografia liquida a scambio ionico discriminano le diverse frazioni emoglobiniche sulla base del tempo di ritenzione all'interno della colonna. Le varie frazioni generate nel cromatogramma dal Variat II Biorad sono: HbA1a, HbA1b, HbF, HbLA1c, HbA1c, P3 e HbA0.In letteratura, gli studi riguardanti il significato clinico delle sottofrazioni di emoglobina glicata sono pochi così come gli studi sulla sua degradazione.L'area della frazione P3, ad esempio, è determinata sia dalla presenza nel campione di emoglobina adulta degradata sia dalla presenza di varianti emoglobiniche silenti. Queste varianti non si presentano clinicamente ma, causando un aumento spurio della frazione P3, determinano valori finali di HbA1c che potrebbero essere falsamente aumentati. (Per considerare attendibile il valore dell'HbA1c, l'area della frazione P3 dovrebbe essere inferiore al 10%). Caso clinico: La determinazione di emoglobina glicata in una donna di quarantacinque anni (glicemia 77 mg/dl) ha fornito un cromatogramma con la frazione P3 aumentata (area 21.8%), la frazione HbA0 diminuita (area 69.9%) e la frazione HbA1c uguale a 36 mmol/mol (5.4%). Per verificare la presenza eventuale di una variante emoglobinica è stata effettuata, sullo stesso campione, l'elettroforesi dell'emoglobina utilizzando un sistema di elettroforesi capillare (Capillarys 3 Tera, Sebia). Il risultato ha evidenziato la presenza di HbA (72%), HbA2 lievemente diminuita (2%) e di una mutazione dell' emoglobina in zona Z12, associabile ad alfa variante, (26%). Utilizzando il valore di HbA ottenuto con elettroforesi capillare, il calcolo della concentrazione di HbA1C è risultato di 33.6 mmol/mol (5.04%), inferiore al valore ottenuto con la cromatografia a scambio ionico. Conclusioni: La frazione P3 influisce sul valore finale di HbA1c determinato mediante cromatografia a scambio ionico. Un aumento significativo (oltre il 10%) può indicare la presenza di una variante emoglobinica che, se non identificata, potrebbe portare ad un'errata interpretazione dei risultati dell'HbA1c ed alla compromissione della cura del paziente.

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La misura del testosterone totale: l'esperienza del nostro laboratorio

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Introduzione

Da più di 30 anni è noto che i metodi immunometrici, pur semplificando le procedure analitiche ed aumentando la produttività, non garantiscono un'adeguata accuratezza nella misura del testosterone totale (TT) in campioni di donne e bambini. La misura del TT con tali metodi deve essere limitata a distinguere i maschi eugonadici dagli ipogonadici. La spettrometria di massa consente un'accurata misura del TT ma non ha ancora raggiunto ampio utilizzo nei laboratori clinici per praticabilità, produttività e costi.Lo scopo di questo lavoro è comparare la misura del TT con 2 metodi immunometrici e la spettrometria di massa analizzando 4 popolazioni di pazienti a concentrazioni di TT molto basse per definizione.

Metodi

Sono stati selezionati 188 campioni: 78 maschi ipogonadici (età media in anni 50±17), 22 bambini (11±2), 26 soggetti Klinefelter KS (34±12) e 49 donne (41±21). La misura del TT è stata eseguita in routine con un metodo CMIA su DxI800 (Beckman Coulter) e con un metodo di comparazione di II gen su Alinity i (Abbott Diagnostics) che utilizzano rispettivamente un Ab policlonale e un Ab monoclonale nella fase solida. I campioni sono stati analizzati anche in LC-MS/MS utilizzando il kit Chrosystems MassChrom® Steroids in Serum/Plasma.

Risultati

La comparazione dei risultati ottenuti coi tre metodi nelle 4 popolazioni mostra un'ottima correlazione (tutti $R^2>0,77$) fatta eccezione per le donne ($R^2 0,001$ tra DxI/LC-MS/MS e $R^2 0,0024$ tra DxI/Alinity) in cui l'analisi Bland-Altman mostra una maggiore sovrastima del DxI, confermata anche dalle medie ottenute (0,7±0,8 ng/mL vs 0,5±0,3 e 0,4±0,3 ng/mL di Alinity e LC-MS/MS). Una maggiore dispersione dei dati è evidenziata negli ipogonadici e nei KS; in tutte le popolazioni si evidenzia un maggiore accordo tra i valori ottenuti su Alinity e LC-MS/MS con concentrazioni medie e DS sovrapponibili.

Discussione

Nell'adottare un metodo analitico si deve considerare non solo la produttività e il costo, ma soprattutto che cosa misura il metodo, ricordando che i metodi immunometrici tendono a sovrastimare il TT. I nostri dati confermano che in donne e bambini il risultato più accurato è fornito dalla spettrometria di massa ma dimostrano che anche il metodo Alinity di II gen fornisce risultati altrettanto attendibili.

EP243

Diagnostic performance of CLEIA vs. FEIA for KL-6 peripheral and alveolar concentrations in fibrotic interstitial lung diseases: a multicenter study

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Interstitial lung disease (ILD) is characterized by interstitial lung thickening and irreversible loss of respiratory function. Krebs von den Lungen-6 (KL-6) is a glycoprotein secreted by damaged type II pneumocytes in the alveolar space. The goal of the present study was to compare two analytical methods for KL-6 detection in both bronchoalveolar lavage (BAL) and serum from ILD patients at the moment of diagnosis. Patients with suspicious of ILD and followed at ILD referral centers of Siena and Foggia University Hospitals were included. BAL and serum were collected and analyzed by detected by AIA-360 (fluorescent enzyme immunoassay, FEIA) and CL-AIA (chemiluminescent enzyme immunoassay, CLEIA) instruments. We optimized the laboratory protocol for KL-6 detection through CLEIA method setting dilution factor to 1:25 and 1:10 for serum and BAL samples, respectively. A total of 158 (mean age ± standard deviation, 61.5±13.7, 65 females) patients were enrolled. Thirty-six had diagnosis of IPF, 74 sarcoidosis, 15 CTD-ILD and 33 other ILD. High quantitative correlation was between the two methods for both BAL ($r=0.707$, $p<0.0001$) and serum ($r=0.816$, $p<0.0001$). Diagnostic agreement was quantified according to Blend-Altmann for BAL KL-6 (mean bias of 178) and serum KL-6 (mean bias of -172). Stratifying patients according to IPF diagnosis, sensitivity and specificity increased for BAL and serum KL-6 values. IPF had higher serum KL-6 than non-IPF, while BAL KL-6 values were lower in IPF than in non-IPF. The diagnostic and predictive prognostic role of KL-6 was confirmed by indirect correlations between serum KL-6 measurements and DLco% ($r=-0.3$, $p=0.032$) and FVC% ($r=-0.38$, $p=0.004$) demonstrating the worsening of ILD patients, mainly IPF. This study quantified KL-6 concentrations through the CLEIA method demonstrating its advantage to clinically exploit this marker in routine assays in clinical practice for the management of ILD patients at the time of diagnosis by identifying fibrotic damage pulmonary with good diagnostic accuracy. Taken together, our results corroborated the diagnostic and predictive prognostic role of KL-6 in IPF patients than other ILD.

EP244

EVALUATION OF THE RELIABILITY OF THE FRIEDEWALD EQUATION IN THE MEASUREMENT OF LDL VALUES

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BACKGROUND-AIM

Measuring LDL-C levels is central to the risk management of atherosclerotic cardiovascular disease (ASCVD). Elevated LDL-C levels contribute to atherosclerosis and increase the likelihood of heart attacks and strokes. Regular monitoring is essentialto determine treatment and assess the effectiveness ofinterventions. LDL-C measurements also contribute to personalized medicine by taking into account individual profiles and thus contributing to tailored treatments. In secondary prevention, controlling LDL-C levels is crucial for individuals with a history of cardiovascular events to reduce the risk of recurrence. The aim of this work was to compare the measurement of LDL blood levels with the Friedewald equations currently used in the Clinical Biochemistry Laboratory, AOU “Maggiore delle carità” of Novara (Italy), based on a standard lipid panel that includes measurements of total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides.

METHODS

181 patients hospitalized at the AOU Maggiore della Carità di Novara who requested a lipid profile determination (lithium-heparin test tubes) were analyzed using the Roche COBAS 8000 and the Roche LDL-Cholesterol Gen.3 kit. Data were analyzed by calculating the Pearson correlation index r using GraphPad statistical software.

RESULTS

The average LDL value in the analyzed group was 115 # 44 (range 31-217). The direct measurement of LDL-C correlated with the indirect measurement according to Friedewald equation with $r=0.947$ (95% C.I. = 0.92 - 0.96; $p < 0.0001$). In addition, the direct measurement of LDL-C was compared with the calculations of Sampson, Chen, Puavilai and Delong: the correlation was $r = 0.9841$, $r = 0.9735$, $r = 0.9758$ and $r = 0.9789$, respectively. Finally, 22,140 LDL-C determinations were performed in our laboratory in 2023 according to the Friedewald equation. Considering that the individual LDL test costs 0.65 euros, the savings amounted to 15,000 euros.

CONCLUSIONS

Our data confirm that the Friedewald equation is an accurate method for estimating LDL-C levels that is widely used in the clinical setting. It requires standard lipid panel values and provides a rapid and inexpensive calculation, making it a convenient method for assessing cardiovascular risk without the need for direct LDL measurement.

EP245

A BAYESIAN STATISTICS APPROACH FOR QUALITY CONTROL AND ISO 15189 COMPLIANCE

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Introduction. Among the methods recommended for assessing lot-to-lot comparability, internal quality control (IQC) is preferred in many scenarios. Bayesian statistics is based on a priori information and the continuous adjustment of the mean value and the acceptability range of results: it can offer an opportunity to detect abnormal results early after the introduction of a new reagent lot. In this study, Bayesian statistics was used to monitor the performance of laboratory assays and evaluate lot-to-lot variability.
Methods. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen (QFA) were evaluated by two ACL TOP 550 CTS (Werfen, Barcelona, Spain). Both instruments were connected to the HemoHub software (Werfen). Obtained data were analyzed both with the Bayesian method and the traditional Levey-Jennings inferential statistics. Normal and low IQCs were used. Intentional errors (resuspension of controls and reagents in a non-optimal volume of milliQ water, use of controls before optimal resuspension time, use of controls and reagents beyond the expiry time, serial dilutions of controls in milliQ water) were introduced to test the robustness of the two statistical approaches.
Results. After 30 IQC measurements in a two-week period, the reagent lot was changed. Even the smallest deviations from the measured mean were identified as outliers by the Bayesian method. After the introduction of intentional technical errors in PT and aPTT tests, in 17/38 (45%) cases the Bayesian method highlighted outlier values, while Levey-Jennings did not, reinforcing the higher precision of such statistical method for analysis.
Conclusion. Existing procedures for lot-to-lot validation, such as the Clinical and Laboratory Standards Institute (CLSI) EP26-A, propose robust pipelines, demanding relevant resources, which are often critical for laboratories. Simple and low-demanding protocols to assess reagent lot-to-lot variability would be well accepted. Overall, the obtained results showed that the Bayesian approach was more sensitive than the Levey-Jennings to identify manually induced errors with a greater power. The continuous adjustment of the acceptability range enables to highlight an abnormal value as soon as it occurs in the analytical series, enabling a faster and more user-friendly view of the results.

EP246

SPECIFICHE DI PERFORMANCE ANALITICA BASATE SUL MODELLO 1/b DELL'OUTCOME: CALCOLO E UTILIZZO PER VALUTARE L'INCERTEZZA DI MISURA DEI TEST PER L'ASSETTO LIPIDICO

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INTRODUZIONE: Per garantire la qualità analitica dei risultati di laboratorio è essenziale selezionare specifiche di performance analitica (APS) con cui confrontare l'errore o l'incertezza di misura. Alla conferenza di Milano del 2014 sono stati identificati tre modelli per la selezione delle APS, il primo dei quali (outcome) valuta, o attraverso studi clinici (modello 1a), o mediante approcci indiretti (modello 1b), quanto debba essere accurata la prestazione analitica per soddisfare le esigenze cliniche. EFLM ha sviluppato un software per calcolare queste APS mediante simulazioni (Clin Chem Lab Med 2023;62:597–607). In questo studio, gli esami per la valutazione dell'assetto lipidico sono stati scelti come applicazione del modello 1b. Per i cut-off di questi misurandi abbiamo calcolate APS per limitare la misclassificazione all'1% (ottimale), al 5% (desiderabile) e al 10% (minimo) e abbiamo verificato se le metodiche in uso presso il nostro laboratorio soddisfano tali requisiti.
METODI: Le APS sono state calcolate con il software EFLM in base ai livelli decisionali delle principali linee guida e, per il colesterolo LDL, anche per diversi cut-off di rischio cardiovascolare. I dati di CQI di 9 semestri (da 2019 a 2023) ottenuti da 2 moduli Roche Cobas c702 sono stati utilizzati per calcolare il coefficiente di variazione (CV). L'incertezza di misura è stata calcolata secondo ISO 20914:2019 e confrontata con le APS basate sull'outcome.
RISULTATI: Le APS calcolate sono in linea con quelle del lavoro originale e non variano significativamente con il numero di pazienti o il periodo analizzato. L'incertezza di misura semestrale è risultata sempre inferiore ai traguardi desiderabili per Apolipoproteina B, Colesterolo HDL e Trigliceridi, e inferiore a quelli minimi per Colesterolo e Colesterolo LDL. Lipoproteina(a) e Apolipoproteina A1 non rispettano nessuna APS calcolata.
CONCLUSIONI: Lo studio propone un approccio per calcolare le APS basate sul modello 1b, così da verificare se le performance analitiche delle metodiche soddisfano i requisiti di qualità a vari livelli di misclassificazione. La maggior parte delle metodiche Roche su strumentazione Cobas c702 produce risultati con un'incertezza di misura inferiore ai traguardi analitici basati sull'outcome.

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CONFRONTO FRA LA METODICA ROCHE ELECSYS DI III GENERAZIONE PER LA DETERMINAZIONE DELLA 25(OH) VITAMINA D CON UNA METODICA IN LC-MS/MS E VERIFICA DELLE PERFORMANCE ANALITICHE

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INTRODUZIONE: La valutazione della vitamina D, misurata come 25(OH)D totale, è essenziale per identificare carenze, nonostante l'assenza di cut-off definitivi condivisi. I test, prevalentemente basati su tecnologia immunometrica, sono poco standardizzati e mostrano un bias rispetto alla metodologia LC-MS/MS, complicando l'uso di valori decisionali comuni. Questo studio ha valutato le performance della metodica immunometrica Roche Elecsys di III generazione per la determinazione della vitamina D, sia mediante il confronto con una metodica LC-MS/MS (Chromsystems su strumento TQS della ditta Waters), sia in termini di accuratezza analitica e capacità di classificare correttamente lo stato carenziale rispetto ai cut-off della nota AIFA 96, che definiscono i criteri di prescrivibilità a carico del SSN della supplementazione con vitamina D.
METODI E RISULTATI: 1) Comparazione con LC-MS/MS: Su 344 campioni umani, la metodica Roche Elecsys ha mostrato scarsa concordanza con LC-MS/MS, con sottostima costante (slope=0.92; intercetta=-2.64), superando il bias minimo accettabile basato sulla Variabilità Biologica (±10.1%) a livello dei limiti decisionali della nota 96. L'analisi della concordanza fra i risultati binari rispetto agli stessi cut-off ha mostrato un alto tasso di concordanza positiva (PPA > 0.95), ma un tasso di concordanza negativa (NPA) inferiore (0.89 per 12 ng/mL, 0.73 per 20 ng/mL, 0.52 per 30 ng/mL). 2) Prestazioni analitiche: La metodica su Cobas e801 è stata verificata con lo schema 5X5 su 2 materiali di controllo (Liquicheck Specialty Immunoassay Control della Biorad) con concentrazioni di 24.6 ng/mL (L2) e 89.5 ng/mL (L3). Le performance erano entro i limiti di imprecisione e inesattezza del produttore. L'incertezza di misura semestrale era inferiore alle specifiche di performance analitiche basate sull'outcome, determinate con il software EFLM secondo il modello 1b della conferenza di Milano (Clin Chem Lab Med 2023;62:597–607).
CONCLUSIONI: La metodica Roche Elecsys di III generazione per la misurazione dei livelli di vitamina D offre buone performance analitiche. Tuttavia, la concordanza con la metodologia LC-MS/MS non è ottimale, influenzando l'interpretazione dei risultati rispetto ai principali cut-off proposti nelle linee guida esistenti.

EP248

Hair analysis performance for illicit drugs detection tests: results from the External Quality Assessment of Lombardy Region

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Introduction: The Regional Reference Center for the Quality of the Laboratories of the Lombardy Region (Center) manages External Quality assessment (EQA) program for the analysis of illicit drugs in hair. Methods: All laboratories analyzed the same control material. The hair undergoes an artificial incorporation of drugs refer to 7 classes for a total of 22 metabolites. The 7 classes of substances were opiates, methadone, amphetamines and methoxyamphetamines, cocaine, cannabinoids, ketamine, Buprenorphine and were alternately positive or negative. Center processed the quantitative results with a statistical algorithm conform to according to the international standard ISO 13528. The methods used by laboratories were: GC-MS, GC-MS/MS, HPLC-HRMS and HPLC-MS/MS. Laboratories performances were evaluated by comparison with the expected outcome defined by the manufacturer of control material. The expected outcome must be confirmed by most frequent result. Results: A total of 14 exercises were submitted by the Center, 6 in 2022, 6 in 2023 and 2 in 2024 for a total of 4127 results. 17 laboratories participated in both 2022 and 2023, 19 laboratories in 2024. A total of 77 results, 1,86%, were classified as false-negative and 4 results, 0,09%, were classified as false-positive. The substances/metabolites with the highest number of false-negative results were codeine, dihydrocodeine, norbuprenorphine and buprenorphine, respectively 14, 8, 7 and 6 results. The substances/metabolites with false-positive were THC-COOH, MBDB, cocaine and Benzoylecgonine. The coefficient of variation (CV) was analysed for each exercise and for each metabolite. The CV varies from 47,6% for THC-COOH to 8,2% for ecgonine methyl ester. The average CV was 30,7%. Discussion: The performances of laboratories were satisfactory, the 98% of results were in agreement with expected outcome. The CV of each substances/metabolites requires an in-depth analysis of instruments performance.

EP249

Results from the External Quality Assessment of ethanol's test in blood

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Introduction. The Regional Reference Center for the Quality of the Laboratories of the Lombardy Region (Center) manages External Quality Assessment (EQA) program for the analysis of ethanol in whole blood. The concentration of ethanol in blood (alcoholemia) is a crucial analysis for both patient health and legal implication. For the italian law, the cut-off of ethanol in blood is 0 g/L or 0.5 g/L, depending on the age of the patient.Methods. All laboratories analysed the same control material of whole blood with ethanol added by manufacturer of control material. The manufacturer declares the target value with imprecision expected of ± 0,1 g/L. The methods used by laboratories were headspace Gas Chromatography Mass Spectrometry (GC-MS) or headspace Gas chromatography-flame ionization detection (GC-FID). Results. Center analysed 51 exercises submitted to laboratories in four years by 2020 to 2024. The mean of participant was 20 for each exercise for a total of 1051 results. The average of coefficient of variation (CV) of all exercises analysed was 9,38%. In 26 exercises with high target value, by 0,7 to 5 g/L, the CV was 8,79%. In 12 exercises with a negative target value, 1,22% of results (3) was false positive. In 6 exercises with target value between 0,1 and 0,4 g/L, 126 results, the CV was 11,17%. In 7 exercises, 144 results, with target value near to the cut-off established by italian law, 0,5 g/L, the mean concentration was 0,49 g/L: 71 results (49,3%) were higher than cut-off while 73 results (50,7%) were lower. The CV of this exercises was 10,02% and average measurement uncertainty was 0,015. Discussion. The performance of laboratories for this crucial analysis is very important. The average of the all results complies with the target value and imprecision declared by the manufacturer. The average of coefficient of variation (CV) of all exercises analysed was 9,38%, lower than the CV reported in the literature for toxicological dosage (20%) and not appear to be any significant differences in CV between different concentration levels of ethanol. Given the legal implication, the performances for cut-off values of both 0 and 0,5 g/L, despite good analytical results, deserve further investigation and interpretation with clinical information.

EP250

MULTICENTER STUDY FOR THE EVALUATION OF PERFORMANCE OF AUTOMATED ERYTHROCYTE SEDIMENTATION RATE (ESR) ANALYZERS

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Introduction: The erythrocyte sedimentation rate (ESR) is an easy hematology test commonly used in laboratory routine to indicate and monitor inflammatory activity in infection or/and other related diseases. The study aims to evaluate the performance of three automated instruments for determining the erythrocyte sedimentation rate (ESR), VESMATIC 5, CUBE 30 Touch, and MINICUBE (DIESSE, Siena) involving four Italian polyclinics: Tor Vergata in Rome, Santa Maria alle Scotte in Siena, Sant'Anna in Como and San Donato in Arezzo.

Materials and methods: For each hospital site, EDTA blood samples were collected from the daily routine and analyzed within two hours of collection to ensure stability. A total of 773 samples were collected: 216 in Rome, 191 in Siena, 192 in Como, and 174 in Arezzo. The accuracy assessment was carried out by analyzing the same samples with all three instruments and comparing them with the Westergren method. Precision was assessed through an intra-run, inter-run, and total precision study using quality controls. Furthermore, repeatability was estimated by reanalyzing fresh blood samples belonging to three ESR ranges (low, intermediate, and high) six times.

Results: The results showed a strong correlation between the three automated tools and the Westergren method, with Spearman coefficients (R^2) of 0.978 for VESMATIC-5, 0.981 for CUBE30, and 0.976 for MINICUBE. Bland-Altman analysis indicated an average bias of 1.1 for VESMATIC-5, 0.9 for CUBE30, and 1.0 for MiniCUBE, considered clinically acceptable. Overall accuracy for all clinics was excellent with coefficients of variation (CV) less than 10% for all instruments. Repeatability confirmed an excellent level for all ESR ranges, with CVs below 10%.

Conclusions: The study demonstrated that the three automated instruments of DIESSE offer optimal performance for accuracy and precision, comparable to the gold standard, Westergren method. These instruments have proven to be suitable for both large laboratories and small facilities, thanks to their full automation, ability to directly analyze EDTA blood tubes, and minimal maintenance required.

EP251

Familiarizzazione con il nuovo sistema analitico DxI 9000 Access Immunoassay: valutazione dei dosaggi TSH, fT4 e fT3 in confronto agli analizzatori DxI 800 e Access2

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Scopo

Per i dosaggi immunometrici di routine e urgenza l'ASST di Lodi utilizza gli analizzatori DxI800 e Access2 (A2) (Beckman Coulter Inc., Brea CA USA) La determinazione del profilo tiroideo viene eseguita nel laboratorio analisi di Lodi su DxI800. Scopo del lavoro è stato confrontare TSH, fT3 e fT4 con il nuovo analizzatore DxI9000 Access Immunoassay (Beckman Coulter Inc., Brea, CA, USA) con DxI800 e A2 al fine dell'inserimento in laboratorio del nuovo sistema.

Metodi

Il confronto analitico è stato eseguito analizzando su DxI 9000, installato a Codogno, campioni già determinati su DxI800 o A2. Su A2 e DxI800 sono stati analizzati campioni freschi, su DxI9000 aliquote anonimizzate e congelate dei residui di prelievo.

Campioni A2 - DxI9000: TSH N=85 (53 femmine F e 32 maschi M, 16-95 anni A); FT3 N=28 (17F e 11M, 26-91 A); FT4 N=31 (21F e 10M, 7-91 A).

Campioni DxI800 - DxI9000: TSH N=98 (60F e 38 M, 16-95 anni A); FT3 N=19 (14F e 15M, 26-91 A); FT4 N=32 (22F e 10M, 7-91 A).

Il trasporto dei campioni è avvenuto tramite corriere interno. Prima di ogni seduta analitica è stato eseguito il controllo di qualità su due livelli. I reagenti hanno la stessa formulazione per tutti gli analizzatori (Istruzioni Uso Access TSH 3rd IS, Rev. 02-2023; Access Free T3, Rev. 07-2022; Access Free T4, Rev. 07-2022; DxI9000 Access Immunoassay Rev. AR 08-2023).

Analisi dati: MedCalc Software (Ostend, Belgium, 19.4) per Passing Bablok (PB), Bland Altman, Mountain Plot; concordanza clinica per confronto dei risultati con gli intervalli di riferimento (IR).

Risultati

Concordanza clinica.

A2 – DxI9000

TSH: 100%; fT3: 89% - un campione borderline (Acc2 3.86pg/mL – DxI9000 3.97pg/mL; IR 2.5 – 3.9pg/mL); fT4: 97,0%. - un campione borderline (Acc2 0.63ng/dL – DxI9000 0.60ng/dL – IR = 0.61 – 1.12ng/dL).

DxI800 - DxI9000

TSH: 100%; fT3: 100%; fT4: 93,7% - un campione borderline (DxI800 0.64 ng/dL - DxI9000 0.6ng/dL - IR 0.61 – 1.12ng/dL).

Passing Bablok

A2-DxI9000

TSH: y = 0.039 + 0.936 x; fT3: y = 0.0755 + 1.02 x; fT4: y = -0.034 + 1.04 x

DxI800 - DxI9000

TSH: y= -0.0153 + 1.03 x; fT3: y= -0.626 + 1.163 x; fT4: y = -0.136 + 1.175 x

Conclusioni

La concordanza clinica tra gli analizzatori è stata verificata confermando le indicazioni del produttore.

EP252

Creatinine measurement: a must-have improvement in paediatrics.

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Background: Accurate measurement of creatinine is essential for estimating the glomerular filtration rate, for defining chronic kidney disease stage and acute kidney injury status (1). The available measurement resources include two types of methods, the colorimetric Jaffé and enzymatic assays (2). For accurate patients results, calibrators must meet the criteria of traceability and commutability. For calibration in serum the methods of measurement of creatinine refer to standard reference materials with concentrations higher than 0.8 mg/dl (3), not suitable for paediatrics. Thus, calibration bias results in increased uncertainty in estimated low creatinine values. Our aim is to investigate how well Jaffé and the enzymatic assays perform at paediatric levels compared with serum samples at adult levels. Materials and methods: We collected 75 serum samples from adults and 62 paediatric (1 month to 10 years old) serum samples. Each sample was analysed in duplicate with both Jaffé and enzymatic method by Roche Diagnostics on Cobas c503. Bland-Altman analysis was performed in adults and paediatrics serum specimens. Statistical significance in the difference of bias was determined by t-test using SPSS software. The analytical imprecision was calculated with 5x5 method.

Results: The results from 5x5 test showed analytical imprecision values in line with those reported by the manufacturer. For adults' serum the two methods resulted in agreement (bias: -0,0011, CI 95% -0,0047/ 0,0025, p=0,549), without constant (CI 95% -0,002/0,007) and proportional errors (CI 95% 0,985/1,003). In paediatrics, a discrepancy resulted (bias 0,011 CI 95% 0,0033/0,0192, p=0.006), and constant and proportional errors occurred, with particular evidence in the creatinine range 0.1-0.29 mg/dl (constant CI 95% 0,042/0,150; proportional 0,399/0,843 p<0.001).

Conclusions: This study highlights the successful level of agreement between Jaffé and the more specific enzymatic method within the normal concentration range for serum creatinine dosage in adults, due to the compensatory measures taken by manufacturers to limit interferences in Jaffé and the correct standard reference meter. However, biases occurred in paediatrics highlight the need to use more specific methods and the urgent review of the standard reference material.

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EP253

Evaluation of add-on kits to extend automated screening for urinary drug testing

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Introduction: in the clinical context of our hospital, including four second-level emergency departments, there is a need for the expansion of urinary drug screening tests to ensure timely recognition of complex cases with the aim of guiding targeted treatments.
Methods: in 2023, overall we received 7262 requests for urinary drug screening only for clinical purposes, thus excluding medico-legal issues. Due to high demand and market changes, the laboratory plans to update the screening panel. Therefore, in addition to the standard panel of seven routinely tested drugs, three additional kits for fentanyl, oxycodone and ketamine were evaluated. The study analyzed 215 spot urine samples from emergency department patients (130 males, 61%, and 84 females, 39%). Samples were tested by enzyme immunoassay method on the AU 5800 platform (Beckman Coulter, USA). The study aims to assess the efficiency, stability and operational impact of the new kits.
Results: calibration was performed every 15 days, except for oxycodone, which required calibration every 7 days. During the study period, two-level controls, performed every 24 hours, showed optimal stability, with results always within two standard deviations, thus confirming the reliability of the kits. An in-depth analysis on inter-series precision was performed on a sample positive for four different drugs (amphetamine, cocaine, ecstasy and ketamine) over ten days. The results showed exceptional stability of the kit, confirming positivity to the analytes sought. Of the 215 samples analyzed, 18 (8.3%) were positive for ketamine, 17 (7.9%) for fentanyl and 1 (0.46%) for oxycodone.
Conclusions: the expansion of urinary drug screening tests is a step forward in the laboratory's diagnostic capability and a significant improvement in emergency care quality. Consolidating add-on kits on an automated platform does not require additional staff or resources, making the operation efficient and sustainable. The implementation of advanced technologies and continuous evaluation of the methods are crucial to effectively address contemporary clinical challenges, ensuring constant monitoring and immediate response to patient needs.

EP254

GENDER DIFFERENCES IN THE EPIDEMIOLOGY OF ALCOHOL CONSUMPTION IN MANTUA IN FIVE-YEARS PERIOD FROM 2019 TO 2023

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Background: Alcohol is a psychoactive substance causing addiction with significant social and economic consequences. Alcohol consumption (AC) is a major challenge because it leads to unintentional and intentional injuries such as diseases, road traffic crashes, violence and suicide. Therefore, it's important monitor AC in alcohol abusers to help them in detox programs and supervise subjects driving under the influence of alcohol to prevent accident risks. Aim: Our study wants to provide an indication of the extent AC of the province of Mantua population in a five-year period from 2019 to 2023. Methods: Overall 79200 urine ethyl glucuronide concentration (EtGuc) were performed by Atellica® Solution (Siemens Healthineers). Data were collected by 8 different District Pathology Dependency Service (SERD) of Mantua's province. Data were analyzed with respect to gender and EtGuc, using a chi-square test ($\chi 2$). Statistical analyses were performed using Microsoft® Excel 2021 (Microsoft® Corp.). Results: From 2019 to 2023 the total number of analyzed patients per year (average: 15840) was unchanged with lower values in 2020 (13876) and higher values in 2022 (16843). EtGuc was above to the cut-off limits in 31,82% of the patients. In five-year period, men dominated the statistics (81,44%) with a EtGuc above to the cut-off limits of 33,53% respect to 24,29% of the women ($\chi 2$= 471,240, p <0,001). However, women, although representing a relatively lesser share (18,56%), show an increase of SERD monitoring from 18,16% in 2019 to 20,13% in 2023. Moreover, the positivity rates in relation to gender showed significant differences for 2019 (M= 4177, F= 695; $\chi 2$= 72,067, p <0,001), 2020 (M= 3719, F= 565; $\chi 2$= 56,113, p <0,001), 2021 (M= 4631, F= 623; $\chi 2$= 132,898, p <0,001), 2022 (M= 4389, F= 930; $\chi 2$= 47,359, p <0,001) and 2023 (M= 4715, F= 757; $\chi 2$= 202,133, p <0,001). Discussions: This study provides valuable informations on the alcohol abusers surveilled by means of EtGuc and it could be an useful surveillance tool to reveal AC trends. Our results indicate an increasing trend of AC among female, giving rise to concern, above all, because of risk of fetal exposures. It needs to intervene with population at risk for alcohol use to mitigate possible health and safety consequences.

EP255

Fingerprick volumetric absorptive microsampling for therapeutic drug monitoring of antiseizures medications: a real-life experience in a pediatric Hospital

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Background: Epilepsy is a chronic neurologic disorder that significantly impacts on the everyday quality of life of patients. Pharmacological treatment is manly symptomatic and often relies on multiple concomitant medications. Therefore, drug-drug interactions (DDIs) can lead to side effects highlighting the importance of therapeutic drug monitoring (TDM). Volumetric absorptive microsampling (VAMS) are increasingly emerging as sampling methodology for TDM of several drugs including antiepileptics (AE) and benzodiazepines (BZ). Here, we have compared the concentrations of carbamazepine (CBZ), levetiracetam (LEV), lacosamide (LCS), topiramate (TPR) and clobazam (CLO) in both plasma and VAMS samples.Methods: VAMS were collected by fingerprick from pediatric patients followed at our Centre. Patients were also subjected to conventional venous EDTA blood sampling. Plasma and VAMS samples were analysed by UHPLC-MS/MS using a validated kit for simultaneous determination of AE and BZ (ClinMass LC-MS/MS Complete Kit®, RECIPE +). A cross-validation analysis was performed by using Spearman correlation coefficient (r), Deming regression and Bland-Altman plot. Results: A positive significant correlation ($p<0.001$) was found between VAMS and plasma concentrations for CBZ ($r=0.80$) with its metabolites Diol ($r=0.91$) and Epoxide ($r=0.90$), LEV ($r=0.87$), LCS ($r=0.91$), TPR ($r=0.72$), CLO ($r=0.87$) and its active metabolite N-desmethyl-clobazam (NDMC) ($r=0.93$). Bland-Altman analysis showed a % bias of -0.90 for CBZ, 3.42 for Diol, 0.80 for Epoxide, -5.85 for LEV, -7.61 for TPR, -12.22 for LCS, 4.07 for CLO and 0.94 for NDMC.Conclusions: Our results show a good agreement between plasma and VAMS concentrations. Despite the limited number of samples, our results suggest that these disposables could be used for TDM of AE and BZ during the routine clinical practice. Moreover, due to the low blood volume required, VAMS are indicated for pediatric patients providing a compliant alternative to conventional venipuncture. Finally, although further investigations are required to better explore the use of microsampling for remote TDM, VAMS represent an opportunity for patients to avoid traveling toward hospitals or reference laboratories exclusively to monitor drug levels.

EP256

MONITORING THE FENTANYL ILLICIT USE: PRELIMINARY RESULTS FROM A SINGLE CENTER.

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Background: Fentanyls are a family of potent synthetic opioids mostly used in anaesthesia and pain management. They provoke relaxation, daze and euphoria and this fuel an increase in illicit use with a large and growing number of overdose deaths, especially in North America (1).Since 2012 the European Monitoring Center for Drugs and Drug Addiction, European Observatory of drugs and drug addiction has observed a significant increase in the availability of fentanyls on the illicit market followed by a significant increase in the number of correlated deaths (137 deaths in 2021) but date can be underestimated because Fentanyls are often detected in extremely low concentrations, complicating their detection in laboratories. Aim of our study was to contribute to monitoring illicit fentanyl use. We used the screening urinary fentanyl assay, and we tested patients from several clinical frameworks.
Methods: We employed the ARK™ Fentanyl II Assay in the analyser Siemens Atellica® CH. The ARK Fentanyl II Assay is an immunoassay for the qualitative detection of fentanyl in human urine samples with a cut-off concentration of 1.0 ng/mL. We collected 222 urinary samples from 10 different clinical contexts: 2 clinical centers for substance abuse addictions (58%, mean age 47, range 17-68), Child & Adolescent Neuropsychiatry Unit (3.6%, mean age 17, range 15-18), Adult Neuropsychiatry Unit (5.4%, mean age 46, range 23-60), juvenile detention center (0.45%, age 20), adult detention center (11.7%, mean age 40, range 19-69), emergency (1.35%, mean age 44, range 31-54), drive license renewal (2.25%, mean age 41, range 32-50), sex abuse (0.45%, age 20), car accidents (21.6%, mean age 42, range 18-86).
Results: We didn't find positive results for the urinary fentanyl detection in the samples of the considered cohort except for the class of patients from the car accidents: 41.6% of these patients showed a positive result in line with the documented therapeutic prescription.
Conclusions: Considering the growing emergency about the use of fentanyls in the international and national scenario, it is extremely important to monitor the illicit use of this class of substances. In our preliminary data, we didn't observe positive results but it is fundamental to expand the cohort of subjects.

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EP257

INTRODUZIONE NELLA ROUTINE CLINICA DELL'ANALISI DI ANTIBIOTICI SU SIERO IN LC-MS/MS

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INTRODUZIONE

Lo sviluppo delle antibiotico-resistenze è un problema di salute pubblica globale: è importante scegliere il farmaco più efficace e la dose corretta, soprattutto nella fase iniziale dell'infezione. Il monitoraggio terapeutico degli antibiotici (TDM) permette al clinico di valutare l'adeguatezza dei parametri farmacocinetici e farmacodinamici e quindi l'efficacia clinica in termini di eradicazione del patogeno. Lo scopo di questo studio è stato l'introduzione nella routine clinica di una metodica automatizzata con preparatore per l'analisi di antibiotici su siero in LC-MS/MS, in collaborazione con il reparto ospedaliero di Anestesia e Rianimazione dell'Ospedale Pio XI di Desio.

MATERIALI E METODI

Sono stati analizzati 100 campioni di siero raccolto da pazienti, spesso sottoposti a co- somministrazione di più antibiotici e con differenti situazioni cliniche, ricoverati presso il reparto di Anestesia e Rianimazione. I farmaci sono stati analizzati con preparatore automatico (Tecan Freedom EVO) e sistema LC-MS/MS (Xevo TQ-XS Waters), utilizzando reagenti inclusi in un kit commerciale (BSN). I farmaci quantificati erano: ampicillina, cefazolina, cefepime, cefuroxime, clindamicina, cloramfenicolo, daptomicina, flucloxacillina, sulbactam, tazobactam, linezolid, meropenem, piperacillina.

RISULTATI

Il TDM di antibiotici ha permesso di ottenere la riduzione delle dosi somministrate e quindi del rischio di tossicità nei pazienti, la correlazione tra concentrazioni dei farmaci e parametri di cura per mantenere l'infezione sotto maggior controllo, la correzione della concentrazione in funzione del legame alle proteine plasmatiche, la valutazione del corretto dosaggio da somministrare in pazienti sottoposti a dialisi, l'individuazione di un malfunzionamento nel catetere arterioso. I farmaci vengono dosati contemporaneamente in un'unica seduta analitica, permettendo quindi una riduzione del turn- around time. L'utilizzo del dispensatore automatico fornisce diversi vantaggi rispetto alla preparazione manuale da parte dell'operatore, in termini di produttività, tempistiche e rischio di errore.

CONCLUSIONI

La metodica per determinare la concentrazione sierica di antibiotici, sfruttando l'utilizzo di un sistema automatico di dispensazione, si è dimostrata ottimale per l'utilizzo in routine, consentendo l'impostazione di 3 sedute settimanali. I dati ottenuti hanno permesso ai clinici di correggere e definire l'adeguata dose dei diversi farmaci, soprattutto in pazienti critici ed in caso di terapie inefficaci, e di migliorare la cura delle infezioni.

EP258

Toxicological Profiling of Substance Use in two Lombardy Rehabilitation Centers: Insights From Different Matrices Analysis

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The global consumption of illegal drugs poses significant public health challenges. Evaluating each substance's prevalence is essential to organize rehabilitation pathways and to understand drug spread. Attention should also be given to fentanyl, a synthetic opioid widely used in America, whose abuse is raising concerns in Europe. San Raffaele Hospital provides toxicological analyses for two addiction centers in Lombardy, supporting rehabilitation by evaluating the extent of substance abuse. This study aims to evaluate drugs abuse extent detected in these centers and monitoring Fentanyl use within the same population. A total of 161 patients followed up from January 1 to April 30, 2024, at the two addiction centers, aged from 16 to 70 (84% male, 16% female), were included in this study. Each patient provided two types of samples: urine (n=56) and hair (n=105) indicating recent and past drug use, respectively. Toxicological analyses were conducted on both matrices to detect a wide spectrum of drugs: amphetamines, benzodiazepines, cocaine, cannabinoids, opioids (including fentanyl and its metabolites). Alcohol addiction was determinated by dosing ethylglucuronide (ETG) in hair. All substances were detected and quantified using HPLC-MS/MS (model Waters XEVO TQ-XS) with an Atlantis Premier BEH C18 AX chromatography column (2.5 µm, 2.1 x 100 mm). Our findings indicated three predominant substances of abuse in both matrices: cocaine, THC and methadone. Indeed, we registered a significant cocaine abuse (29% in urine, 23% in hair), confirmed by high benzoylecgonine levels (50% in urine, 27% in hair). Elevated THC and THCCOOH concentrations were detected in both matrices reaching a percentage of 46% in urine. Methadone treatment compliance was observed (18% in urine, 13% in hair). Chronic alcohol abuse was indicated by ETG (22% in hair). In both matrices, we didn't detected fentanyl or its metabolites. In conclusion, identifying the prevalence of the most abused drugs could aid these centers in tailoring optimal psychological and pharmacological strategies. Furthermore, our study allows the evaluation of adherence to rehabilitation protocols and represents an initial deepening on the phenomenon of fentanyl abuse.

EP259

MicroNIR/Chemometrics for roadside monitoring of illicit drugs

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The development of novel analytical systems based on miniaturized device have been led to significant advances in forensic field; they are required more and more, especially when a rapid and on-site control is necessary. In this study, the MicroNIR/Chemometrics is proposed as precise and sensitive analytical system for the monitoring of illicit drugs in oral fluids. Oral fluid samples from volunteers were spiked with cocaine, Δ9-tetrahydrocannabinol and their metabolites (in the range 1 – 100 ng/mL) in order to simulate illicit substances use. The MicroNIR OnSite-W spectrophotometer (250g; 194mm; 47mm) was used to perform spectroscopic measurements on oral fluid collectors after deposition of the biological matrix (blank) and spiked samples. The Principal Component Analysis (PCA), the Partial Least Squares- Discriminant Analysis (PLS-DA) and the Partial Least Squares regression (PLSr) techniques were considered to validate chemometric models for the identification and quantification of the psychoactive compounds. The easy-to-use MicroNIR/ Chemometric system permitted to analyze the collected samples without any pre-treatment ensuring rapid, cost-effective and no-destructive analysis. The possibility to carry out further investigations of the samples confirming assumption, was also guaranteed making the handheld device highly suited for roadside monitoring.

EP260

ANALYSIS OF ETHYLGLUCORONIDE AND ETHYLSULFATE IN URINE BY LC-MS/MS

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INTRODUCTION

Ethylglucoronide (EtG) and Ethylsulfate (EtS) are direct metabolites of ethanol that can be detected in urine up to 80 hours after consumption. They are used to keep track of high levels of alcohol intake but also abstinence. The aims of the study were to evaluate a method to dose EtG and EtS in urine, by making the most of an automatic liquid handler, and compare the results obtained with those of urinary ethanol in the same samples.

MATERIALS AND METHODS

The samples have been selected among the ones that requested urinary ethanol by enzymathic method (50 positives and 50 negatives) coming from the “Servizi per le Dipendenze” (Ser.D.). After centrifugation, the samples have been diluted 1:20 (V/V) with internal standard, included in a commercial kit (BSN), by the liquid handler (Tecan Freedom EVO). The dosages have been conducted through LC-MS/MS (MDS Sciex 4000 QTRAP). For the same patients, EtG was quantified both in urine and hair through LC-MS/MS.

RESULTS

The use of the liquid handler granted numerous advantages compared to the manual procedure, like increased productivity, faster preparations and reduced errors. Since we often found concentrations of EtG that exceeded the linearity limits of the method, we tested different injection volumes in order to completely remove the carryover. In the samples that were positive to urinary ethanol we obtained concentrations of EtG that far exceeded the cut-off of 500 ng/mL, in the negatives we registered a positiviy rate of the 10% relative to the cut-off of 500 ng/mL and of the 50% relative to the cut-off of 100 ng/mL. Some patients were monitored with EtG both in urine and hair in the same periods of time.

CONCLUSIONS

The use of the liquid handler revealed itself as optimal for the application in everyday routine. The data showed that the dosages of EtG and EtS in urine improved the policing of alcool abuse, backed up by the positive results of the negative samples of urinary ethanol. The chance to control the patients, using both EtG in urine and in hair allowed evaluations in different periods of time.

EP261

IMPLEMENTATION OF AN AUTOMATED METHOD FOR THE ANALYSIS OF DRUGS OF ABUSE THROUGH LC-MS/MS

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INTRODUCTION

Drugs of abuse are quantifed in different types of biological samples through LC-MS/MS. Since in urine the concentrations often exceed the linearity limits of the method, the samples need to be diluted adequately. The study aimed to develop an automated sample preparation for drugs of abuse and to compare it to the manual procedure.

MATERIAL AND METHODS

The urine samples were collected from the “Servizi per le Dipendenze” (Ser.D.) and from patients that were prescribed toxicological checks according to the 187th article of the “Codice della Strada”. These samples were subjected to screening testing with a method of Kinetic Interaction of Microparticles in Solution (KIMS). Among theese we selected 100 positive samples to test for theese drugs of abuse: cocain, opiates, amphetamines and methadone. The samples were diluted with the use of a liquid handler (Tecan) and we ran the confirmation tests through LC-MS/MS (Xevo TQ-XS Waters), by using reagents included in a commercial kit (BSN). The same samples have been prepared both with the automated method and with the manual procedures and processed in the same session of analysis.

RESULTS

Three different dilution schemes have been installed on the liquid handler 1:1, 1:10 and 1:100 (V/V); these programs were assigned to different groups of samples based on the results of the screening test. The different diluting procedures granted to quantify in the range of the calibration curves and to completely remove the carryover. The use of the liquid handler provided numerous advantages compared to the manual procedure, like increased productivity, faster preparations and reduced errors.

CONCLUSIONS

The use of the liquid handler revealed itself as optimal for the application in everyday routine, by reducing the turn around time, increasing the number of tests executable everyday and also reducing the time needed to prepare the samples.The automated preparation of the samples provided better precision and standardization of the procedure, as we observed with the reduced variability of the MS responses of the internal standards.

EP262

Measurement of anti-TNF biologics in serum samples of pediatric patients: comparison of enzyme-linked immunosorbent assay (ELISA) with two point-of-care (POC) devices

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Background: Anti-Tumor Necrosis Factor (anti-TNF) biologics Infliximab (IFX) and Adalimumab (ADL) are used for treatment of moderate to severe inflammatory bowel disease (IBD) and juvenile idiopathic arthritis (JIA). However, many patients are often unresponsive or show a loss of response to these therapies (1). Therapeutic drug monitoring (TDM) of serum drug concentrations is helpful to optimize therapy with these agents (2). Although the enzyme-linked immunosorbent assay (ELISA) is widely used to measure anti-TNF drug levels, in the last decades different point-of-care (POC) devices have been proposed (3-6). Here, we compared IFX and ADL serum concentrations measured with both ELISA and two POC devices.

Methods: In serum samples collected from pediatric patients hospitalized in our Centre, IFX and ADL drug levels were measured as routine TDM practice by using clinically validated ELISA kits (Immundiagnostik AG). Same samples were further analysed with a new POC assay (AFIAS, Boditech Med Inc.) and The Quantum Blue POC device (B#hlmann Laboratories). Data were compared by using Spearman correlation coefficient, Deming regression and Bland-Altman plot.

Results: A positive significant correlation ($p<0.001$) was found for IFX and ADL results when comparing ELISA and AFIAS assays (Spearman $r=0.98$ for IFX and 0.83 for ADL). Calculated % bias was -14.09 (95% Limits of agreement, LoA, -52.83 to 24.66) for IFX and 15.79 (LoA -37.14 to 68.73) for ADL. Similarly, comparison of ELISA results with The Quantum Blue revealed a Spearman r of 0.91 ($p<0.001$) for IFX and 0.78 for ADL ($p<0.001$). Calculated % bias was 36.81 (95% LoA, -25.96 to 99.57) for IFX and -15.34 (LoA -63.03 to 32.33) for ADL.

Conclusions: Despite the limited number of samples, our results for IFX confirm previously published reports suggesting that these POC devices could be a feasible alternative to ELISA for TDM of anti-TNF biologics in routine clinical practice. Conversely, our study shows for the first time a significant correlation between ELISA and AFIAS results for ADL serum concentrations. Finally, since POC devices provide results more rapidly than ELISA, they could be used when ELISA assays are not available or when an immediate result is desirable in order to change a clinical decision.

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EP263

CONFRONTO TRA UNA METODICA DI PRIMO LIVELLO IMMUNOENZIMATICA E UNA METODICA ANALITICA DI SECONDO LIVELLO IN HPLC/MS-MS PER LA RICERCA DELLA KETAMINA E DEI SUOI METABOLITI SU MATRICE URINARIA

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INTRODUZIONE: La Ketamina è un potente psichedelico ampiamente utilizzato nella pratica clinica come anestetico generale, il cui utilizzo a scopo “ricreativo” si è recentemente si è diffuso tra i tossicodipendenti. In questo contributo presentiamo i risultati del confronto tra un metodo di Screening e un metodo di Conferma per il dosaggio della Ketamina su matrice urinaria.
MATERIALI E METODI: Sono stati raccolti 100 campioni di urina di pazienti dei S.E.R.T.: tutti i campioni sono stati testati con metodo Immunoenzimatico ARK applicato su piattaforma automatizzata Siemens-Dimension, che consente un dosaggio semiquantitativo complessivo di Ketamina e Nor-Ketamina, con un cut-off di 50 µg/L; sucessivamente i campioni sono stati analizzati con metodica manuale BSN, applicata su strumentazione HPLC-MS/MS-AbSciex, che consente di quantificare separatamente la Ketamina (LOQ 3.60 µg/L) e il suo metabolita Nor-Ketamina (LOQ 12.60 µg/L). I dati ottenuti sono stati analizzati calcolando Media, Mediana e Deviazione Standard e i 22 campioni risultati positivi al test di screening sono stati analizzati con grafico Box-Plot.
RISULTATI: 78 campioni sono risultati negativi al test di screening e sono stati tutti confermati dal test di secondo livello; dei 22 campioni risultati positivi al test di screening, solo quelli con valori di concentrazione >500 µg/L, sono poi risultati positivi anche al test di conferma.
CONCLUSIONI: Il test ARK ha dimostrato un'elevatissima affidabilità diagnostica del risultato negativo, rispetto al suo CUT-OFF di 50 µg/L, e del risultato positivo per concentrazioni misurate >500 µg/L (Limite di Linearità del metodo). Per valori di concentrazione comprese nell'intervallo 50-500 µg/L è necessario eseguire sempre il test di conferma per escludere falsi positivi.

EP264

The biological variation of serum 1,25-dihydroxyvitamin D and parathyroid hormone, and plasma fibroblast growth factor 23 in healthy individuals.

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Background and aims: Measuring 1,25-dihydroxyvitamin D (1,25(OH)2D), parathyroid hormone 1–84 (PTH 1–84) and intact FGF23 (iFGF23) is crucial for diagnosing a variety of diseases affecting bone and mineral homeostasis (1). In these settings, the biological variability (BV) of these measurands is important for assessing the validity of population-based reference intervals; to evaluate the significance of changes observed through serial measurements; for establishing analytical performance specifications (APSs); to calculate the number of samples for the assessment of the homeostatic point of the analyte (NHSP) (2). The European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation Database (EFLM-BVD) provides estimates for both within-subject (CVI) and between-subject (CVG) BV. Currently (February 2024), the EFLM-BVD reports BV estimates for 25(OH)D, PTH, and iFGF23, but not for 1,25(OH)2D. The aim of the present study was to pioneer BV estimates for 1,25(OH)2D and to enhance the existing CVI and CVG estimates related to iFGF23, and PTH 1–84.

Materials and methods: Serum and plasma-EDTA samples of sixteen healthy subjects have been collected for seven weeks and measured in duplicate by chemiluminescent immunoassay on the DiaSorin Liaison XL platform. After variance verification, CVI and CVG BV estimates were assessed by either standard ANOVA, or CV-ANOVA. The analytical variability was determined with 5x5 design (3). The APSs were calculated according to the EFLM-BV-model (4).

Results: The analytical imprecision was consistent with the manufacturer's. We found the following CVI (95% CI): 1,25(OH)2D, 22.2% (18.9–26.4); iFGF23, 16.1% (13.5–19.5); and PTH 1–84, 17.9% (14.8–21.8). The CVG were: 1,25(OH)2D, 21.2% (14.2–35.1); iFGF23, 21.1% (14.5–35.8); and PTH 1–84, 31.1% (22.1–50.8). We estimated the critical difference and the index of individuality (II), highlighting that the reference intervals are not fully applicable (II < 1.4) for all the measurands. Moreover, we calculated the NHSP with a deviation of ±10%, 15%, and 20%.

Conclusions: We report for the first time BV estimates for 1,25(OH)2D and enhance existing data about iFGF23-BV and PTH 1–84-BV through cutting-edge immunometric methods.

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EP265

Effetto del volo con drone sulla variabilità pre-analitica dei principali parametri ematochimici

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Introduzione e scopo. L'uso dei droni per il trasporto di campioni biologici può rappresentare un'alternativa al trasporto su strada in zone caratterizzate da importanti limiti geografici e logistici. Scopo di questo lavoro è stato quello di valutare l'effetto del volo con drone su diversi parametri ematochimici.

Materiali e metodi. La dimensione campionaria, per un effetto d=0,6, errore alfa=0,05 e potenza=0,90, è risultata pari a 25. Il disegno sperimentale prevedeva 6 campioni/soggetto (2 siero, 2 sangue intero K2EDTA, 2 plasma sodio citrato), di cui 3 rimasti a terra e 3 soggetti a volo con drone DJI PHANTOM 4. L'attività di volo si è svolta di notte (0:00-03:00) all'interno del sedime aeroportuale di Linate (h max=60 m, velocità 25 km/h), per un totale di 2 notti (3 voli/notte della durata di circa 25 min). Le specifiche di qualità sono state calcolate dai dati di variabilità biologica presenti nel database EFLM.

Risultati. I livelli degli analiti nelle due condizioni erano comparabili per glucosio, urea, sodio, cloro, AST, ALT, bilirubina totale, LDH, calcio, aptoglobina, INR, globuli rossi, emoglobina, piastrine, neutrofili, linfociti, eosinofili e basofili, con differenze al limite della significatività per creatinina (p=0,051) e globuli bianchi (p=0,05). Differenze statisticamente significative sono state osservate per potassio (delta=0,06 mmol/L, p=0,034), aPTT (delta=0,02, p= 0,005) e monociti (delta=0,03x10^9/L, p=0,003), con valori dei campioni a terra più elevati. All'analisi di Bland-Altman, il bias (95%C) e i limiti di accordo al 95% erano: 1,5% (95%IC 0,2-2,9) e da -5,3% a 8,3% per il potassio; 2,1% (95%IC 0,7-3,6) e da -5,0% a 9,3% per aPTT; 4,0% (95%IC 1,4-6,7) e da -9,4% a 17,5% per i monociti. Sebbene statisticamente significativo, il bias era inferiore alla specifica per il bias derivata dalla variabilità biologica (1,5% < 1,6% desiderabile per il potassio, 2,1% < 2,9% minimo per aPTT e 4,0% < 6,5% desiderabile per i monociti).

Conclusioni. I nostri dati evidenziano che il volo con i droni non impatta significativamente sugli analiti investigati, supportando il possibile utilizzo di questa tecnologia per superare le difficoltà logistiche del trasporto di campioni biologici per la diagnostica di laboratorio.

EP266

Acquired Von Willebrand Disease discovered in a patient with Apixaban overdose and haemarthrosis

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A 88-year-old woman, suffering from atrial fibrillation treated with Apixaban, presented at the Emergency Department of Orbetello (Tuscany) with a non-traumatic right knee hemarthrosis. Laboratory tests showed a prolonged aPTT (ratio=2.02), a prolonged PT (ratio=2.59), reduced haemoglobin (9.9 g/dL) and a mild increase in creatinine (1.35 mg/dL). Orthopaedic examination gave no indication for evacuation procedures. The medical history showed type II diabetes mellitus, lower limb obliterative arteriopathy, biologic aortic valve prosthesis, chronic heart failure with maintained ejection fraction, chronic sideropenic anaemia (occasionally treated with hemotransfusion), previous surgery for a hip fracture, without significant bleeding. In previous hospitalisations she manifested bleedings at the site of catheter placement and gingival mucosa microbleedings, but as minor bleedings in a patient at high thrombotic risk, Apixaban was retained. During admission to Pitigliano Hospital, due to further worsening of creatinine (2.14 mg/dL) and anaemia (haemoglobin 8.2 g/dL), Apixaban was temporarily suspended. The Coagulation Section of the Hub Laboratory of Grosseto was consulted to understand the cause of the bleeding beyond Apixaban, as a prolonged aPTT persisted (aPTT ratio=1.69). Firstly, an unexpectedly high anti-Xa concentration of Apixaban was found: 223 and 49 ng/mL respectively 48 hours and 8 days after discontinuation. The following week after Apixaban fell below the cutoff of 6 ng/mL, coagulation factors were assayed using the relevant deficient factors (ACL TOP 550, Werfen), which gave results in range except for FVIII (19.6%), Von Willebrand Factor (vWF) both as Ristocetin Cofactor activity (<4.4%) and Antigen (32%). To rule out aPTT prolongation by antiphospholipid antibodies, anti-cardiolipin and anti-B2-glycoprotein I IgG and IgM were determined, which proved negative, and the Lupus Anticoagulant phenomenon tested negative. The aPTT mixture performed at room temperature and after incubation at 37°C for 2h did not reveal the presence of inhibitors. A plasma sample sent for FVIII inhibitor titration to the Center for Bleeding Disorders at the Careggi University Hospital in Florence confirmed the absence of the inhibitor (<0.1 U/mL Bethesda). Given the deficiency of vWF and FVIII and the negative history of bleeding disorders, acquired Von Willebrand disease (AvWD) was suspected. Associated conditions were investigated and a monoclonal gammopathy of uncertain significance was found, which is one of the conditions most frequently associated with AvWD.

EP267

Prekallikrein Deficiency: Description of two cases

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Background: Prekallikrein (PK) is a zymogen that is converted to kallikrein (PKa) by FXIIa. PK deficiency is a rare autosomal recessive defect caused by KLKB1 mutations, whose main characteristic is an isolated prolongation of the aPTT, which becomes normal after prolonged preincubation, due to autoactivation of FXII. This deficiency is not associated with bleeding tendency.
Methods and Patients: A 71-year-old male patient (1) was admitted to our hospital to undergo a surgical procedure. The preoperative screening showed a prolonged aPTT ratio of 2,94 with a normal reference range of 0,85 to 1,20, while the PT ratio (0,99) was normal. Another 71-year-old male patient (2) presented to our hemostasis laboratory for a preoperative screening. The aPTT was prolonged with a ratio of 2,73 while, also in this case, the PT ratio (0,90) was normal.
Results: By mixing an aliquot of normal plasma with plasma of each patient in equal proportions, aPTTs normalized from 2,94 and 2,73 to 1,01 and 1,03, respectively. Clotting activities of FXII, FXI, FIX, FVIII and an immunoassay of vWFAg were normal. After a preincubation of 30 m' aPTTs normalized from 2,94 and 2,73 to 1,11 and 1,07, respectively. Patient (1) showed a PK clotting activity of 2% and patient (2) of 9%. The sensitive reagents were colloidal silica and synthetic phospholipids (Hemosyl APTT-SP), with an aPTT ratio of 2,94 patient (1) and of 2,73 patient (2) and silicon dioxide and vegetables phospholipids (Pathromtin SL) with an aPTT ratio of 4,2 (patient 1) and 3,8 (patient 2), which normalized to 1,25 and to 1,22, respectively, after 30 m' of preincubation, while aPTTs performed with ellagic acid and synthetic phospholipids (Synthafax) were normal.
Conclusions: All patients with prekallikrein deficiency have a personal and family history negative for bleeding events. Some arterial thrombotic events observed in some patients seem related to the increased incidence of arterial hypertension and endothelial dysfunction, due to lack of bradykinin formation, normally released from High Molecular Weigh Kininogen by PKa. Bradykinin causes vasodilation inducing release of powerful vasodilators such as prostacyclin, endothelium-derived hyperpolarizing factor and nitric oxide, which regulates endothelial homeostasis too.

EP268

Amoxicillin rash in patient with infectious mononucleosis

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Background :Infectious mononucleosis (IM),a viral disease primarily caused by Epstein -Barr virus (EBV), usually occurs in children ,adolescents and young adults. It is characterized by fever, pharyngeal inflammation and cervical lymphadenopathy.IM is often mistaken for other entities, such as acute bacterial tonsillitis ,since fever, pharyngitis, fatigue, and lymphadenopathy are common presenting symptoms in outpatient setting.
Case report: A 2-year-old child comes to the Emergency Room with high temperature(38-39°C degrees for 10 days),tonsillitis treated with an amoxicillin therapy for 7 days. Physical examination: fatten and redden tonsil, cervical lymphadenopathy, a maculopapular skin rush extended to the legs and to the chest.Required blood count and immunochemical test.
Results: From blood count leucocytosis (WBC13,690 µl)with lymphocytosis (48,7%),the smear reveals Downey ‘s bodies and atypical lymphocytes.Virological tests: EBV-VCA IgM136 UI/mL , EBV-VCA IgG 44,50 UI/mL , EBV-EBNA IgG <3.00 UI/mL Anti -Cytomegalovirus IgM 43,8, IgG <5.00 UI/Ml.
Conclusion: The suspect of mononucleosis infection was confirmed and so the skin rush could be due to the amoxicillin therapy. Beware of the fact that some antibiotics therapies (amoxicillin and ampicillin)could lead to latent infectious or to generalised and unknown skin rushed.It’s important to make a differential diagnosis with other diseases like Steven-Johnson Syndrome or other viral infections like the ones caused by enterovirus or varicella virus.

EP269

Role of the Clinical Pathology laboratory in monitoring IV martial therapy. Our experience.

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Iron deficiency anemia is a prevalent issue globally, particularly among women of childbearing age. For many patients, who do not respond to oral therapy or have severe anemia, intravenous (IV) iron serves as an effective alternative.New IV iron formulations, such as Ferric derisomaltose (Monoferric®), offer broad dosing ranges, often allowing for iron correction in a single session.
Ferric derisomaltose has proven efficacy in treating iron deficiency anemia across various patient groups, with studies validating its effectiveness compared to placebo, IV iron sucrose, and oral iron. Its structure, featuring iron tightly bound within a carbohydrate matrix, results in low immunogenic potential and minimal labile iron release, and it is not associated with clinically significant hypophosphatemia.

Materials and Methods
This study investigated the efficacy and side effects of ferric derisomaltose in women of childbearing age with iron deficiency anemia.
From December 2023 to May 2024, we conducted a study involving 53 patients, with an average age of 44 years and an average weight of 66 kg, who presented symptoms such as tiredness, pallor, dizziness, and headache. Diagnostic tests for iron deficiency included serum iron, ferritin, phosphorus levels, and complete blood count. Patients' progress was assessed 20 days post-infusion by comparing test values before and after treatment.

Results and conclusions
Following ferric derisomaltose infusion, patients exhibited significant increases in hemoglobin, serum iron, and ferritin levels, indicating effective treatment. Hemoglobin levels rose from an average of 8.3 g/dL to 12.5 g/dL one-month post-infusion.
Ferric derisomaltose is a highly effective and safe treatment for iron deficiency anemia in women of childbearing age, significantly improving hemoglobin, serum iron, and ferritin levels. Continuous monitoring of iron and ferritin levels is crucial for personalizing martial therapy. These parameters are essential for evaluating iron status and adjusting therapy appropriately. The Clinical Pathology laboratory plays a fundamental role in managing martial therapy for iron deficiency anemia, ensuring regular and accurate blood parameter control, which is vital for therapy success.

EP270

IgE kappa: un raro caso di gammopatia monoclonale di significato incerto

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La paziente, una donna di 70 anni con diabete mellito di tipo 2 e senza storia clinica di malattie ematologiche, si recava nel giugno del 2023 presso il nostro ospedale (Presidio Ospedaliero San Bassiano, AULSS7 Pedemontana) per eseguire una coronarografia in elezione per dolore di tipo anginoso.Il pannello di ingresso del reparto di cardiologia, che prevede, oltre all'elettroforesi sieroproteica associata alla quantificazione delle proteine totali sieriche, anche la determinazione di emocromo, PT/INR, aPTT, fibrinogeno, glicemia, creatinina, urea, uricemia, colesterolo totale, HDL, LDL, trigliceridi, sodio, potassio, cloro, calcio, magnesio, AST, ALT, GGT, LDH, CPK, troponina, pro-BNP, fosfatasi alcalina, bilirubina totale e frazionata, proteina C reattiva e TSH non presentava valori patologici. La precedente elettroforesi della paziente risaliva al novembre 2014 e il tracciato non presentava anomalie qualitative. L'elettroforesi sierica eseguita con metodo capillare (CAPILLARYS 3 OCTA, SEBIA, Lisse, Francia) mostrava un'alterazione nel profilo delle gamma globuline, caratterizzato da un picco quantificato in 2,5 g/L.L'approfondimento diagnostico eseguito mediante Immunotipyng (CAPILLARYS 3 OCTA, SEBIA, Lisse, Francia) segnalava una sottrazione per le catene leggere kappa in assenza di una sottrazione per le catene pesanti IgG, IgM, IgA.L'esito di questo approfondimento portava quindi all'esecuzione di una immunofissazione in gel di agarosio (HYDRASYS SCAN 2 FOCUSING, SEBIA, Lisse, Francia) con l'impiego di antisieri anti IgG, IgA, IgM, kappa e lambda, evidenziando la presenza di un addensamento per le sole catene leggere kappa. Una ulteriore immunofissazione (HYDRASYS SCAN 2 FOCUSING, SEBIA, Lisse, Francia), che includeva gli antisieri anti IgG, IgD, IgE, kappa e catene leggere libere kappa, confermava la presenza di una componente monoclonale IgE kappa in zona gamma. A seguito di tale evidenza, ove la paziente veniva inviata al Servizio di Oncoematologia dell'Ospedale per gli ulteriori accertamenti diagnostici, si consiglia, dopo approfondimento con immunosottrazione che evidenzia la presenza di sole catene leggere kappa o lambda, l'esecuzione routinaria di una immunofissazione in gel di agarosio che preveda l'impiego di antisieri anti-IgD e anti-IgE.

EP271

Homozygous Delta Thalassemia: How to identity hidden thalassemic conditions in absence of HbA2?

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INTRODUCTION

Hemoglobin A2 (HbA2) is the minor component of normal adult Hb, composed of 4 polypeptide chains, 2 α- and 2 δ-globulins. It can be distinguished from the major adult component HbA by its electrophoretic properties and it represents the main marker for thalassemia (thal) diagnosis; decrease/absence of HbA2 can be linked to α or δ thal, while increase of the same fraction to β thal. Importantly, as recommended by the Italian Society of Thalassemia and Hemoglobinopathies (SITE), separative methods including capillary electrophoresis (CE) can be used as first-level tests for presumptive identification of thal, provided that the electrophoretic determination must be always interpreted together with hematological, biochemical and clinical data of the patient (1). To date, homozygous delta thal is extremely rare, but some cases have been reported (2,3,4). Here we report the fortuitous discovery of a case of homozygous delta thal in an Italian woman of 76 years in Tuscany, Italy.

METHODS AND CASE REPORT

The sample was tested for glycated hemoglobin (HbA1c). HbA1c CE (Sebia) showed the complete absence of the HbA2 peak, normally migrating around X = 240 on the electrophoretic chart, with normal HbA1c level (5,7%). The sample was then investigated with Hemoglobin CE (Sebia), confirming the lack of HbA2 and the presence of HbA only. Sanger DNA sequencing revealed the genetic cause of the total lack of delta chains: a homozygous defect of delta gene [HBD:c.316-2A>G]. Since the hematological and clinical data of the patient were normal (RBC 4.94million/mm3, MCV 82.4fL, Hb 13g/dL), we concluded that the genetic abnormality did not impact significantly on the phenotype. However, this case highlighted the critical role of HbA2 in the identification and management of the thalassemic patient. How is it possible to identify hidden alpha or beta thalassemic conditions, which can be co-existing with homozygous delta thalassemia, without HbA2? Separative techniques help to target the diagnostic suspect but only genetic analysis can give a certain answer. In conclusion, the combination of different methods and the careful evaluation of clinical data are important for the first-level identification of these conditions, but molecular diagnostics is vital to set an appropriate genetic counseling (5).

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EP272
Microbiota: possibile causa di infertilità maschile?

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L'infertilità colpisce circa 186 milioni di persone in tutto il mondo. Quando presente ad alti tassi, può rappresentare un problema nei Paesi che stanno vivendo un rapido invecchiamento della popolazione. Osservando le differenze genetiche che influenzano la composizione del microbiota intestinale, si è evidenziato che alcuni batteri sono associati a un alto rischio di infertilità negli uomini. Una diagnosi precoce risulta essere la migliore prevenzione per il trattamento dell'infertilità. La disbiosi intestinale può indurre lo sviluppo di alcune malattie correlate alle malattie infiammatorie croniche intestinali come gastrite, ulcera peptica, sindrome del colon irritabile, cancro dello stomaco e del colon. La fertilità dipende da vari aspetti. I fattori di rischio sono: aumento della concentrazione di leucociti nel liquido seminale; varicocele di grado avanzato; riduzione dei livelli del testosterone; fattori metabolici, in particolare l'obesità viscerale; fumo di sigaretta; alcol; malattie a trasmissione sessuale; uso inappropriato di farmaci. Sono stati reclutati 23 pazienti con età compresa tra i 30 e 60 anni. Si è proceduto con l'analisi del liquido seminale e con l'analisi delle feci. Con lo spermiogramma si è analizzata la quantità, la morfologia e la motilità degli spermatozoi a tempo zero e tempo 2 ore. Contestualmente si è proceduto con l'analisi chimico-fisica delle feci e la coprocultura per valutare l'assorbimento intestinale e la presenza di infezione. In tutti i pazienti è stata effettuata anche la valutazione della concentrazione di PSA libero e totale e del testosterone per escludere problemi prostatici. Dei campioni di sperma analizzati 10 pazienti presentavano morfologia alterata e 7 motilità ridotta. Ai pazienti è stato chiesto di effettuare una analisi per la valutazione del microbiota intestinale. Le analisi hanno identificato nei pazienti con motilità ridotta basse concentrazioni di Bacteroidaceae.id.917, genere.Bacteroides.id.918 e Enterobacteriales.id.3468 e alte concentrazioni di Allisonella.id.2174 I dati erano concordanti per tutti i pazienti con ridotta motilità spermatica. Questo dato incuriosisce ponendo le basi per una ricerca a larga scala per poter così capire come il microbiota intestinale possa influenzare l'infertilità.

EP273
Presenza di un composto emoglobinico: contributo di due metodi separativi

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Le emoglobinopatie comprendono un gruppo di disordini, generalmente a trasmissione autosomica recessiva, caratterizzati da alterazioni nella sintesi di una o più catene globiniche (talassemie) o dalla sintesi di emoglobine strutturalmente anomale (varianti dell'emoglobina). Tali disordini possono essere identificati eseguendo l'assetto emoglobinico in elettroforesi capillare (CE) o con metodo HPLC. Una paziente, di anni 65 e di nazionalità italiana, è pervenuta presso il nostro laboratorio con richiesta di assetto emoglobinico ed emocromo (Hb=12,8 g/dL; MCV=60,5 fL; MCH=18,1 pg; HCT=42,8%). All'elettroforesi capillare (Capillarys 3, Sebia, Francia), il tracciato mostra una HbA=9,1%, HbA2=4,5% ed HbF=86,2%. Lo stesso campione, processato con tecnologia HPLC (Trinity 9210 Resolution, Menarini, Italia), ha riportato HbA=9,8%, HbA2=4,8% ed una variante HbS-like= 70,2%. I due metodi, seppur contrastanti in termini di identificazione di variante, hanno permesso di indirizzare la genotipizzazione verso l'analisi dei geni beta globinici. Si è posta, dunque, l'ipotesi diagnostica di doppio difetto a carico delle catene beta globiniche, che spiega l'aumento della frazione HbA2 e la presenza di una frazione aggiuntiva all'assetto emoglobinico. Il risultato dell'analisi genetica effettuata alla paziente conferma un doppio difetto a carico dei geni beta globinici che determinano il quadro clinico ematologico. Nello specifico, accanto al difetto beta+ talassemico IVS-I-110 (G->A); HBB: c.93-21G>A di più frequente riscontro evidenziata su un allele, l'analisi ha identificato una variante più rara a carico dell'altro allele del gene HBB, la c.23A>G p. (Glu8Gly), nota come HbG-San José. In letteratura, ad oggi, esistono pochissimi casi del composto Hb G St.Josè/ Beta Tal. associati di solito a fenotipi clinicamente poco alterati. È stata eseguita analisi genetica anche alla figlia della paziente, la quale risulta essere "portatrice sana" di beta-talassemia. Il caso qui rappresentato contribuisce a confermare l'importanza di poter valutare e confermare il pattern Hb mediante due diversi metodi separativi soprattutto quando si osservano risultati quali-quantitativi anomali.

EP274

Diagnostica molecolare in allergologia: il ruolo chiave per la gestione di casi di sensibilizzazione multipla

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Donna di 39 anni primo accesso in PS per dispnea, tosse ed eritema al volto dopo uso di candeggina. Positività per IgE specifiche classe 1-2 per inalanti comuni; per alimenti: classe 4 per frumento (42.30 kU/L), classe 3 per semi di sesamo e arachidi, classe 2 per semi di soia e classe 1 per nocciola e noce. Confermata oculorinite primaverile mai trattata, normale assunzione di sesamo e arachidi. Prick test: positività per farina di frumento e piselli. Si invia a domicilio con terapia per oculorinite ed esclusione di sesamo e arachidi dalla dieta. A 41 anni rientro in PS per angioedema del volto, dispnea e rash eritematoso insorti 15 minuti dopo l'assunzione di Oki Task. Invio a visita allergologica si evidenziano: dermatite da contatto, tosse e rinite in relazione ad uso di farina. Negati disturbi correlati all'ingestione di farinacei, riferito eritema diffuso dopo assunzione di piselli. Ricerca IgE specifiche per alimenti: riconfermati i valori, in aggiunta IgE specifiche classe 4 per avena, classe 3 per glutine, granoturco, riso integrale e piselli. IgE sp Gliadina=0.04 kU/L. IgE CCD=0.17 kUI/L. Per approfondimento si esegue microarray test ISAC che risulta negativo (v < 0.3 ISU_E), eccetto Der f 2 0.5 ISU_E, Der p 2 0.9 ISU_E, Cor a 1.0101 0.5 ISU_E e Tri aA_TI 0.3 ISU_E (al limite). Conclusioni: caso di sensibilizzazione multipla in allergia alla farina in particolare da inalazione. Mediante utilizzo della diagnostica allergologica molecolare si evidenzia la presenza di sensibilizzazione ad alto titolo verso cereali, in particolare frumento, e a titolo moderato verso leguminose e sesamo (IgE-Profilline negative e IgE PR-10 e CCD molto basse). Le IgE estrattive per frumento sono dirette maggiormente verso le proteine idrosolubili (albumine e globulina) e in minor parte verso il glutine, non IgE sp per gliadina, ma in particolare verso la porzione gluteninica e in minima parte verso le IgE Tri aA_TI. Le IgE rilevate per leguminose presentano specificità non ancora disponibili. Una maggiore disponibilità di diagnostica molecolare permetterebbe un miglior supporto del laboratorio al clinico, soprattutto nell'ottica di una medicina di precisione, offrendo una gestione personalizzata del paziente ad esempio evitando l'esclusione di alimenti ove non necessario.

EP275

MALARIA INFECTION REVEALS HEMOGLOBINOPATHY: A PEDIATRIC SICKLE CELL ANEMIA CASE REPORTS.

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BACKGROUND-AIM

In sickle cell anemia, which is caused by a hemoglobin gene mutation, abnormal hemoglobin S (HbS) is produced, causing red blood cells to take on a sickle shape. This leads to vaso-occlusive crises, hemolysis and severe anemia. Heterozygous individuals with sickle cell disease (SCD) have a higher survival rate and better protection against Plasmodium, inhibiting the growth of the malaria parasite. However, HbS heterozygotes can still contract malaria and develop significant anemia due to hemolysis.

METHODS

The case involves two Nigerian siblings, aged 2 and 1, admitted to the pediatric emergency room in December 2023 with fever after a 5-month stay in Nigeria. They returned to Italy a week ago. The boy's complete blood count, performed on the BC 6800 Plus analyzer (Mindray), showed mild monocytosis (monocytes=1.58x109/L), lymphocytopenia (lymphocytes=3.74x109/L), normochromic microcytic anemia (hemoglobin=75 g/L; MCV=63.2 fL;
MCH=20.4 pg; MCHC=32.3 g/dL). The girl's complete blood count showed thrombocytopenia (platelets=97x109/L), normochromic microcytic anemia (hemoglobin=77 g/L; MCV=65.3 fL; MCH=21.2pg; MCHC=32.5 g/dL).
In addition, the BC 6800 Plus displayed a specific flag for malaria-infected red blood cells (iRBC# = 0.01x109/L) in both
siblings, leading to further investigation by blood smears and optical microscopy.

RESULTS

The blood smear examination revealed gametocytes in the brother's sample and ring forms in the sister's sample.
Additionally, the presence of target cells and microcytosis raised suspicion of hemoglobinopathy or thalassemia.
The thin smear showed Plasmodium falciparum (parasitemia: 8-10%), confirmed by a rapid malaria test (Paramax-3,
Effegiemme). High performance liquid chromatography (BioRad HPLC) revealed the presence of HbS in both children,
27.5% in the brother and 33.5% in the sister. The boy's and girl's HbA2 levels were 3.1% and 3.2%, respectively. Molecular
genetic analysis was suggested.

CONCLUSIONS

This case illustrates the importance of the complete blood count, an inexpensive test available in all laboratories,
not only for the diagnosis of leukemias and blood diseases but also for detecting malaria infections and diagnosing hemoglobinopathies.

EP276

SEBIA Capillarys 3 DBS discovered a new peack in a patient with HbS hemoglobin variant

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Introduction

The correct use of the Sebia instrument capillary zone electrophoresis (CZE) (Sebia Capillarys 3 DBS) in addition to high-performance liquid chromatography (BioRad HPLC Variant II β-thalassemia short program) was essential for the discovery of a new peak in a Voxelotor-treated patient with HbS disorder.

Materials and methods

A 24-year-old African patient with sickle cell disease was hospitalized for SCD-related vaso-occlusive crisis. Complete blood count showed normochromic microcytic anemia: Hb = 94 g/L (RR: 135–175); MCV = 64.1 fL (RR: 80.5–99.7); MCH = 22.0 pg (RR: 26.6–33.8); MCHC = 344 g/L (RR: 315–363), elevated RDW = 28.7% (RR: 11.8–14.8), and increased absolute value and percentage of reticulocytes (Ret# = 0.37 × 1012/L [RR: 0.02–0.09]; Ret% = 8.82% [RR: 0.60–2.71]). Total iron concentration was 63 μg/dL (RR: 65–175); transferrin was 276 mg/dL (RR: 215–365); and ferritin was 148 ng/mL (RR: 22–322). Biochemical tests showed an elevated LDH of 915 U/L, total bilirubin of 1.40 mg/dL, and a slightly elevated aspartate aminotransferase of 43 U/L with a normal alanine aminotransferase of 29 U/L. The patient was treated with phlebotomy and quantification of the HbS variant was performed.However, in February 2024, a new peak was detected by Sebia instrument Capillarys 3 DBS.

Results

A new peak was detected by Sebia instrument (CZE) (Sebia Capillarys 3 DBS), near the HbS peak, which appeared branched: Hb S peak eluted at 211 sec (49.5%), and new peak at 220 sec (7.9%). For confirmation, the analysis was repeated using HPLC. Hb S variant eluted at 3.43 minutes and accounted for 90.8% of total Hb, confirming the homozygous genotype, and the new peak eluted at 3.21 minutes and accounted for 12.4% of total hemoglobin. The hypotheses were pharmacologic interference or the presence of a new hemoglobin variant.

Discussion

The laboratory learned from the physicians that a few weeks earlier he had started treatment with Voxelotor, an oral sickle hemoglobin inhibitor that reversibly binds to an α-globin chain, preventing HbS polymerization under conditions of reduced oxygen tension and risk of sickle cell formation. This case clearly demonstrates the importance of Sebia Capillarys 3 DBS in addition to HPLC for the proper screening of hemoglobinopathies.

EP277

Thyroglobulin measurement: comparison between two immunometric methods.

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Thyroglobulin (Tg) is a high-molecular-weight glycoprotein (660 kD), the principal constituent of the colloid contained within the central cavity of the follicle bounded by thyrocytes, essential for the synthesis of thyroid hormones such as Triiodothyronine (T3) and Thyroxine (T4), and stored in the thyroid gland. Small quantities of Tg can enter the bloodstream during synthesis stimulated by TSH hormone and transport towards the follicles, making it possible to detect low concentrations of Tg in the blood of healthy subjects or in pathological conditions such as differentiated thyroid carcinoma (DTC). In the latter case, Tg becomes an important tumor marker for monitoring the follow-up of patients with DTC after thyroidectomy and ablation with radioiodine (I131). Indeed, detectable serum levels of Tg post-intervention indicate persistence or recurrence of the carcinoma, while undetectable levels indicate remission.

Following thyroid ablation, Tg levels should be close to zero; therefore, the use of ultrasensitive immunometric methods has become necessary, offering greater sensitivity and specificity for better diagnostic accuracy and monitoring in case of minimal variations in Tg concentration. Moreover, it is necessary to consider the interference due to the presence of anti-Tg autoantibodies that could cause falsely low or negative measurements, accompanying the analysis with a quantitative assay to validate the Tg result as established by the ATA Guidelines.

For reliable and precise measurement, it is advisable to use the same immunological assay for the continuous monitoring of patients, as indicated by the guidelines of the American Thyroid Association (ATA).

Therefore, it was deemed useful to verify the comparability of laboratory results obtained with two different ultrasensitive Tg methods: Tg Access Assay (Beckman-Coulter, Fullerton, CA) and Elecsys Tg II (Roche Diagnostics, GmbH, Sandhofer Strasse) using the Operational Protocol of the SIBioC study group (M.Vidali et al., 2019).

In particular, from the analysis of Tg concentrations of 136 patients with DTC who underwent total thyroidectomy analyzed according to the ATA guidelines cut-offs, a concordance between the Access and Elecsys immunological tests of 97% emerged with a Cohen's kappa index of 0.94.

EP278

AN UNUSUAL CASE OF ERYTHROCYTE SEDIMENTATION RATE (ESR) DOSAGE IN A SUBJECT WITH DIFFUSE LARGE B CELL LYMPHOMA (DLBCL): INSIGHTS FROM A CLINICAL CASE ON A PROBABLE INTERFERENCE.

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Background : The erythrocyte sedimentation rate (ESR) is a nonspecific marker of inflammation, widely diffused in hematology laboratories to monitor inflammatory statuses, response to therapies and oncologic diseases. The gold standard for the determination of ESR is the Westergren method but there are various automated instruments, they are modifications of the Westergren method. The prognostic value of elevated ESR in patients with Diffuse Large Cell B Lymphoma (DLBCL) has not been explored, but elevated ESR indicates a higher risk Non-Hodkin Lymphomas (NHLs).

Case presentation : The total count blood test of a patient admitted at Policlinic of Rome Tor Vergata was performed (MINDRAY BC-6800 PLUS) and shows anaemia and moderate thrombocytopenia. White Blood Cells (WBC) in the normal range (7,9 x 10(3)), with a normal leukocyte formula (74, 5% neutrophils 14,2 % lymphocytes 11,4% monocytes 0,2 % eosinophils 0,10 % basophils). The peripheral venous blood smear (SPV) confirms the absence of morphological anomalies and platelet aggregates (MINDRAY MC-80). The morphological analysis of the marrow aspirate was performed with negative results. Lymph node biopsy confirms the diagnosis of DLBCL one of the most prevalent non-Hodgkin lymphomas (NHLs). Serum protein electrophoresis is characterized by a monoclonal peak in the gamma zone (IgM-k component). During therapies, the patient was hospitalized for Sepsis (Klebsiella infection and reactivation of the Cytomegalovirus), and ESR was undetectable with the instrument used routinely (ALIFAX TEST 1). The same was instead determined using different instrument, DIESSE CUBE 30 TOUCH which determined the ESR value 7 mm/h, while with the Westergren method, the ESR was 40 mm/h.

Conclusion and Discussion : Jaundice, lipemia, haemolysis and viscosity are interfering factors that must be evaluated in each laboratory analysis. In this case, the significant increase in blood viscosity (by the presence of the monoclonal peak) interferes with automated methods that are unable to detect the ESR. The gold standard in this case is least affected by this interference because the dilution with citrate limits the effect of viscosity and it is the preferred method for measuring

EP279

A case of cryoglobulinemia type I presenting with pseudothrombocytosis

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Cryoglobulins are circulating immunoglobulins that precipitate at temperatures less than 37°C and redissolve on warming. They have been reported in various infectious, autoimmune and hematological diseases. Falsely elevated leukocyte count is one of the major artifacts caused by the presence of cryoprecipitates. Very few works report abnormal platelet counts in the presence of cryoglobulins. We report the case of a cryoglobulinemia type I manifesting with pseudo-thrombocytosis. A 58-year-old woman affected by MGUS (monoclonal gammopathy of uncertained significance) IgG kappa was admitted to our hospital for a hematological check-up. Her last blood count performed by an external laboratory revealed an elevated platelet count (1.700.000/µL). The patient reported asthenia, pharyngitis, osteoarticular pain mainly in the large joints and the first metacarpal. Blood count was repeated by our laboratory using the automated hematology analyzer Sysmex XN-9000, with platelet counting carried out in the optical, impedance and fluorescence channels. Platelet count obtained with impedance channel was 1.039.000/µL, while optical and fluorescence channels gave a platelet count of 314.000/µL and 341.000/µL, respectively. Peripheral blood smear examination revealed the presence of amorphous faint blue-gray deposits between the cells that has led to the falsely elevated platelet reading carried out with the impedance method. The clinical and laboratory data may us assume that these deposits could be attributable to the presence of cryoglobulins. Cryoglobulinemia was confirmed by the detection of a cryoprecipitate (cryocrit 40%) in the patient's serum maintained at 4°C for 3 days, and which dissolved already at room temperature. Immunofixation of the precipitate revealed the presence of a monoclonal component IgG kappa and diagnosis was made of cryoglobulinemia type I (according to Brouet's classification). A first finding of thrombocytosis must be confirmed by optical or fluorescence platelet counting and morphological examination. In the case of interference the presence of cryoglobulins must be assumed because anomalous protein precipitates can falsely be counted as platelets on automated cell analyzers.

EP280

DITHIOTREITOL TREATMENT REDUCES PARAPROTEIN INTERFERENCES IN AUTOMATED CHEMISTRY METHOD IN A PATIENT AFFECTED BY WALDENSTROM DISEASE: A CASE REPORT.

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Background: Paraproteins are a well-known issue in clinical chemistry, since different automated methods could be interfered and spurious results on many analytes are possible. We documented a case report of paraprotein interference for biochemical analyses in a patient affected by Waldenstrom Macroglobulinemia (WM) with a marked monoclonal component using Dithiotreitol (DTT) to inactivate IgM antibodies.
Materials and method: a 70-year-old man with medical history of WM has been reported with an IgM monoclonal component of 39.2 g/L. The patient performed regular controls and during his usual follow-up electrolytes, glucose, urea, creatinine, total cholesterol, triglycerides, high density lipoprotein cholesterol, total protein, AST, ALT, GGT, ALP, cholinesterase, total and conjugated bilirubin and albumin were requested. None of the previous analytes was measured because of a technical error due to high paraprotein level. It is well known that sulphydril compounds as DTT may inactivate IgM immunoglobulin. The same patient plasma sample was treated with a DTT solution 0.2 M by adding 10 uL of DTT to 390 uL of plasma in order to obtain a DTT concentration of 0.005 M. The sample was incubated in a water bath at 37 °C for 2 hours and then measured on Cobas PRO c503 (Roche).
Results: previous clinical chemistry tests were repeated. All biomarkers, except for ALP, total cholesterol, high-density lipoprotein cholesterol, triglycerides and uric acid, were correctly determined without any technical alarm demonstrating the usefulness of DTT to inactivate IgM in the sample.
Conclusions: In conclusion, a so marked paraproteinemia without a preanalitical sample treatment could not allowed a biochemical longitudinal evaluation for patient. DTT demonstrates good properties to eliminate IgM interference on many laboratory tests and it could be considered a valuable practice when similar conditions are present in our laboratory. Further evaluations are mandatory, especially a repeatability and precision protocol are pivotal to demonstrate the absence of any kind of spurious results.

EP281

Riscontro mediante elettroforesi capillare di Hb Nunobiki [# 141(HC3) Arg>Cys] con affinità aumentata per l'ossigeno

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Lo studio delle varianti emoglobiniche contribuisce alla conoscenza dei meccanismi di espressione, sintesi, stabilità e funzionalità delle catene globiniche. Nella quasi totalità dei casi, le varianti emoglobiniche richiedono una accurata caratterizzazione molecolare e funzionale al fine di poterne prevedere i fenotipi ematologici e clinici. In questo lavoro descriviamo un caso di variante emoglobinica riscontrata in un soggetto maschio di anni 61 pervenuto alla nostra osservazione, con richiesta di assetto emoglobinico. Sebbene gli esami ematochimici sono risultati nella norma (HB=14,9 g/dL; MCV=90,6 fL; MCH=29,7 pg; HCT=45,5%), l'elettroforesi delle emoglobine (Capillarys 3, Sebia, Francia) ha evidenziato una frazione emoglobinica anomala in zona Z12. Il confronto con la tecnologia HPLC (Trinity 9210 Resolution, Menarini, Italia) conferma la presenza di sospetta variante emoglobinica che eluisce più velocemente dell'emoglobina HbA. Un campione di sangue del paziente è stato anche sottoposto ad elettroforesi delle emoglobine glicate con la strumentazione CAPILLARYS 3 (Sebia, Francia); l'elettroferogramma conferma una chiara frazione variante, seppur non interferente con la determinazione dell'HbA1c. È stato consigliato al paziente approfondimento diagnostico con indagine molecolare, che ha rivelato la presenza di una variante delle catene alfa, nota come Hb Nunobiki [a 141(HC3) Arg>Cys]. Tale variante è stata riscontrata per la prima volta nel 1985 in un soggetto maschio giapponese con eritrocitosi. La variante Hb Nunobiki è nota per essere la prima variante emoglobinica scoperta che presenta una cisteina al C-terminale della catena alfa globinica. Questa caratteristica comporta un cambiamento di carica elettrica che rende la variante visibile con i principali sistemi separativi in uso. Questo studio ha lo scopo di sottolineare l'importanza dell'utilizzo di metodi separativi ad alta risoluzione per lo studio di emoglobinopatie anche in test di screening in pazienti senza apparenti alterazioni ematologiche o sospetti clinici. Sono numerose, infatti, le alterazioni emoglobiniche silenti che possono produrre nella progenie, se combinate con altre alterazioni, difetti emoglobinici patologici.

EP282

A case of macro-luteinizing hormone

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Introduction

Luteinizing hormone (LH) is a 28 kDa glycoprotein hormone secreted by the anterior pituitary gland in response to hypothalamic gonadotropin-releasing hormone. LH secretion is necessary for normal sexual function and is regulated by the interaction of positive and negative feedback mechanisms involving hormone secretion from the pituitary, hypothalamus, and gonads. Here, we report a patient with clinically inexplicable elevations in serum LH and present the results of the examinations.

Methods

LH concentration was measured using Dimension Vista® System (Siemens Healthineers, Erlangen, Germany) and UniCel DxI 800 Access Immunoassay System (Beckman Coulter, Brea, CA, US). Follicle-stimulating hormone (FSH) concentration was measured by Dimension Vista® System.

Results

We present the case of a 51-year-old menopausal woman with remarkably high LH value measured using Siemens Healthineers System (>150 IU/L; analytical measurement range: 0.2-150 IU/L; menopause reference interval: 8.6-61.8 IU/L) although FSH concentration was within the reference interval (115.7 IU/L; menopause reference intervals 12.7-132.2 IU/L). Pituitary MRI showed no tumorous lesion. Thus, we performed the following investigations: dilution linearity test, determination of LH concentration with an alternative method and 25% polyethylene glycol (PEG) precipitation test. Dilution linearity was maintained. LH dosage using Beckman Coulter System was 206 IU/L (menopause reference interval 10.8-61.4 IU/L). After precipitation with PEG, the recovery rate of monomeric LH levels was 30.8%. The recovery rate of LH in 40 control women aged 48 and 80 years was higher (average=63.7±11.4%; 2.5-97.5 percentile=48.3-84.3%). These results suggest the presence of macro-LH, resulting in a falsely high LH value.

Conclusions

Interferences in immunoassay are a well-known phenomenon, however to date only few cases of macro-LH have been reported in literature. Here, we demonstrated a case of macro-LH interference using PEG precipitation underlining the fact that an alternative dosage method is not enough. In conclusion patients' presenting unexpectedly elevated hormones values indicates the need of further investigations to detect the presence of macro-complexes.

EP283

First observation in Italy of the Hb Nantes variant [β34(B16)Val → Leu)] by capillary electrophoresis method

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Hemoglobin Nantes, a variant with autosomic dominant transmission, is a very rare hemoglobinopathy associated with an increased oxygen affinity.These type of hemoglobin variants can be accompanied by erythrocytosis and are characterized by a decreased p50 value.The phenotype of "hemoglobin variants" can range from clinically silent to having severe clinical manifestations (e.g., severe anemia, sickling, polycythemia, etc.).Hb Nantes variants, as well as other variants with high affinity for oxygen, must be diagnosed early because, although well tolerated in young people, they often lead to thrombotic complications in older patients.Hb variants are detectable by laboratory techniques used for the measurement of hemoglobin species, for example, capillary zone electrophoresis and high-performance liquid chromatography (HPLC).Some variants impair the glycosylated hemoglobin (HbA1c) diagnosis on account of their instability (shortened erythrocyte survival), or the quantification process by interfering with the analysis method.Here we described a case of a Caucasian 18-year old male that undergoing screening blood tests in order to become a blood donor; the routine test showed an erythrocytosis and pO2 21.8 mmHg. The family history revealed that the father and his paternal grandmother had erythrocytosis, and they have a past diagnosis of Hb Nantes variant.Capillary electrophoresis performed with Capillarys 3 Octa (Sebia) showed an atypical pattern with a peak partially overlapped HbA1 migration zone, with HPLC we confirmed the presence of abnormal hemoglobin. However, neither method was able to quantify the variants. HbA1c analysis performed with Capillarys 3 Octa (Sebia) better revealed the abnormal double peak. Subsequently, an NGS analysis was conducted that confirmed the presence of the Nantes variant [β34(B16)Val → Leu] in the heterozygous state. The dizygotic twin brother showed no increased affinity for oxygen, HPLC and capillary electrophoresis revealed no abnormal peaks and NGS confirmed the absence of Hb variants. Our work demonstrated for the first time that it is possible to highlight the Hb Nantes variant using capillary electrophoresis and that the accuracy of Hb variant identification can be greatly improved by combining separative methods.

EP284

Individuazione di una variante emoglobinica. Comparazione tra Elettroforesi capillare e Cromatografia ad alta prestazione

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Una paziente di origine nordafricana di 39 anni esegue presso il nostro Centro un test di Screening per la Microcitemia. All' HPLC, oltre alle bande relative all' Hb A e A2 viene rilevata una banda (area 22,1 %) con un tempo di ritenzione di 4,83 minuti (determinazione eseguita mediante HPLC Biorad). La foresi eseguita su acetato di cellulosa conferma la presenza della banda anomala. Considerata l'area geografica di provenienza della paziente, viene eseguito il test di falcizzazione per verificare l'eventuale presenza di Hb S, che da esito negativo. Poichè la paziente presenta un quadro di sospetta anemia sideropenica (Hb 10.1; MCV 58; MCH 17.9; RDW 19.9), viene eseguito anche il dosaggio della ZnPP (zinco protoporfirina eritrocitaria) che risulta aumentata. Il campione viene quindi sottoposto ad elettroforesi capillare su Capillarys 2 Flex Percing Sebia che conferma la presenza di una bande anomala (area 26,1 %) che migra insieme alla HbA2. Con l'obiettivo di separare la banda della variante dalla banda dell' HbA2, il campione viene sottoposto a migrazione in elettroforesi capillare con metodica per la determinazione della emoglobina glicata (caratterizzata da un maggior grado di risoluzione). L'esito conferma la presenza di una variante che in base al punto di migrazione il software del Capillarys identifica come HbE. La variante emoglobinica non coincide con l' area geografica di provenienza della paziente. Infatti la HbE è una variante presente soprattutto in India e nel sud est asiatico. Per verificare l'esatta natura della variante (HbE o eventuale variante emoglobinica caratterizzata da un analogo pattern di migrazione elettroforetica), la paziente viene indirizzata presso un Centro specializzato (Ospedale microcitemico di Cagliari) per la caratterizzazione molecolare (indagine in corso). La metodica per la determinazione della emoglobina glicata, può risultare utile in quei casi in cui la separazione tra varianti emoglobiniche risulta difficoltosa con l'utilizzo della metodica tradizionale.

EP285

Modifiche morfologiche transitorie dei leucociti in seguito a colpo di calore

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Un uomo di 23 anni viene ricoverato in Rianimazione in stato di incoscienza provocato da colpo di calore durante un allenamento all'aperto per maratona. I valori di laboratorio all'accesso evidenziano scompenso elettrolitico, danno epatico e renale acuto (AST 6632 U/L; ALT 1447 U/L; creatinina 2,38 mg/dL), rabdomiolisi (creatin-chinasi 6500 U/L), acidosi lattica severa (LDH > 2500 U/L; pH 7,42). L'esame emocromocitometrico mostra neutrofilia (9,50x109/L) e linfocitosi (4,01x109/L) non marcate, globuli rossi (GR, 5,51x1012/L), emoglobina (15,4 g/dL), MCV (83,1 fL) nella norma.

La lettura dello striscio periferico evidenzia la presenza di rari elementi immaturi (mielociti, eritroblasti) e di una morfologia bizzarra a carico dei leucociti. Si osserva marcata ipersegmentazione dei neutrofili con nuclei 'botroidali' (> 50% dei neutrofili), ovvero formati da cluster in grappoli che ramificano a partire da un unico stelo (1). Una conformazione nucleare analoga si osserva anche in alcuni linfociti. L'osservazione dello striscio successivo, dopo due giorni dal ricovero, mostra una totale risoluzione delle anomalie citologiche. L'ipersegmentazione nucleare dei neutrofili, accompagnata da macrocitosi dei GR, è classicamente associata a deficit di folati e vitamina B12. In assenza di macrocitosi dei GR la caratteristica disposizione dei nuclei multi-lobulari è stata descritta in pazienti con ipertermia o colpo di calore, uso di anestetici o farmaci neurolettici, consumo di cocaina e metamfetamine (2). I meccanismi alla base delle formazione dei nuclei 'botroidali' ipertermia-associati sono sconosciuti; le ipotesi includono modifiche pre-apoptotiche, variazione di osmolarità intracellulare, danno alle membrane cellulari (3). Modifiche 'botroidali' in più del 50% dei neutrofili su uno striscio periferico possono essere sufficienti per fare diagnosi di colpo da calore, in assenza di sepsi e con screening tossicologico negativo (4). Riconoscere questi quadri morfologici caratteristici è pertanto utile nell'esclusione di eventuali patologie di natura infettiva/ematologica e nella corretta gestione del paziente.

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EP286

IDENTIFICAZIONE DI UNA VARIANTE EMOGLOBINICA RARA IN CORSO DI SCREENING PRECONCEZIONALE: LA NOSTRA ESPERIENZA

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L'emoglobinopatia è un'alterazione ematologica dovuta ad un difetto genetico della globina, un'apoproteina che costituisce le emoproteine emoglobina e mioglobina. I difetti possono essere strutturali (con alterata funzione e stabilità dell'emoglobina), di sintesi (talassemie) o misti, e la condizione si può associare ad anemia.La ricerca di varianti attraverso l'assetto emoglobinico può essere effettuata con elettroforesi capillare o con metodo HPLC. Se viene identificata la presenza di una variante anomala, il paziente viene rimandato ad un Centro di riferimento per lo studio delle emoglobinopatie, che eseguirà il sequenziamento genico.Presso il nostro laboratorio si è presentato un soggetto maschio di 40 anni e di nazionalità italiana con richiesta di assetto emoglobinico per screening preconcenzionale. L'analisi eseguita in HPLC (Variant II, Biorad, USA) evidenziava una frazione del 3,1% che eluiva dopo l'emoglobina A2 (Retention Time = 3,52 min), nella stessa zona di un'eventuale emoglobina S. Dopo aver escluso la presenza di tale variante e verificato che il picco non fosse dovuto ad un trascinamento di un campione precedente, l'analisi è stata ripetuta in elettroforesi capillare (Capillarys 3 Tera, Sebia, Francia). Il tracciato mostrava un picco anomalo (0,7%) che in parte si sovrapponeva all'emoglobina A2, nella zona di un'eventuale emoglobina C.Le ipotesi diagnostiche erano di una delta variante o di un'alfa variante instabile. Al paziente è stato dunque suggerito di rivolgersi ad un Centro di riferimento per lo studio delle emoglobinopatie.L'analisi molecolare ha deposto per emoglobina Shuangfeng, una rara variante instabile data dalla mutazione del codone 27 (GAG→AAG, Glu→Lys) della catena globinica alfa2.I parametri ematologici del paziente erano nella norma (Hb=14,3 g/dL, MCV=90,8 fL, Hct=41,7%) ma il tratto è trasmissibile e l'eventuale associazione con alfa-talassemia potrebbe produrre un fenotipo "HbH like", con episodi acuti di anemia emolitica in presenza di infezioni, esposizione a farmaci o a sostanze ossidanti, e splenomegalia.Da qui l'importanza che lo studio dell'assetto emoglobinico riveste nell'ambito dello screening preconcezionale e il contributo fornito dall'elettroforesi capillare a tale tipologia di indagine.

EP287

Infezione da HCV e gammopatia monoclonale di significato indeterminato (MGUS): Caso Clinico

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[1]

Introduzione Secondo l'OMS nel mondo sono circa 80 milioni le persone colpite da HCV con età media ~60 anni. La gammopatia monoclonale di significato indeterminato (MGUS) è una discrasia plasmacellulare premaligna che precede costantemente il mieloma multiplo (MM) con un rischio di progressione dell'1% all'anno. Sono sempre più chiari i fattori genetici e immunologici responsabili della progressione dal clone di plasmacellule aberranti alla MGUS e al MM conclamato. L'effetto principale dell'HCV è il danno epatocitario immunomediato. L'HCV provoca la stimolazione cronica antigenica delle cellule B e la produzione di anticorpi. Qui descriviamo il caso di una donna di 55 anni affetta da epatite C cronica ad eziologia sconosciuta, con anche una diagnosi di MGUS. Materiali e metodi L'RNA dell'HCV nel plasma umano e relativo genotipo è stato determinato con il sistema Cobas Taqman Roche. I livelli proteici sono stati visualizzati mediante elettroforesi capillare delle proteine sieriche ed immunofissazione. Il dosaggio Ig monoclonali e totali, le catene leggere libere kappa/lambda nel siero e nelle urine sono stati effettuati con Architect ABBOTT. Risultati La PCR quantitativa ha rilevato la presenza del virus HCV con una viremia di ~106 UI/ml e genotipo 2a/2c. Dopo sette mesi di terapia antivirale con Sofosbuvir e Ribavirina, la paziente ha eradicato il virus. L'elettroforesi delle proteine ha rilevato un picco monoclonale in zona gamma, che l'immunofissazione ha identificato come IgG di tipo #. Lo stato della malattia è stato poi monitorato mediante, la quantificazione delle Ig monoclonali, delle catene leggere totali e libere kappa/ lambda nel siero e nelle urine. Conclusioni La MGUS è considerata una condizione precursore benigna che può progredire verso una malattia linfoproliferativa o un mieloma multiplo. La MGUS è associata a un'aspettativa di vita ridotta e, in una minoranza di casi, a una serie di condizioni di comorbilità. Diversi studi suggeriscono una correlazione tra il meccanismo patogeno nella MGUS e nel MM con l'infezione virale (HCV, HIV e EBV), di conseguenza la riduzione dell'antigene target apre nuove opportunità per il trattamento della MGUS e del MM. Se il bersaglio delle Ig monoclonali viene eliminato, la stimolazione cronica dell'antigene scompare, portando al controllo delle plasmacellule clonali.

EP288
INTERPRETAZIONE DEL SEDIMENTO URINARIO: IL RUOLO CLINICO DEGLI SFEROPLASTI

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Introduzione

L'infezione del tratto urinario (UTI) è tra le infezioni batteriche più comuni ed è considerata una minaccia per la salute pubblica dato il crescente tasso di resistenza agli antibiotici tra gli uropatogeni. Escherichia Coli e Klebsiella Pneumoniae sono tra gli agenti eziologici più frequenti. Sotto l'influenza di dosi subottimali di antibiotici ß-lattamici questi batteri possono perdere la loro tipica forma a bastoncino e acquisire quella di sferoplasti, indicando una possibile resistenza all'antibiotico. Qui descriviamo il caso di un paziente che ha sviluppato sferoplasti, identificati durante l'analisi del sedimento urinario.

Case Report

Paziente di 91 anni giunge in Pronto Soccorso presso il Presidio Ospedaliero di Desio con febbre e riferita ematuria. Gli esami di laboratorio riportavano: WBC 60,5 x 10^3/mmc (Intervallo di Riferimento (IR) 4-11 x 10^3/mmc), PCR 246 mg/L (IR < 5 mg/L), PCT 158.80 ng/mL (IR <0,5 ng/mL). La radiografia del torace mostrava un vasto addensamento parenchimale basale dx + versamento pleurico bibasilare. Viene ricoverato nel reparto di Medicina Interna con diagnosi di insufficienza respiratoria lieve in polmonite e trattato con ceftriaxone disodico e azitromicina diidrato. Durante il periodo di degenza sono stati effettuati: emocoltura, urinocoltura ed esame chimico-fisico e del sedimento urinario. All'esame microscopico del sedimento sono stati osservati batteri e leucociti, indici di infezione, e sferoplasti, indicatori di terapia antibiotica inefficace. Difatti l'urinocoltura e l'emocoltura sono risultate positive per Klebsiella Pneumoniae resistente ai carbapenemici (KPC).

Conclusioni

Molto poco è stato pubblicato in letteratura riguardo alla presenza di queste particolari forme batteriche aberranti nelle urine. Infatti questi elementi possono essere erroneamente classificati come lieviti o eritrociti. Pertanto, una formazione adeguata dei professionisti del laboratorio sul riconoscimento degli sferoplasti e sul loro significato clinico è importante per supportare il medico specialista nella scelta di una terapia antibiotica appropriata. L'esame del sedimento urinario può così assumere un ruolo di maggiore rilievo, fornendo risultati che vanno oltre il livello di screening.

EP289
CASO CLINICO: DIAGNOSI DI ANEMIA FALCIFORME SU STRISCIO EMATICO DI UN BAMBINO CON PARVOVIRUS B19

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INTRODUZIONE
Il Parvovirus B19 (B19V) è un virus a DNA appartenente alla famiglia Parvoviridae, genere Erythrovirus. E' l'agente eziologico della V malattia o Megaloeritema infettivo. Nei soggetti affetti da anemia falciforme (AF) l'infezione da B19V può causare gravi episodi di anemia; il virus, replicandosi nei precursori degli eritrociti presenti nel midollo osseo, determina un' alterata produzione non compensata da emazie con emivita normale.
CASO CLINICO
Maschio di 4 anni, di origine africana, giunge presso il pronto soccorso pediatrico dell'Ospedale "Moriggia Pelascini" di Gravedona (CO). All'esame obiettivo si rilevano febbre, pallore cutaneo, labbra fortemente esangui, marcata astenia accompagnata da sospette anomalie scheletriche e ritardo nell'accrescimento. I genitori riferiscono un episodio febbrile circa un mese prima trattato empiricamente con Azitromicina.
RISULTATI
Gli esami ematochimici mostrano Hb 2.3 g/dL con piastrinosi e leucocitosi. L'assetto marziale rileva ferro 178 ug/dL, ferritina 1492 ng/mL, transferrina 1.97 g/L, vitamina B12 1020 pg/mL e folati 3.98 ng/mL. L'osservazione dello striscio periferico evidenzia anisopoichilocitosi delle emazie, con rari drepanociti, trombocitosi e linfociti attivati. Sono effettuati: type&screen, test di Coombs diretto e indiretto entrambi negativi; trasfusione di concentrato di globuli rossi di 5 mL/kg/h. Nel sospetto clinico legato a grave anemia, presenza di drepanociti e origine etnica, è stata eseguito il sickling test (test di falcizzazione), risultato positivo. Data età e clinica del soggetto, è stata effettuata l' indagine sierologica per il Parvovirus B19, positiva per IgG (5,1 U/mL) e IgM (>48 U/mL). Lo studio delle emoglobine patologiche mediante elettroforesi capillare (EC) evidenzia: Hb F 26%, Hb S 71,9% e Hb A2 2,1% confermando la diagnosi di AF.
CONCLUSIONI
L'infezione acuta da Parvovirus ha causato una forte anemia in un soggetto omozigote per AF, condizione fino a quel momento non nota. Grazie all'osservazione di drepanociti nello striscio ematico, è stato eseguito il test di falcizzazione, permettendo una diagnosi precoce di AF, confermata poi da dati strumentali.

EP290

L'importanza dell'uso combinato di due metodologie diverse per la valutazione di interferenti nel dosaggio dell'emoglobina glicata.

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L'emoglobina glicata (HbA1c) è un importante marcatore biochimico utilizzato nella diagnosi e nel monitoraggio del diabete in quanto è in grado di fornire una stima della concentrazione media di glucosio nel sangue nei 2-3 mesi precedenti.La determinazione di HbA1c può essere influenzata dalla presenza di interferenze sia analitiche, sia legate alle condizioni cliniche del paziente che possono rendere i risultati dell'analisi non attendibili o di difficile interpretazione.Nel laboratorio di ematologia specialistica del LUM di Bologna, dove si esegue la determinazione della Hba1c con metodo HPLC si è presentato il caso di una donna di 36 anni ,originaria del Marocco, in stato di gravidanza, con richiesta di dosaggio di HbA1c per alterata glicemia.La glicemia è risultata essere pari a 117.4 mg/dL, fruttosamina= 214micromol/L, HB= 9,5 g/dL e gli indici eritrocitari alterati (MCV=72 fL,MCH=21.7,MCHC=30.9).Il campione è stato analizzato mediante il sistema diagnostico in HPLC D-100 (Biorad) che ha rilasciato un risultato (allarmato '' A1c peak shape talling'') di HbA1c pari a 34 mmol/mol.Come da procedura interna il dato non viene refertato in quanto verosimilmente alterato dalla presenza di una variante.L'approfondimento diagnostico in HPLC Biorad Variant II e Dual kit ha evidenziato la presenza di HbF= 2,9% e di HbA2= 4,7% compatibile con un tratto beta-talassemico e di HBA1c= 2,9% (9 mmol/mol).Segue valutazione con un secondo metodo in elettroforesi capillare (Capillarys di Sebia) che ha confermato la presenza del tratto beta-talassemico e ha evidenziato la presenza di una variante in Z12zone pari al 71% (analisi molecolare in corso) che non era stata individuata con metodo HPLC.Anche il valore di HbA1c fornito dall' elettroforesi capillare di 35 mmol/mol ,causa interferenza da variante ,risulta essere non refertabile.Da questi risultati si evidenzia l'importanza dell'utilizzo di differenti approcci metodologici nella valutazione delle varianti emoglobiniche e della loro possibile interferenza sulla corretta quantificazione dell'HbA1c.

EP291

La variabilità dei livelli delle piastrine nella gestione clinica del disturbo bipolare.

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Background Il Disturbo Bipolare (BD) è una condizione psichiatrica caratterizzata da significative fluttuazioni dell'umore, inclusi episodi maniacali e depressivi. Ricerche recenti indicano che parametri piastrinici come la conta piastrinica, il volume medio piastrinico (MPV), il piastrinocrito (PCT) e l'ampiezza di distribuzione delle piastrine (PDW) potrebbero servire come biomarcatori per il BD. Questo abstract rivede i valori misurati di questi parametri nei pazienti con BD, con l'obiettivo di comprenderne la potenziale utilità clinica.Obiettivo: Indagare le differenze nei parametri ematologici e biochimici tra pazienti con Disturbo Depressivo Maggiore (MDD) e Disturbo Bipolare (BD) utilizzando metodi statistici non parametrici, e valutare la presenza di tendenze che potrebbero suggerire una differenza statisticamente significativa con un campione più grande.Metodi: È stato condotto uno studio comparativo analizzando l'emocromo completo (CBC) e i parametri biochimici in pazienti con diagnosi di MDD e BD. Poiché l'analisi della distribuzione dei dati nel campione ha evidenziato la presenza di variabili con distribuzione parametrica e non parametrica, sono stati utilizzati il test di MannWhitney U e l'ANOVA per valutare le differenze tra i due gruppi. I parametri valutati includevano conta piastrinica (PLT), colesterolo, creatinina, glicemia, ematocrito (HCT), lipoproteine ad alta densità (HDL), emoglobina (HGB), linfociti, emoglobina corpuscolare media (MCH), concentrazione media di emoglobina corpuscolare (MCHC), volume corpuscolare medio (MCV), monociti, volume medio piastrinico (MPV), neutrofili, rapporto neutrofili/linfociti (NLR), rapporto piastrine/linfociti (PLR) e procalcitonina (PCT).Risultati: Gli studi hanno riportato che, durante gli episodi depressivi, la conta piastrinica tende a essere più bassa rispetto ai parametri presenti nei soggetti con episodi maniacali, con valori che generalmente variano da 150 a 200 K/µL. Durante gli episodi maniacali, infatti, sono state osservate conte piastriniche elevate, con valori che variano da 250 a 300 K/µL per microlitro, mentre negli stati eutimici si registrano conte piastriniche normali, circa 200 a 250 K/µL. Durante le fasi maniacali, sono stati notati valori elevati di MPV che variano da 9,0 a 11,0 femtolitri (fL), mentre durante gli episodi depressivi, i valori di MPV sono generalmente compresi tra 7,0 e 9,0 fL. I valori di PCT nei pazienti con BD sono stati riportati in un intervallo da 0,15% a 0,30%, sebbene i dati siano limitati e necessitino di conferma con un campione più grande. Valori di PDW più elevati nei pazienti con BD variano da 15% a 18%, con aumenti notati durante gli episodi maniacali rispetto agli stati depressivi.Conclusioni: I valori misurati dei parametri piastrinici nei pazienti con BD mostrano variazioni significative con gli stati dell'umore, suggerendo il loro potenziale come biomarcatori. L'elevato MPV e PDW durante gli episodi maniacali e le conte piastriniche alterate nei diversi stati dell'umore evidenziano la necessità di ulteriori ricerche per chiarire queste relazioni e la loro rilevanza clinica. La comprensione di questi parametri può migliorare l'accuratezza diagnostica e informare le strategie di trattamento per il Disturbo Bipolare, migliorando in ultima analisi la cura dei pazienti.

EP292

Coagulation assays complications associated with Paraproteins in Monoclonal Gammopathy

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In MGUS and Multiple Myeloma, the presence of excess monoclonal protein (M-component or paraprotein) can affect laboratory coagulation tests. We present a case concerning a 79-year-old woman. In January 2024 she comes to our hospital with fatigue and anaemia. The bone marrow biopsy was compatible with a diagnosis of MM with a monoclonal component IgG/k 4,5 g/dl. The course of hospitalization was complicated by the occurrence of spontaneous abdominal muscle hematoma with severe anemization. Patient's initial coagulative assays showed prolonged Prothrombin Time (PT ratio = 1.5) and activated Partial Thromboplastin Time (aPTT ratio = 1.36), with normal platelet levels. In order to explain first level coagulation abnormalities PT and aPTT reflex were requested. First level coagulation assays showed normal PT and confirmed aPTT prolongation (ratio = 1.52). To differentiate between factor deficiency and inhibitors against specific clotting factors or lupus anticoagulant (LA) interference, we performed aPTT on a plasma sample mixed with normal plasma. In our patient, the aPTT mixture corrected completely suggesting factor deficiency for this patient. Further laboratory investigations revealed a negative LA, and factor VIII, XII and vonWillebrand (vWF) deficiencies (VIII:C 11%, XII:C 46%; vW Ag 9,6% vW Ricof 6,1%). We conducted factor VIII and XII tests on three dilutions of the plasma sample to determine linearity and parallelism and we obtained a "parallelism" CV of 1.5% and 1.2% for factor VIII and XII respectively confirming the two factor deficiencies for this patient. Because the presence of paraprotein in patient's plasma we supposed interactions between monoclonal protein and coagulation factors that caused reduced levels of factor VIII, XI and vWF and prolong aPTT time. Thrombin Generation Time (TGT) performed in this patient showed slightly reduced Peak Height and Endogenous Thrombin Potential suggesting a mild bleeding profile (compatible with acquired vWF). Often, in patients with excess of monoclonal protein, abnormal haemostasis tests result, are not related to clinical apparent haemostatic complication. This case report shows valuable insights into, how coagulation parameters, can be affected by paraproteins.

EP293

Hemostasis tests panel portfolio and reagent in emergency circumstances: the importance of mixing test, different aPTT reagents and availability of a DOACs removal pre-analytical system.

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We report on a 81-year-old man with a hematoma in the left upper limb and an extremly prolonged activated partial thromboplastin time (aPTT ratio = 3.47). Patient undergoing therapy with Dabigatran for Atrial Fibrillation. In order to explain whether prolonged aPTT was determined by Dabigtran drug accumulation and eventually other coagulation abnormalities, by using "stat haemostasis tests", Dabigatran measurement and an aPTT reflex tests were requested. We performed a thrombin time (TT) and a dilute TT (dTT) in this patient, and we find a dTT=33 ng/ml, and a slightly prolonged TT (TT ratio = 1.22), results incompatible with Dabigatran drug accumulation and such a prolonged aPTT. Direct Oral Anticoagulants (DOACs) are known to interfere with first and second level coagulation tests. In order to resolve DOACs interference we pre-treat sample with DOAC stop reagent (Activated Charcoal based compound). dTT and TT on DOAC stop treated plasma were respectively dTT < 20 ng/ml and TT ratio = 1.01, proving Dabigatran removal from our plasma sample. First level coagulative assays on pre-treated plasma showed normal PT ratio = 1.01 and prolonged aPTT ratio = 3.37. To understand, in emergency circumstances, whether the cause of the prolonged aPTT, was an antibody or an inhibitor, we performed the aPTT mixing test and we obtained a prolonged aPTT mixing time (ratio = 1.32) suggesting the presence of an inhibitor in this patient. To understand the type of inhibitor, Lupus Anticoagulant (LA) or anti-factor antibody, we performed the aPTT with a second reagent very sensitive to factor deficiency and anti-factor inhibitors and with very low sensitivity to LA and we obtained a prolonged aPTT ratio (aPTT Actin ratio = 2.99). By using "stat first level coagulation assays" (TT, dTT, two different aPTT and aPTT mix) and with availability of a pre analytical system to remove DOACs from plasma sample, in less than 2 hours we ruled out Dabigatran overdose and confirmed the presence of an intrinsic factor inhibitor in this patient. Routine second level coagulation tests performed next day classified this patient as an acquired hemophilia with low levels of factor VIII (VIII:C = 2.5%) and a strong positivity to anti factor VIII antibodies (8.5 U Bethesda/ml).

EP294

Early onset colorectal cancer: a novel nonsense pathogenic DNA variant in SMARCA4 gene

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Colorectal cancer (CRC) is the third leading cause of death worldwide, with increasing incidence in low- and middle-income countries. Due to the increasing number of new screening programs, we are seeing a rising number of Early-Onset Colorectal Cancer (EOCRC) in patients younger than 50 years old, accounting for 10-12% of all CRC cases. To understand the involved mechanisms, there is a growing need to study the younger population through early genomic analysis, particularly in familial cases, and to investigate the genomic predisposition using a combination of new technologies and strategies of molecular biology. Herein, we present a case of a 43-year-old woman, who went to the hospital in May 2021 for abdominal pain. After some investigations, she was diagnosed with left colon cancer. Given the patient's young age and familiarity with oncological diseases, she was included in our research program, which foresees a scheduled collection of blood and tissue samples. Whole blood was collected the day before surgery, and small pieces of tumor and healthy mucosa were taken during the surgery. For all the samples collected, we performed a combination of molecular and cellular analyses: (I) a customized multi-gene panel (n=56 genes) to search for putative susceptible variants for CRC onset, (II) comparison of the variants found in the above-mentioned panel in (I) in genomic DNA extracted from blood, tumor tissue, and adjacent healthy mucosa, (III) study of microsatellite instability, (IV) analysis of copy number variants via multiplex ligation-dependent probe amplification (MLPA), and (V) stabilization of patient-derived organoid (PDO). A mutation in the SMARCA4 gene was found in all the samples analyzed with a multi-gene panel. The variant c.3854T>A, p.Leu1285X, is a nonsense variant that results in the production of a premature stop codon at exon 27 and is not reported in clinical databases such as ClinVar or dbSNP. The SMARCA4 gene is involved in damage repair mechanisms and, if mutated, is associated with the development of several types of tumors. Furthermore, we have stabilized PDOs from the same patient's tumor tissue, thereby confirming the presence of this previously unreported pathogenic mutation. This clinical case highlights the necessity to incorporate individuals younger than 50 years old in screening programs, which should also include genetic tests for predisposition to early-onset malignancies.

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EP295

Comparative analysis of Copy Number Variations suggests etiological and biological overlap between intellectual disability and schizophrenia: a case report

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Several genes and pathways may have pleiotropic effects on psychopathology by affecting neurodevelopment. Dosage alterations of multiple genes affected by deletions or duplications may be the cause of cross-disorders associated with CNVs that reveal common molecular pathways, such as those related to neuronal cell adhesion, synapse function, and neuronal development. CNVs associated with schizophrenia (SCZ) are also associated with other neuro-developmental disorders (NDDs), including intellectual disability (ID) and autism spectrum disorder (ASD). To support this hypothesis, we report the case of a male patient previously diagnosed with moderate ID that developed into SCZ using array-CGH. We identified a duplication of 472.6 Kb on chromosome 15q13.3 involving the CHRNA7 gene, which encodes the α7 subunit of the nAChR. CHRNA7 is a significant candidate gene for neurodevelopmental disorders included in the SFARI database. Indeed, CNVs of the CHRNA7 gene have been detected in patients with neurological phenotypes such as cognitive deficits, language impairment, ASD, ID, and SCZ, with varying degrees of penetrance and severity, suggesting that the human brain is sensitive to the dosage of CHRNA7. Furthermore, a deletion of 1.9 Mb was identified in the 22q11.21 region. Individuals with 22q11.2 deletion syndrome have borderline intellectual functioning and the genes within 22q11.2 may be responsible for the pathogenesis of SCZ. These include the COMT gene, which encodes for a postsynaptic enzyme critical for dopamine metabolism, and the PRODH gene, which encodes a mitochondrial protein that catalyzes the first step of proline degradation. Transcriptional and behavioral interaction PRODH-COMT has been documented; PRODH-deficient mice showed selective up-regulation of COMT. According to this model, 22q11 hyperprolinemic individuals with low COMT activity showed less efficient in compensating for dopaminergic hyperactivity and are therefore at higher risk of developing a cognitive and/or psychiatric phenotype. NDDs can be defined a continuum of neurodevelopmental disorders that reflect the relative burden of rare harmful mutations, the magnitude of their effects, and the timing of their impact on brain development and on the consequent functional results.

EP296

Creatinine measurement in a patient with hypergammaglobulinemia: A case of analytical interference with Roche COBAS instruments

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Introduction

Accurate creatinine determination is essential for the assessment of renal function. However, conditions such as hypergammaglobulinemia (HGG) may interfere with some measurement methods.

Materials and Methods

The case of a 64-year-old woman undergoing oncologic follow-up in a nephrology outpatient clinic is presented. Her clinical history revealed MGUS IgM kappa with absent Bence Jones. In October 2023, the plasma creatinine measured with Siemens ADVIA 1800 Chemistry (RR: 0.51 to 0.95 mg/dL) was 0.6 mg/dL. In April 2024, the measurement with the Roche Cobas 8000 core Chemistry Analyzer showed a creatinine value of less than 0.05 mg/dL. Both instruments use enzymatic methods.

Results

Measurement of plasma creatinine using the Roche Cobas 8000 core Chemistry analyzer showed abnormal negative values of -1.79 mg/dL and -2.25 mg/dL in two evaluations. Dilution of the sample at 1:10 and 1:20 resulted in values of 4.06 mg/dL and 3.39 mg/dL, corrected for the respective dilution. IgM were found to be 3513 g/L (RR: 0.03-0.32 g/L) in line with HGG. To rule out IgM macro-complexes interference, creatinine was measured after a 1:2 dilution with PEG 6000, in order to precipitate IgM, resulting in 1.04 mg/dL corrected for the dilution. The primary sample was then sent to Analytical Laboratory of the Alessandria University Hospital, where a creatinine value of 0.83 mg/dL was obtained using the Siemens Advia Chemistry XPT Analyzer. In addition, the measurement of cystatin C with the Siemens BN II Nephelometer showed a value of 1.13 mg/L (RR 0.53 to 0.95 mg/L).

Discussion

The abnormalities observed in the creatinine determination indicate analytical interference due to HGG using Roche Cobas 8000, but IgM precipitation with PEG almost completely abrogated interference. The Siemens Advia Chemistry XPT Analyzer provided more reliable creatinine measurements in the presence of HGG. Measurement of cystatine is a valid alternative for the assessment of renal function in case of analytical interferences.

EP297

Importanza delle informazioni pre-test nell'interpretazione degli assetti emoglobinici ai fini di una corretta conclusione diagnostica

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Le emoglobinopatie comprendono i difetti qualitativi e quantitativi delle catene globiniche dell'emoglobina. L'elettroforesi capillare e il sistema HPLC sono metodi utilizzati in fase di screening per separare e quantificare le varie frazioni dell'emoglobina. Il caso esaminato è quello di una donna italiana di 82 anni inviata in pronto soccorso per dispnea e anemia, con anamnesi di diabete mellito, IRC e anemia cronica microcitica in assenza di carenza marziale (Hb 75 g/L; Hct: 25%; MCV 65 fL; MCH 20 pg; RDW 15,8%; ferritina 168 ng/mL). In seguito al ricovero è pervenuta al laboratorio una richiesta di assetto emoglobinico. Il tracciato elettroforetico (Capillarys3 Tera/Octa, Sebia, Francia) mostrava una HbA2 (4,4%) compatibile con eterozigosi per beta-talassemia, ed un picco anomalo (4,3%) migrante tra l'emoglobina A e la A2, nella zona compresa tra D ed S, che lo strumento identificava come variante "borderline". Il profilo elettroforetico relativo al dosaggio della HbA1c eseguito 10 giorni prima del ricovero non mostrava un profilo atipico e riportava una HbA1c del 6,8%. Si è quindi segnalato un probabile trait beta-talassemico associato alla presenza di una variante anomala, da verificare mediante un test molecolare. L'analisi genetica ha portato al riscontro esclusivo della presenza di una mutazione C>T non senso al codone 39 del gene beta-globinico allo stato eterozigote, frequente nei portatori di beta-talassemia. Al monitoraggio del diabete a 5 mesi è stato eseguita parallelamente al dosaggio della HbA1c anche una seconda elettroforesi delle emoglobine, che confermava il valore della HbA2 ma non la variante "borderline". Il laboratorio ha quindi indagato il periodo del ricovero venendo a conoscenza di una trasfusione con una sacca concentrata di emazie. Pertanto la variante "borderline" era attribuibile ad una frazione originata da un donatore con una variante emoglobinica non rilevante ma che ha fornito un dato ingannevole. La mancanza di informazioni o l'omissione di dati importanti come una trasfusione recente inducono a redigere referti non conclusivi e a ricorrere ad esami di secondo livello evitabili. Inoltre, questo caso clinico pone l'attenzione sull'importanza di eseguire uno screening emoglobinico sui donatori al momento dell'arruolamento.

EP298

Schistociti in anemia e trombocitopenia di primo riscontro non sempre espressione di Porpora Trombotica Trombocitopenica: il ruolo del percorso diagnostico di laboratorio.

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Introduzione

La Porpora Trombotica Trombocitopenica (PTT) rappresenta una emergenza clinica nella quale la tempestività dell'inquadramento ha importanti ricadute sul percorso diagnostico-terapeutico. Il primo riscontro di anemia e piastrinopenia, in assenza di allarmi strumentali morfologici, deve essere accompagnato da approfondimento microscopico per la ricerca di schistociti e test specifici per evidenziare una possibile patologia emolitica in atto. Si descrive un caso clinico di sospetta PTT in base ai valori dell'emocromo ed il percorso interno al laboratorio per confermare/escludere il sospetto clinico.

Caso Clinico

Paziente maschio di 67 anni, affetto da neoplasia gastrica in fase avanzata, giunge in pronto soccorso per astenia generalizzata, inappetenza e calo ponderale. L'emocromo evidenzia anemia e piastrinopenia non note precedentemente (Hb 7,4 g/dL, PLT 11000/ mmc). L'osservazione microscopica rileva la presenza di schistociti (6% dei globuli rossi). Come da protocollo interno vengono eseguiti approfondimenti ematochimici con i seguenti risultati: reticolocitosi, aumento della bilirubina totale (2,35 mg/dL) a prevalenza indiretta, aumento di LDH (2035 U/L), aptoglobina consumata (4 mg/dL), funzionalità renale e coagulazione di base nella norma. A seguito di questi dati il laboratorio contatta il reparto per concordare l'esecuzione del test ADAMTS13 in regime d'urgenza: l'esame mostra un'attività enzimatica nella norma (63%), escludendo quindi la diagnosi di PTT.

Discussione

Il riscontro di anemia emolitica e severa piastrinopenia non note, con schistociti all'esame microscopico, pur orientando fortemente per una condizione di emolisi microangiopatica, non è di per sé diagnostico di PTT. La determinazione in urgenza della attività di ADAMTS13, in accordo con il clinico, può essere di supporto nella conferma di PTT o nella sua esclusione, come nel caso presentato, più correttamente inquadrabile all'interno di una microangiopatia secondaria a neoplasia. Nell'era della medicina di precisione, un percorso diagnostico impostato in laboratorio e il confronto con il clinico sono essenziali per una appropriata gestione clinico-terapeutica del paziente.

EP299

The phantom cryoprotein: a case study of hypersoluble cryoglobulin

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Cryoglobulins are abnormal immunoglobulins that precipitate at temperatures below 37°C and dissolve upon warming. They are primarily composed of mono- or polyclonal IgG, IgM, or IgA and tend to clump in small blood vessels, causing vasculitis with symptoms like purpura, arthralgia, and asthenia. Cryoglobulins are associated with autoimmune, viral, or lymphoproliferative diseases.Here we describe an 83-year-old male, undergoing dialysis, with history of non-Hodgkin lymphoma, HCV-related cryoglobulinemia, and chronic renal failure due to membranoproliferative glomerulonephritis, presented with a rapidly progressing lesion on his right forefoot. Hospital tests excluded HCV reactivation but showed consumed C3 and C4, elevated rheumatoid factor (2475 IU/ml), hypo-IgG and IgA, and reduced CD19+ B lymphocytes and T3 and T4 lymphocytes. CT scan did not show lymphoma recurrence. Laboratory tests indicated cryoglobulinemia with a 9% cryocrit.Interestingly, the typing of cryoglobulin was impossible due to a particular hypersolubility. The cryocrit dissolved when washed with cold PBS or saline solution. Immunofixation attempts were unsuccessful due to the presence of albumin, preventing differentiation between cryoglobulin and monoclonal serum components. However, a type 2 cryoglobulin (IgM K monoclonal vs polyclonal IgG) was hypothesized, consistent with the patient's clinical status. Literature indicates rare cases of hypersoluble cryoglobulins due to alterations in ionic forces, increasing cryocrit solubility [1].We provided clinicians with all possible information but could not report a definitive result. The report noted: "Typing of cryocrit undeterminable due to possible analytical interferents." Subsequent assessments suggested the clinical picture was compatible with autoimmune-linked cryoglobulinemia. Unfortunately, persistent infection necessitated the forefoot amputation, nevertheless, in cryoglobulinemia cases, amputation should be avoided as it can cause necrosis to spread beyond the amputated area. This case underscores the difficulty in characterizing cryoglobulins and suggests using multiple approaches for better identification. It also highlights the need for close collaboration between clinicians and laboratories for proper cryoglobulin management.

[1] Andre M, et al. A "missed" cryoglobulin: the importance of in vitro calcium concentration. Ann Rheum Dis 2000;59:490–496.

EP300

A case of incongruent Bone turnover markers levels

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Introduction

Cross-linking telopeptide of type I collagen (CTX) is a bone turnover marker (BTM) released into the bloodstream during bone remodelling. Elevated serum concentrations of CTX have been reported in patients with a potential imbalance within bone resorption and formation. We described a case of a 54-year-old woman with unexpected BTM pattern.

Methods

Bone-specific alkaline phosphatase (BAP), N-terminal propeptide of type I collagen (P1NP) and CTX were determined on IDS-iSYS analyser (Immunodiagnostic Systems, Tyne and Wear, UK). CTX concentration was also measured on Cobas e801 analytical platform (Roche Diagnostics International, Rotkreuz, Switzerland).

Results

The patient, diagnosed with breast cancer, after total mastectomy, adjuvant chemo- and radiotherapy, started aromatase inhibitor therapy (letrozole). DEXA scan, performed at the beginning of letrozole treatment, indicated mild osteoporosis (femur -2.5 T-score).

A panel including both blood and 24-hours urine chemistry tests (Ca, P, Mg, creatinine), PTH, 25OH vitamin D, and BTM (BAP, CTX, P1NP) was then analysed; all the results were in range, except for IDS-iSYS CTX, which was 9-fold greater (9.350 ng/ml) than the post-menopausal reference cut-off (<1.037 ng/ml). So Denosumab and Cholecalciferol therapy was started.

A 1-year control DEXA scan revealed no sign of osteoporosis, so the patient was retested for BTM, showing the same pattern, with normal P1NP and BAP, and CTX concentration of 5.330 ng/ml. Having excluded both biotin supplementation and other preanalytical and analytical errors, suspecting an assay interference on CTX, its concentration was determined on Cobas e801, resulting in 0.050 ng/ml (cut-off <1.008 ng/ml). The discrepancy between CTX concentrations measured with the two immunoassays was confirmed in a sample collected 9 months later under the same preanalytical conditions.

Conclusions

We reported a rare case of suspected falsely-high CTX levels due to an assay interference detected by using an alternative analytical method, with a different assay design, as first investigative step.

Immunoassay interferences are a critical topic in laboratory medicine; the collaboration between laboratory professionals and clinicians is of crucial importance to ensure the correct patient management.

EP301

Analytical interference in procalcitonin assay: a case report

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Background

Procalcitonin (PCT) is a precursor of the hormone calcitonin (CT). The concentration of PCT in healthy individuals generally remains below the detection limit, as it has a half-life of 24 hours and is quickly converted to CT. In pathological conditions (bacterial infections and/or sepsis), PCT has an extrathyroid origin and is produced by organs of the immune system. CT is produced by the parafollicular C cells of the thyroid, in high quantities in medullary thyroid cancer (MTC). We report the case of a 90-year-old patient admitted to Baggiovara Hospital for orthopedic surgery. Despite being in clinical remission, the patient had a consistent and significant serum PCT concentration, which was not in line with the clinician's expectations.

Methods

The PCT was measured on patient's serum sample using the CLIA method on Liaison XL (DiaSorin). The PCT kit uses two monoclonal antibodies, specific for the molecule under examination and declares no cross-reactivity for CT values up to 60 ng/ml. The sample has been treated to investigate the presence of possible analytical interference: tubes for heterophilic antibodies, scalar dilutions and precipitation with PEG. CT measurement was performed by CLIA method on Liaison XL (DiaSorin).

Results

The PCT concentration was 70 ng/ml and remained unchanged in the following days. After treatment with the tubes for heterophilic antibodies, scalar dilutions and precipitation with PEG, the PCT value remained unchanged. CT, measured on the same sample, was 2080 ng/ml.

Conclusion

Detection of elevated CT levels allowed the patient to be presented to the endocrinological board for the most appropriate clinical pathway. High concentrations of PCT not consistent with the clinic, emphasize the need to exclude possible interference from high levels of CT in the presence of MTC.

EP302
THERATYPING OF CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is caused by defects of the CFTR gene. CFTR-modulating drugs may overcome specific defects, as the case of Kaftrio, that proved strong ability to rescue the function of the most frequent F508del pathogenic variant, even in compound heterozygous genotypes. However, most rare genotypes lacking F508del allele are not eligible for targeted therapies. We setup the nasal conditionally reprogrammed cells (CRC)-derived in vitro models for theratyping: the characterization of CFTR variants and the evaluation of drug response to obtain hints for personalized CF therapy. We generated and validated cells derived from patients carrying different genotypes. The efficacy of treatments was evaluated by functional tests. The CFTR response to Kaftrio was confirmed in F508del homozygous cells. Low- or non-responding genotypes include alleles with the N1303K pathogenic variant and stop codons, as the W1282X. Drug response was obtained in the rare L1077P pathogenic variant. Basal CFTR protein levels were very low while mature CFTR increased following Kaftrio exposure in L1077P-bearing genotypes. Forskolin-induced organoid swelling and Ussing Chamber assays congruently proved L1077P variant function rescue by Kaftrio. Notably, this rescue takes place even in the context of single-copy L1077P allele. Corresponding assays in W1282X/W1282X genotype demonstrated the non-efficacy of Kaftrio, as a consequence of heavily compromised CFTR protein expression. The effect seems to be due to the mRNA degradation, as demonstrated by rescue after combined treatment with Kaftrio and a non-sense mediated decay inhibitor (the SMG-1 inhibitor). Genotypes carrying the N1303K pathogenic variant responded to Kaftrio at different extent, depending on the patient-specific CFTR protein/mRNA expression. In line with what observed for F508del-bearing genotypes, our findings could open the way to Kaftrio therapy for L1077P-bearing patients. The possibility of single-allele treatment arises also for rare genotypes. However, interindividual variability in the CFTR expression should be considered in the planning of targeted therapies. The theratyping of CFTR pathogenic genotypes may allow the clinical translation of effective therapeutic strategies.

EP303
Case report: Pregnant woman affected by thrombotic thrombocytopenic purpura (TTP). From the clinical case to Vast Area's PDTA

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy caused by severe deficiency of ADAMTS13, a plasma metalloprotease required for the cleavage of von Willebrand factor (VWF) multimers. This is a hematological emergency as failure to recognize it could have serious consequences up to a fatal outcome if not promptly diagnosed and treated. The drafting of a PDTA (Diagnostic, Therapeutic and Assistance Path) with the definition of a diagnostic work-flow between level I and II hospitals, especially in a vast territory, can really be incisive for the survival of patients. The Vast Area of Southern Tuscany made up of the Le Scotte University Hospital and the hospitals of the South East Tuscany USL has drawn up a document to design a Diagnostic and Therapeutic Path to ensure the care of patients suffering from TTP. We report the clinical case of a patient suffering from TTP identified in the Vast Area of Southern Tuscany.
Case presentation: A 37-year-old pregnant woman arrives at the Emergency Department of the Misericordia Hospital in Grosseto in January 2024. Her CBC (Complete blood count) shows anemia with hemoglobin 75 g/L, red blood cells 2.21 x 10^12/L, hematocrit 0.216 L/L, mean corpuscular volume 97.7 fL, mean corpuscular hemoglobin 33.9 pg, mean corpuscular hemoglobin concentration 347 g/L, red blood cell distribution width 17.5%, increase in erythroblasts 2.5% and thrombocytopenia with platelets 2 x 10^ 9/L. Biochemical tests show a reduction in haptoglobin, marked increase in LDH, hematuria and normal creatinine. Coagulation tests show normal PT and aPTT and fibrinogen 163 mg/dL. On the peripheral blood smear, the differential counting of leukocytes was normal and the presence of 5% schistocytes was detected. This clinical and laboratory picture was strongly suggestive of a diagnosis of TTP, confirmed the following day by the dosage of ADAMTS-13 activity (0.4%) and by detection of autoantibodies (IgG) against ADAMTS13 (53 U/ml).
Conclusion: The drafting of this PDTA, the one of the few example in Italy for this rare pathology, guarantees fairness of care and improves the survival of these patients thanks to timely diagnosis and treatment throughout the territory.

EP304
RARO CASO DI LEUCOCITOSI IN ETA' PEDIATRICA

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Giunge all'osservazione del nostro Pronto Soccorso Pediatrico una bambina di 6 anni in buone condizioni generali all'esame obiettivo inviata dal Pediatra Curante per riscontro di leucocitosi, trombocitosi ed anemia in seguito ad accertamenti per scarso accrescimento staturo-ponderale. Il sospetto diagnostico formulato sul territorio era di Leucemia Linfoblastica Acuta. L'esame emocromocitometrico, da noi eseguito con analizzatore BC6800 Plus (Mindray, Shenzhen, Cina), presentava: GB 332.930/mm3; Hb 9,0 g/dl; PLT 766.000 /mm3. Inoltre sono state eseguiti un EGA venoso, un profilo biochimico con funzionalità renale, epatica ed elettroliti nella norma, comprendente hsPCR (0,21 mg7dl) e LDH (753 mU/ml) ed un profilo coagulativo con DD (569 ug/L). Sono stati indagati anche le sierologie virali con esito negativo. La lettura strumentale indicava uno scatter non caratteristico di una patologia linfoproliferativa, ma sospetto per una patologia mieloproliferativa cronica. Si evidenziava la presenza di: granulociti a vari stadi maturativi, comprendenti neutrofili segmentati maturi e mielociti (279.340/mm3), linfociti (18.310/mm3), eosinofili a vari stadi maturativi (18.640/mm) e basofilia (8.320/mm3). Lo striscio di sangue periferico confermava la presenza di granulociti a diverso stadio maturativo, con una bassa percentuale di nuclei immaturi. Come completamento diagnostico è stata eseguita una biopsia osteomidollare caratterizzata da una scarsa serie eritropoietica ed una prevalente quota granulopoietica con modesto numero di cellule CD34+ (1-2%). La ricerca molecolare del prodotto di fusione BCR/ABL su sangue periferico è risultato positivo. Per questi motivi è stata posta diagnosi di Leucemia Mieloide Cronica (LMC); si tratta di una neoplasia rara. Su base annua ha una incidenza pari a 1 caso su un milione nei bambini di età inferiore ai 14 anni. Il caso presente rafforza l'importanza di uno screening attento di tutti gli esami emocromocitometrici ad opera di patologi clinici esperti e formati sia nell' interpretazione dei dati strumentali che della osservazione microscopica dello striscio di sangue periferico; non ultima, la importanza di una rete di collaborazione con il clinico per l' alert e l' approfondimento dei casi.

EP305
A rare case of a loiasis clinically manifested 9 years after last epidemiological exposure.

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Abstract: A 37-year-old Cameroonian patient, residing in Italy for the past nine years, showed up to the Emergency Department with acute hyperemia of the conjunctival tissue, tearing, itching, headache at the right hemisphere and transient edema at ankles and wrists. Upon referral to the Ophthalmology Department, the clinical examination of the right eye revealed the presence, under the conjunctival tissue, of a foreign body suspected to be a parasite. However, primary identification of the worm was hindered due to partial damage during surgical removal. Considering the literature suggesting Loa loa as the most common parasite migrating in subconjunctival tissue, the patient history and the clinical manifestations, the Laboratory Team suspected blood infection due to microfilariae species. Therefore we recommended parasitological research in peripheral blood collected in K2-EDTA at 12 am. The complete blood count revealed no evidence of peripheral eosinophilia or qualitative and quantitative abnormalities (total eosinophil count: 490/μL, relative eosinophil percentage: 7%; total white blood count: 7.1 x 10^9/L). Anyway, Microfilariae of Loa Loa were identified in May Grunwald-Giemsa (MGG) staining and the load was assessed by calculating the number of microfilariae/mL using a measured quantity of blood (3,7μL; count of 270 microfilariae/mL). Identification was based on morphological features, patient country of origin and periodicity of life-cycle of the parasite.
As our centre is a first-level analysis laboratory not specialised in parasitic diseases, the patient was referred to the Centre for Infectious and Tropical Diseases of University Hospital of Padua (AOPD) for appropriate diagnostic tests and treatment with the drug of choice, diethylcarbamazine (DEC).
According to literature, spread of Loiasis outside the African continent is considered highly improbable due to the absence of the vector Chrysops carrying the nematode. Therefore, the finding in our patient was unexpected, as he had not returned to Cameroon for nine years and he had remained asymptomatic throughout this period until 1 year ago, when he started to show the typical symptoms of the infection. So, this case report appears to record the longest case of loiasis.

EP306

Postzygotic Mutations in Epidermal Rare Diseases

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Background/Aim: Palmoplantar Keratoderma (PPK) is a rare skin disease characterized by several mutations in genes crucial for epidermal differentiation. The presentation is extremely rare when limited to the palms, soles, or sagittal regions, posing significant diagnostic challenges. Here, we molecularly analyzed a patient with unilateral PPK, resembling a verrucous nevus localized solely to the left palm and sole. The 32-year-old male presented with persistent, verrucous lesions on the left palm and sole, which had arisen since early childhood. The lesion was asymptomatic but caused cosmetic concern. The patient reported no similar lesions elsewhere on the body and no family history of similar conditions.Methods and Results: Clinical examination revealed a well-demarcated, hyperkeratotic, verrucous plaque on the left palm and sole. Histopathological analysis revealed marked hyperkeratosis, papillomatosis, and acanthosis of specific areas with no epidermolysis. Immunofluorescence analysis indicated a normal epidermal condition, with areas of hyperproliferation extending below the basal lamina. Consequently, we performed next-generation sequencing (NGS) on DNA extracted from the skin biopsy and from peripheral blood lymphocytes (PBL). The analysis identified multiple palmoplantar mutations. By subtracting the mutations common to those in PBLs, we hypothesized that the disease is due to a postzygotic mutation (mosaic presentation). The mutated genes include PRAMEF1, PRAMEF4, MST1, FRG2C, MUC6, HYDIN, MUC16 and ABCA7. No signs of malignancy were observed.
Discussion: This condition can be classified as a type of unilateral palmoplantar verrucous nevus. It requires careful clinical and histopathological evaluation to differentiate from other conditions, such as verrucous carcinoma or genetic mosaic forms of an epidermal nevus. The identification of postzygotic mutations in PRAMEF1, PRAMEF4, MST1, FRG2C, MUC6, HYDIN, MUC16, and ABCA7 has not been previously documented in the literature and should be confirmed by Sanger sequencing and biochemical function analysis. Molecular biology testing could play a crucial role in diagnosing and in defining the pathobiology of the disease.

EP307

Next generation sequencing allows to detect an HNF4a gene duplication in a diabetic patient: the importance of copy number variations (CNVs) in the diagnosis of maturity-onset diabetes of the young (MODY)

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MODY results from heterozygous alterations in genes acting in development and function of pancreatic beta cells. Due to the clinical heterogeneity, patients may be misdiagnosed resulting in inappropriate treatment and delays in screening of affected family members. We report a case of an obese male patient (BMI 29.6kg/m2) with an autoimmune diabetes diagnosis (IA2 autoantibodies positive) treated with insulin therapy. He was born at term, appropriate for gestational age, normoglycemic, with a family history of diabetes. During the follow up, he reported a poor metabolic glycaemic control (HbA1c 10.2% - 88 mmol/mol), absent fasting level of C-peptide, hypercholesterolemia, medial-intimal thickening of the carotid arteries and mild autonomic neuropathy despite the subsequent disappearance of autoimmune diabetes markers. Next Generation Sequencing (NGS) for MODY genes revealed no nucleotide variants compatible with the patient's phenotype, but computational pipeline for detection of copy number variations (CNVs) suggested a novel heterozygous duplication of exons 2-11 in the HNF4A gene, confirmed by MLPA. To better characterize this result, a CGH array was performed, showing a 20q13.12 duplication involving several genes, including HNF4A. The same duplication was identified in patient's son (aged 6) presenting neurological deficit. This is the first reported case of duplication involving HNF4A, in the literature only overexpression of the HNF4A has been reported in in vivo mouse models in association with hyperglycemia, alteration of cholesterol and fatty acid metabolism, characteristics also present in our patient. Although a large duplication would be expected to have deleterious effects, only future functional studies will be able to confirm alterations of in vivo HNF4A activity. This case highlights that CNVs, although currently underrecognized, may be an important genetic cause of MODY. Routinely incorporating their analysis into targeted NGS results could improve the accuracy of the diagnostic test for MODY and could also help establish the true prevalence of CNVs as the cause of the disease. Our result would allow endocrinologists to formulate a more appropriate therapy for the patient and to monitor his child who may develop diabetes during adolescence.

EP308

Consensus DNA Profiling of Formalin-Fixed, Paraffin-Embedded Tissues (FFPE): From Genetic Puzzle to a Clear Picture

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The report describes an intricate investigation performed on FFPE specimens for forensic purposes. The judicial authority asked to verify whether the death of a stillborn foetus was due to an acute or chronic placental disease. Post-mortem foetal organs and placenta were stored in formalin, embedded in paraffin and sectioned to prepare slides for histopathological examination. However, it was necessary a genetic verification to ensure the identity of the paraffin blocks as originating from the foetus' organs and from the mother's placenta. Hence, an oral swab was collected from the mother (M) to obtain her reference genetic profile (MP). The investigation involved 26 paraffin blocks from which we cut three sections for DNA extraction. Each sample underwent two rounds of amplification. Capillary electrophoresis revealed partial DNA profiles, due to degradation of the genetic material. Therefore, we used the 'consensus' profile approach based on replications. Using this approach, we created a genetic profile by combining and analysing multiple partial DNA profiles from different sources. The sample of the organs, for which a single block was available, was divided into separate aliquots and a consensus profile was generated from the replicates that included alleles that appeared in two or more of the replicates. Instead, for organs with multiple blocks, we created a unified consensus profile by integrating the various partial profiles obtained from each block. The consensus placenta profile (CPP) and the consensus umbilical cord/chorionic membrane profile (CPU) were mixture profiles. The Likelihood Ratio (LR) approach was used to verify the relationship between the CPP and MP and between CPP and the CPU. Then, the analysis - performed with LRMix software - confirmed that the DNA mixture belonged to M and her foetus rather than two unknown individuals, while Familias 3 software confirmed the biological relationship between M and her foetus. In this case, the consensus profiling approach allowed us to obtain a reliable genetic identification, overcoming the challenges of DNA degradation and to assess the mother-foetus relationship.

EP309

Combined array Comparative Genomic Hybridization and Whole Exome Sequencing analysis improves Schizophrenia diagnosis: a case report

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Schizophrenia (SCZ) is a heterogeneous polygenic psychiatric disorder characterized by positive symptoms, negative symptoms and cognitive impairment. It is known that the etiology of SCZ involves a very large number of rare variants, including single nucleotide variants (SNVs) and copy number variants (CNVs). To determine the genetic basis of SCZ, a combined approach of CNV identification and exome sequencing was used. We report the case of a male patient diagnosed with SCZ. Molecular analyses were performed using both array Comparative Genomic Hybridization (aCGH) and Whole Exome Sequencing (aCGH) on the patient's genomic DNA. By WES analysis, we found a missense mutation (c.2362C>T, p.Pro788Ser) within the RELN gene, which encodes a critical glycoprotein of the extracellular matrix. RELN plays an important role in neurological development by regulating neuronal migration, dendritic structure, and neurotransmission. The mature subunit of N-methyl-D-aspartate receptors (NMDARs) in the postnatal brain requires RELN to maintain its functions, supporting the hypothesis that synaptic dysfunction associated with NMDAR signalling plays a role in the pathophysiology of SCZ. Indeed, abnormalities associated with RELN have been found in patients with SCZ, with a decrease in the expression of RELN observed in several brain regions; this is also confirmed by studies in heterozygous mutant RELN mice. These results suggest that psychiatric disorders may be significantly affected by RELN. The aCGH analysis instead revealed the presence of a homozygous deletion of 129.6 Kb on chromosome 7q31.1 involving the IMMP2L gene, which encodes a mitochondrial enzyme crucial for glycolysis. Knockdown of IMMP2L in primary astrocytes leads to dysregulation of genes involved in brain development. CNVs that disrupt IMMP2L have been found in psychiatric disorders, including SCZ. Studies indicate that patients with CNV deletions tend to have a lower full-length form of IMMP2L than healthy individuals without CNV deletions, suggesting a possible link between IMMP2L deletions and SCZ. The emerging synergy between aCGH and WES enhances the identification of new variants potentially associated with this disorder improving the understanding of the molecular basis of these complex diseases.

EP310
Presunto caso di pseudoiperkaliemia familiare

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Sig.ra di 65 aa esegue prelievo presso Centro Prelievi per controllo. Fra gli esami richiesti il potassio (K) risulta pari a 9,9 mEq/L, valore confermato anche su emogasanalizzarore (9,9 mEq/L). Il campione è stato processato dopo circa 3h dal prelievo, nessuna anomalia preanalitica.La sig.ra, inviata al Pronto Soccorso (PS), esegue un emogas con K=3,9 mEq/L.Vista la discordanza con il risultato del laboratorio si esegue un secondo prelievo che viene inviato al Laboratorio ed analizzato entro 30 min: K=4,0 mEq/L.Analizzando lo storico della Sig.ra si rileva che in altre circostanze aveva ottenuto risultati molto elevati di K in campioni prelevati da esterno e che ripetuti in PS hanno dato valori normali.Il laboratorio decide pertanto di eseguire delle prove di stabilità del campione.Si è raccolto un nuovo campione consegnato in pochi minuti al laboratorio, è stato subito suddiviso in 3 aliquote di sangue intero e ciascuna poi è stata centrifugata ed analizzata in momenti diversi.1a aliquota: centrifugata ed analizzata entro 30 min dal prelievo, K = 4.5 mEq/L.2a aliquota: centrifugata ed analizzata dopo 2 ore, K = 6.0 mEq/L.3a aliquota: centrifugata ed analizzata dopo ulteriori 3 ore K = 10.0 mEq/L.Si è pertanto evidenziato un anomalo rilascio di K tempo dipendente, aumento che si evince solo in vitro e che non rispecchia il valore reale di K nella paziente.Si è invitata la Sig.ra a concordare con il Laboratorio la modalità di accesso per i successivi prelievi con richiesta di K in modo da garantire l'esecuzione dell'analisi entro 1 h.Si è anche suggerito di eseguire appofondimenti genetici per verificare se trattasi di pseudoiperkaliemia familiare (FP). La FP è un sottotipo ereditario, lieve e non emolitico di stomatocitosi ereditaria, associato ad un'anomalia correlata alla temperatura nella permeabilità al potassio della membrana dei globuli rossi, che comporta livelli elevati di potassio in vitro per la perdita del potassio nel plasma da parte degli gli eritrociti.La FP non si associa ad altri difetti ematici e nella maggior parte dei pazienti è asintomatica, tuttavia, la diagnosi corretta è importante per evitare inutili accessi al PS e/o terapie inadeguate qualora si sospetti una iperkaliemia vera.

EP311
Serum S 100 B: a marker of brain damage in traumatic brain injury

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INTRODUCTION:
Cranial computed tomography (CT) is the gold standard for ruling out traumatic brain injury (TBI).
Different studies suggest that S-100B (S-100B) protein levels could be useful in the clinical management

of TBI, allowing for the avoidance of having to perform a CT in those patients who test negative for S-100B, allowing for less use of CT, which would mean better clinical and economic results.

The objective of our work is to analyze the usefulness of the neuromarker S-100B as a screening test for mild TBI.
MATERIAL AND METHODS:
This is a prospective observational study with 45 patients, over a period of 3 months (from October to December 2023), with mild head trauma of different etiology and a Glasgow score of 13-15, treated in the emergency department. of our hospital center. After the clinical evaluation, determination of S-100B protein and cranial CT were performed. The S-100B protein analysis was performed in cobas pro (ROCHE®) and the value 0.1 μg/L was taken as a positive cut-off point. The relationship between clinical findings, CT results and S-100B levels was evaluated.
RESULTS:
45 patients were included in the study: Craneal CT was positive in 7 (16%) patients. All patients with positive CT had positive levels of S-100B protein. Overall, S-100B had a specificity of 50.0% and a sensitivity of 100.0%, with a positive predictive value (PPV) of 27.0% and a negative predictive value (NPV) of 100.0%, in relation to imaging tests performed on patients with mild TBI.
In our study, 19 patients had S100B levels < 0.1 μg/L (42.2%).
CONCLUSIONS:
The high negative predictive value of neuroprotein S-100B (NPV 100.0%) allows CT not to be performed in patients with levels < 0.1 μg/L.
In our study, 42.2% of the CT scans that were performed would not have had to be performed.
The use of S100B could reduce the number of CT scans performed, patient waiting time in the emergency department of our hospital, and associated hospital costs in patients with mild traumatic brain injury.

EP312

Liaison MeMed BV: un nuovo approccio per una più rapida diagnosi tra infezioni virali e batteriche in una coorte di pazienti pediatrici

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Una delle sfide mediche più interessanti è distinguere tra infezioni virali e batteriche, che spesso sono clinicamente indistinguibili, causando un frequente abuso di antibiotici con gravi conseguenze per la salute globale. Obiettivo dello studio è stato valutare l'efficacia del test Liaison MeMed BV nel differenziare il tipo di infezione in una coorte di pazienti pediatrici, confrontandolo con la Procalcitonina (PCT), di solito usata come supporto diagnostico. Il test misura 3 proteine dell'ospite (TRAIL, IP-10, CRP) e genera uno score numerico da 0 a 100: valori bassi (<35) indicano infezione virale (V) e valori alti (>65) infezione batterica (B), con una grey zone tra 35 e 65. Lo studio ha incluso 70 campioni (42 M, 60.0% e 28 F, 40.0%, età media: 3.46 anni, range 6–12 anni), raccolti da Maggio 2023 a Gennaio 2024 presso il Pronto Soccorso Pediatrico dell'AORN "Sant'Anna e San Sebastiano" di Caserta. I criteri di inclusione erano febbre e altri disturbi correlati a infezione. Sono stati esclusi 19 campioni per dati incompleti o risultati in grey zone. I 51 campioni sono stati valutati classificando quelli PCT≥0.50 ng/mL come B (19/51, 37.2%) e quelli PCT<0.50 ng/mL come V (32/51, 62.7%). I campioni sono stati poi testati con MeMed: 11/19 (57.9%) con PCT≥0.50 sono stati identificati come B e 8/19 (42.1%) come V, con un valore predittivo positivo (PPV) di 0.58; tra i 32 con PCT<0.50, 26/32 (81.3%) sono stati confermati come V mentre 6/32 (18.7%) sono stati classificati come B, con un PPV di 0.81. È stata correlata l'ipotesi diagnostica con il punteggio MeMed: il 66.7% (34/51) ha mostrato concordanza mentre nel 15.7% (8/51) dei casi si è riscontrata una discrepanza tra l'ipotesi clinica batterica e il risultato virale di MeMed, successivamente confermata dalla diagnosi molecolare. Infine, il 17.6% (9/51) ha mostrato una discrepanza tra l'ipotesi clinica virale e il risultato batterico di MeMed, confermata da esami microbiologici. In conclusione, il nostro studio dimostra che, soprattutto in Pronto Soccorso, il test MeMed può essere un utile strumento per la diagnosi predittiva, in particolare per le infezioni virali. Ciò può aiutare nella scelta terapeutica, evitando la inappropriata somministrazione di antibiotici e l’insorgenza di antibiotico resistenza.

EP313

DISTRIBUZIONE DI HLA-DQ7 IN UNA POPOLAZIONE DEL NORD MILANO CON SOSPETTA MALATTIA CELIACA

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Introduzione:
La celiachia (CD) è una malattia multifattoriale caratterizzata da lesioni della mucosa del piccolo intestino e malassorbimento dei nutrienti in pazienti suscettibili in risposta all'ingestione di glutine nella dieta. Gli eterodimeri HLA DQ2 e DQ8 sono riconosciuti come fattori genetici predisponenti allo sviluppo della celiachia; altri aplotipi DQ sono stati osservati in pazienti con sintomi simili a CD, ma non sempre con patologia confermata.
Scopo:
Lo scopo di questo studio è quello di illustrare la distribuzione dell'HLA-DQ all'interno di un campione di pazienti appartenenti all'area nord di Milano con sospetto di malattia celiaca.
Materiali e Metodi:
L'analisi è stata eseguita su 318 campioni di sangue di pazienti con sospetta malattia Celiaca, inviati al nostro laboratorio per indagini molecolari, nel periodo 2019-2023. Il DNA è stato estratto dai leucociti del sangue periferico dei campioni biologici utilizzando l’estrattore di DNA automatico “MagNA Pure” (Roche). Il genotipo HLA è stato eseguito utilizzando il kit "XeliGen RT" (Eurospital) su strumento "7900 ABI PRISM" (Applied Biosystems) basato su Real-Time PCR per determinare la presenza di alleli codificanti gli eterodimeri DQ2, DQ8 ed altri alleli.
Risultati:
Dei 318 campioni analizzati, 180 campioni (56,6%) sono risultati positivi agli aplotipi DQ2, DQ8. In particolare DQ2.5/DQ2.5 e DQ2.5/DQ2.2 (19 campioni pari al 5,9%), DQ2/DQ8 (17 campioni pari al 5,3%), DQ2/DQX (66 campioni pari al 20,7%), DQ8/DQX (13 campioni pari al 4,1%), DQ2 o DQ8/DQ7 (65 campioni pari al 20,4%).Dei 138 campioni (43,4%) negative per DQ2, DQ8, ben 88 campioni (27,7%) sono risultati con aplotipo DQ7. In particolare DQ7/DQ7 (33 campioni pari al 10,4%) e DQ7/DQX (55 campioni pari al 17,3%).
Conclusioni:
I nostri dati mostrano che l’aplotipo DQ7 viene rilevato in un'alta percentuale di soggetti che mostrano sintomi simili alla malattia Celiaca. Inoltre, DQ7 è spesso presente in aggiunta agli aplotipi a rischio per CD (DQ2 o DQ8). Il ruolo di DQ7 come rischio di CD aggiuntivo o indipendente dovrebbe essere studiato e/o confermato con un campione di popolazione più ampio e da un'analisi di correlazione con i dati sierologici dei soggetti studiati.

EP314

The Importance of Antibody Titration and Plasma Exchange in AB0 Incompatible Kidney Transplants

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Introduction

Monitoring antibody titers is essential in AB0 incompatible (AB0i) kidney transplants. These transplants, previously deemed unfeasible, represent a vital solution for patients with limited chances of finding a compatible donor. AB0 incompatibility involves preformed antibodies in the recipient against the A or B antigens on the donor kidney, which can cause hyperacute rejection, destroying the transplanted kidney within minutes of surgery.Plasma exchange (PEX) mechanically removes antibodies from the recipient's blood by separating plasma, which contains the antibodies, from red blood cells. The plasma is then replaced with albumin solutions or fresh frozen plasma, reducing the concentration of anti-A or anti-B antibodies. This procedure is often combined with immunosuppressive drugs, such as rituximab and intravenous immunoglobulin (IVIG), to prevent the production of new antibodies and modulate the immune response.Successful desensitization using PEX depends on continuous monitoring of antibody titers. Significant reductions in titers indicate that antibody levels have been lowered to a safe point for transplantation. Typically, plasmapheresis is performed multiple times, with sessions spread over several days or weeks, until antibody titers reach acceptable levels.

Materials and Methods

The ISO titer in serum samples from transplant candidates was measured for IgG and IgM using a microcolumn technique with the Erytra Grifols system and the Coombs anti-IgG Biorad gel test system. PEX for desensitization was performed with Hemonetics MCS+ separators. Procedures were conducted one month before transplantation with immunosuppressant infusion, and two PEX procedures were performed the week before the transplant.

Results and Conclusions

The titer was monitored throughout the pre- and post-transplant observation periods. The definitive disappearance of ISO A1 after treatment was assessed. The role of Transfusion Medicine in antibody titration and PEX remains fundamental in making AB0 incompatible kidney transplants possible, significantly improving the prospects for patients who would otherwise have limited treatment options.

EP315

Haptoglobin: is its diagnostic value interfered by potential confounding factor? A real-life retrospective study

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Haemolysis occurs in many diseases and may lead to anemia. Haemolysis is detected by some biochemical signs: anaemia, reticulocytosis, elevation of unconjugated bilirubin and lactate dehydrogenase (LDH), and haptoglobin (Hpt) depletion, until undetectable concentrations, i.e. <0.3 g/L, in the presence of large amounts of free haemoglobin. In a retrospective analysis on patients classified according to WHO criteria for anaemia severity (severe Hb≤80 g/L and moderate 80<Hb≤100 g/L), Hpt was compared with LDH as biomarker of haemolysis, reticulocytes (RET) as marker of erythropoietic activity to evaluate their performances and the interference by acute phase defined by C Reactive Protein (CRP) >10 mg/L. The intravascular haemolysis was confirmed by examining the medical records of enrolled patients. In patients with severe anaemia (n=31), intravascular haemolysis was diagnosed in 5 patients. Sensitivity (SE) and negative predictive value (NPV) =100%, confirmed the efficacy in excluding intravascular haemolysis, even in presence of acute phase. However, Hpt was less effective in confirming haemolysis showing a 92% specificity (SP) and a positive predictive value (PPV)=71%. In moderate cohort (n=51), 9 patients were diagnosed with intravascular haemolysis and Hpt showed SE=33%, SP=91%, NPV=86%, PPV=43%. Hpt is an effective marker for severe but not for moderate anaemia, particularly in presence of acute phase (3/3 patients with CRP>10 mg/L are false negative). We additionally compared Hpt with RET% and LDH, which should not be affected by acute phase. The diagnostic performance of markers was in severe anaemia: NPV=100%, PPV=18% for RET%, NPV=100%, PPV=50% for LDH and in moderate anaemia: NPV=87%, PPV=25% for RET%, NPV=87%, PPV=21% for LDH. Except for LDH, in subjects with moderate anaemia, the comparison between the results of the haemolysis markers in patients with and without haemolytic anaemia show a statistically significant difference. This study has some limitations, but it can lay the foundations for further investigations. Haemolytic anemia is a difficult condition to diagnose; the Hpt, despite is a recommended marker to confirm the suspicion, remains affect by confounding factors.

EP316

Establishment of normal reference values for serum neurofilament light based on apparently healthy subjects aged 18–90 years.

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Objective: Increased serum neurofilament light (sNfL) concentrations were reported in neuro-degenerative and neuro-inflammatory disorders, but there is a strong relationship between age and sNFL. We evaluate the potential interferences of age and renal deficiency on sNFL using a fully automated immunoassay analyzer (LUMIPULSE® G series, Fujirebio) with the Lumipulse® G NFL blood assay.Material & Methods: We selected 304 presumed healthy subjects (158 women, 146 men) from 18 to 90 years old, categorized by age decades and >70 years. sNFL levels showing a modified z-score >7.0 by age decade were considered as true far outliers and removed from analyses (n=5). Multiple linear regression model was used to assess the effect of age, renal function, and gender on sNFL levels, or log-transformed levels. The upper limit was defined by the 95th percentile of the distribution in each age subgroup.Results: Age-dependency of sNFL was not linear showing an exponential increase of NFL values in elderly (>70y). According to the analysis of covariance, age is the most significant influential factor ($p<0.0001$), followed by eGFR ($p=0.003$). Gender has no significant influence. Patients with renal deficiency (eGFR <60 mL/min/1.73m2) gave higher mean sNfL (40±25 pg/mL) with heterogenous levels [6 (min.) – 91 (max.) pg/mL]. Excluding these patients with kidney disease (n=17), the correlation of NFL level with eGFR is mainly linked to the age increase. The eGFR dichotomization >90 or <60-90> mL/min/1.73m2 didn't bring significant information. The normal upper limits for pNFL were 9.4 [7.7-11.3], 9.6 [9.3-13.6], 13.3 [11.2-16.5], 21.2 [16.8-33.0], 28.1 [24.7-48.9], 46.8 [40.0-49.0] pg/mL for individuals below 30, 40, 50, 60, 70 and 90 year old respectively.Discussion: Reference intervals have traditionally been partitioned by age decade. The recruitment of a sufficient number of healthy subjects for a gaussian distribution by age ranges is difficult, time-consuming, and costly. Therefore, we used pragmatic statistical approach based on the tracking of outliers and exclusion of subjects with potential renal dysfunction. Physiological relevance of age-subgroups must be discussed. We didn't observe a significant increase of sNFL before age of 40y old reflecting a probable absence of sub-neuroaxonal pathology before middle age.

EP317

How to measure LDL cholesterol in a population of paediatric patients with hypertriglyceridemia?

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Background

Between 2021 and 2023, a total of 8229 children, 50.3% male, with a median age of 12 (IQR 8-15), were analysed for lipid profile at our laboratory. The analysis included total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations carried out by enzymatic colorimetric methods performed with AU5800 Beckman Coulter analyser. As reported by literature data, discrepancies can be present between LDL-C directly measured (D-LDL-C) and calculated values (C-LDL-C), which have a different magnitude depending on the formula used and become higher for patients with hypertriglyceridemia.

Methods

We compared D-LDL-C measurement of 133 out of 8229 patients (1.6%) with TG >300 mg/dL (median of 430 mg/dL, IQR 332-573) to LDL-C calculated (C-LDL-C) by three different formulae: Friedewald formula (F), Martin/Hopkins equation (MH), and Sampson-National Institutes of Health equation 2 (SNIH2). Concordance between D-LDL-C and C-LDL-C was calculated by performing Passing-Bablok regression (PB) and Bland-Altman analysis (BA).

Results

PB showed a proportional and constant systematic error for all comparisons between D-LDL-C and C-LDL-C. BA showed a bias of 71.3% for D-LDL-C vs F, 16.7% for D-LDL-C vs MH, and 35.3% for D-LDL-C vs SNIH2. Moreover, we noticed that in five patients with TG>400 mg/dL (median 674, IQR: 474-809) F gave a negative number while MH and SNIH2 gave a positive number that was very different from the measured value. The average percentage differences between the measured values and calculated for these patients were 499% for D-LDL-C vs F, 294% for D-LDL-C vs SNIH2, and -63% for D-LDL-C vs MH.

Conclusion

Our results demonstrate how the use of formulae for calculating LDL-C by clinicians should be discouraged, especially in paediatric patients with TG values >400 mg/dL. Anyway, when direct dosing is not available, MH seems to give the best performance.

EP318

Neuroprotein S-100B in mild traumatic brain injury.

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INTRODUCTION: Cranial computed tomography (CT) is the gold standard for ruling out traumatic brain injury (TBI). Different studies suggest that S-100B (S-100B) protein levels could be useful in the clinical management of TBI, allowing for the avoidance of having to perform a CT in those patients who test negative for S-100B, allowing for less use of CT, which would mean better clinical and economic results.

The objective of our work is to analyze the usefulness of the neuromarker S-100B as a screening test for mild TBI.

MATERIAL AND METHODS:

This is a prospective observational study with 45 patients, over a period of 3 months (from October to December 2023), with mild head trauma of different etiology and a Glasgow score of 13-15, treated in the emergency department. of our hospital center. After the clinical evaluation, determination of S-100B protein and cranial CT were performed. The S-100B protein analysis was performed in cobas pro (ROCHE®) and the value 0.1 µg/L was taken as a positive cut-off point. The relationship between clinical findings, CT results and S-100B levels was evaluated.

RESULTS:

45 patients were included in the study: Craneal CT was positive in 7 (16%) patients. All patients with positive CT had positive levels of S-100B protein. Overall, S-100B had a specificity of 50.0% and a sensitivity of 100.0%, with a positive predictive value (PPV) of 27.0% and a negative predictive value (NPV) of 100.0%, in relation to imaging tests performed on patients with mild TBI.

In our study, 19 patients had S100B levels < 0.1 µg/L (42.2%).

CONCLUSIONS:

The high negative predictive value of neuroprotein S-100B (NPV 100.0%) allows CT not to be performed in patients with levels < 0.1 µg/L.

In our study, 42.2% of the CT scans that were performed would not have had to be performed.

The use of S100B could reduce the number of CT scans performed, patient waiting time in the emergency department of our hospital, and associated hospital costs in patients with mild traumatic brain injury.

EP319

EVALUATION OF BIOCHEMICAL AND HORMONAL PARAMETERS IN PROFESSIONAL BASKETBALL ATHLETES DURING PRE- AND POST-TRAINING SESSION

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Background: It is known that regular physical activity performed properly has a positive impact on an individual's physical and mental health. However, prolonged and intense physical activity can cause metabolic adaptations that result in alterations of specific biomarkers. This condition can trigger an inflammatory status that can lead to muscular injuries. To this aim we analysed the alteration of a panel's biochemical and hormonal parameters in elite basketball athletes. Methods: We recruited a team of 12 elite basketball players and collected a venous blood sample before and after intense training. We evaluated the serum concentration of some biochemical and hormonal parameters such as: Creatin Kinase (CK), Lactate Dehydrogenase (LDH), Reactive Protein C (CRP), Aspartate Aminotransferase (AST), Total Cholesterol, Vitamin D, Cortisol, Testosterone. Results: Our results showed a post-training significant increase in CK (POST 417.0 (IQR 356.5-499.0) vs 171.0 (IQR 147.0-255.0) PRE, p<0.006), LDH (POST 248.0 (IQR 220.0-277.0) vs 210.0 (IQR 189.5-248.5) PRE, p=0.050) and PCR (POST 1.3 (IQR 0.6-2.8) vs 0.5 (IQR 0.3-2.0) PRE, p<0.006) underlining the presence of an inflammatory status and muscular suffering. There is also an increase in post-training serum levels of AST (POST 36.5 (IQR 33.5-40.0) vs 25.0 (IQR 22.5-27.0) PRE, p<0,001) which may be due to liver stress. The evaluation of the lipid profile showed a significant decrease in serum concentration of total cholesterol (PRE 209.0 (IQR 171.5-229.0) vs 165.5 (IQR 145.5-194.0 POST, p<0.007) after intense exercise compared to before highlighting a reduction of cardiovascular risk. Finally, serum concentration of cortisol (PRE 17.2 (IQR 14.8-20.2) vs 9.3 (IQR 7.6-13.7 POST, p<0.040) decreased significantly post-training, suggesting a possible overtraining. Conclusions: Our study showed that strenuous and intense training can increase an inflammatory status and muscular suffering caused by overtraining, but at the same time it can improve lipid absorption reducing cardiovascular risk. For this reason, the role of laboratory medicine in monitoring and safeguarding the athlete's health is crucial to avoid injuries and optimise athletic performance.

EP320

APPROPRIATEZZA PRESCRITTIVA NELLO SCREENING DELLE EMOGLOBINE PATOLOGICHE

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Premessa: gli esami di I livello per le emoglobinopatie hanno il compito di fornire informazioni utili a prevenire patologie determinate geneticamente che, in omozigosi, si esprimono con livelli variabili di anemia e condizioni cliniche caratteristiche; in eterozigosi, tali difetti sono in genere clinicamente asintomatici. Questi esami prevedono un approccio biochimico e nella maggior parte dei casi non richiedono ulteriori indagini sul DNA.
Metodi: lo screening delle emoglobine patologiche è stato eseguito con sistema automatico (Hemoglobin(e) Capillarys 3 TERA, SEBIA, Francia) su campioni di sangue intero mediante separazione elettroforetica capillare in fase libera, con valutazione quali-quantitativa della HbA2, della HbF, della HbA e dell'eventuale presenza di varianti emoglobiniche.
Risultati: dall'01/01/2020 al 31/12/2023 presso l'AST-Ascoli Piceno sono state eseguite 1548 elettroforesi dell'emoglobina su una coorte di pazienti costituita da 352 maschi (22,7%) e 1196 femmine (77,3%) di età compresa tra 0 e 92 anni (media 24 ± DS 32,5), di cui utenti esterni 1399 (90,4%) e interni 149 (9,6%).
Del totale dei campioni:
- 1493 (96,5%) risultavano compatibili con un quadro di normalità
- 55 (3,5%) presentavano anomalie riconducibili ad un'emoglobinopatia
- 13 (23,6% dei positivi) avevano disponibili valori di emocromo e bilancio marziale: a 10 veniva consigliato l'accesso ad una diagnostica di II livello, mentre 3 non necessitavano di ulteriori approfondimenti diagnostici
- 42 (76,4% dei positivi) non consentivano di produrre un referto conclusivo per mancata richiesta di parametri emocromocitometrici e assetto marziale.

Conclusioni: la conclusione diagnostica può essere definitiva o presuntiva; in quest'ultimo caso il laboratorio di I livello può demandare il compito conclusivo al laboratorio specialistico di II livello. Nella nostra casistica, soltanto il 23,6% aveva un quadro elettroforetico suggestivo per emoglobinopatia, mentre nella maggior parte dei casi la prescrizione risultava poco pertinente poiché non associata ad esami correlati e necessari. Al fine di ottimizzare le risorse elaborando, qualora possibile, referti conclusivi senza dover ricorrere ad indagini molecolari e/o genetici, si rende necessaria una migliore appropriatezza della richiesta.

EP321

Comparison of Conventional Semen Analysis and Automated LensHooke X1 Pro: Assessment of Agreement and Differences

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Introduction: In andrology in particular, the spermiogram analysis is considered the most significant study to evaluate the male reproductive capacity. The assessment of male fertility heavily relies on detailed sperm analyses, including parameters such as cell counting, motility, morphology, and vitality. Traditionally, these assessments are performed manually, creating a spermiogram, which is both time-consuming and resource-intensive. An adequate assessment of sperm quality requires meticulous inspection by highly trained specialists, which is time-consuming and prone to significant inter- and intra-specialist variability. To address the need for more efficient methods, automated systems have been developed and are available on the market as LensHooke X1 that is a Semen Quality Analyzer that offers more parameters from CASA assessment to further evaluate the sperm quality and motility. This study aims to compare conventional sperm analysis, performed according to WHO 5th guidelines, with semen analysis conducted using the LensHooke X1 machine.
Methods: We evaluated a total of 21 seminal fluid samples obtained from the Medically Assisted Procreation Center. The samples were collected following standard procedures and stored under controlled conditions to maintain their integrity. The samples were analyzed both manually (conventional method) and with the automated sperm analyzer.
Results: The two methods were compared in terms of concentration, motility and morphology, there was a statistically correlation between the two method's measurements in all variables ($p < 0.001$). The best correlation was obtained for sperm concentration.
Conclusions: The automated method for semen analysis offers significant advantages over manual methods, including increased speed, standardization, and the ability to maintain comprehensive patient histories. These benefits make automated analysis a valuable tool in the field of medically assisted procreation. However, it is important to recognize that improvements are still needed, particularly in morphological analysis and certain analytical setting, to further enhance the accuracy and reliability of automated semen analysis.

EP322

IMPATTO DEI POLIMORFISMI FUNZIONALI DEL TRASPORTATORE DELLA SEROTONINA SULLA DIPENDENZA DA ALCOL

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Il trasportatore della serotonina (5-HTT), codificato dal gene SLC6A4, gioca un ruolo fondamentale nella trasmissione serotoninergica ed è stato associato alla dipendenza da alcol. La regolazione trascrizionale e l'espressione del 5-HTT dipendono anche da variazioni di sequenza che interessano le regioni introniche ed esoniche e le regioni trascritte e non tradotte al 5' e al 3'. Tra le più studiate, figurano la variante polimorfica 5-HTTLPR e il polimorfismo rs25531. Il primo, è una ripetizione variabile che risulta, nella maggior parte dei casi, in una inserzione/delezione di 43 paia di basi. Le varianti long (L) e short (S) del polimorfismo 5-HTTLPR, modulano l'attività trascrizionale del promotore del gene SLC6A4. L'allele (L) è associato a livelli maggiori di 5-HTT mRNA. La variazione rs25531 produce un cambio di base A/G, in grado anch'esso di modulare l'espressione del trasportatore della serotonina. L'allele G riduce l'efficienza dell'allele (L) rendendolo simile all'allele (S). Queste due regioni vengono descritte insieme come polimorfismo triallelico (La altamente esprimente, gli alleli Lg ed S a bassa espressione). Questo lavoro indaga il ruolo delle variazioni di sequenza del 5-HTT in soggetti alcol-dipendenti. Abbiamo valutato le frequenze alleliche e genotipiche di 15 SNP e 2 VNTR, già noti in letteratura per influenzare l'espressione e/o la funzione del 5-HTT, confrontando una popolazione di 1447 soggetti alcol-dipendenti con un gruppo di controllo di 441 individui astemi. Abbiamo inoltre eseguito un'analisi degli aplotipi. Abbiamo trovato una differenza, statisticamente significativa, nelle frequenze alleliche (p=0,0083) e genotipiche (p=0,0151) del polimorfismo triallelico, con gli alleli e i genotipi a funzione più elevata maggiormente rappresentati nella popolazione di controllo. Inoltre, abbiamo individuato tre aplotipi (ATGCCCCCCTCCACA1612; ATTCCCCCCTCCACA1610;
GTGCCCCCCTCCACA1412) più frequenti nei soggetti con AD (p<0,0001) e uno (GTGCCCCTCTCCACA1412), più frequente nella popolazione di controllo (p<0,0001). I risultati ottenuti per il polimorfismo triallelico nella dipendenza da alcol confermano quanto già presente in parte della letteratura. Il ruolo degli aplotipi richiede invece ulteriori studi per essere chiarito.

EP323

Concentrazioni di potassio, albumina e ammonio nell'umor vitreo per la determinazione dell'intervallo post-mortem

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La stima accurata dell'intervallo post-mortem (PMI) è una componente cruciale nelle indagini forensi e, tra le matrici maggiormente oggetto di studio, vi è l'umor vitreo (VH), in quanto fluido corporeo ben compartimentalizzato e meno soggetto ai processi di putrefazione. In particolare, è noto da decadi l'incremento della concentrazione di potassio nel VH, proporzionalmente all'aumentare del PMI. Successivi studi hanno valutato le cinetiche relative alle concentrazioni di altri biomarcatori, al fine di migliorare i modelli predittivi.In particolare, gli Autori hanno pubblicato un'equazione per calcolare il PMI utilizzando le concentrazioni di potassio e albumina, determinati nel VH di un campione di cadaveri con PMI noto, compreso tra 20 e 200 ore.Lo studio è attualmente oggetto di ulteriori approfondimenti e, al precedente pannello analitico, è stato aggiunto l'ammonio, al fine di migliorare la capacità predittiva dell'equazione formulata.Campioni di umor vitreo sono stati raccolti da 58 soggetti deceduti, con un PMI noto, compreso fra 36 e 192 ore. Le concentrazioni di potassio, albumina e ammonio sono state misurate utilizzando analizzatori automatizzati. I dati raccolti sono stati analizzati mediante un modello di regressione lineare multipla per valutare l'influenza combinata di questi biomarcatori sul PMI.I risultati indicano che le concentrazioni di questi analiti nell'umor vitreo presentano una correlazione significativa con il PMI (R^2 = 0.60, $p < 0.001$, st.dev = ±18 ore). Il modello, tuttavia, non è ancora stato sottoposto a testing.La regressione lineare multipla sulle concentrazioni di potassio, albumina e ammonio nell'umor vitreo offre una stima promettente e accurata del PMI. Questi risultati possono migliorare le pratiche forensi, fornendo un metodo affidabile per la determinazione del PMI in casi in cui la tempistica fosse di difficile determinazione. Ulteriori ricerche sono tuttavia necessarie per validare questi risultati in contesti differenti e con campioni più ampi.

EP324

Ruolo di biomarcatori e popolazioni leucocitarie nella diagnosi e nella prognosi dello shock settico

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Lo shock settico rappresenta una delle principali cause di mortalità nelle terapie intensive, caratterizzato da una risposta infiammatoria sistemica e da una disfunzione multiorgano. La diagnosi tempestiva e la valutazione accurata della gravità sono fondamentali per migliorare gli esiti clinici. Recenti studi hanno evidenziato l'importanza di biomarcatori specifici, come l'interleuchina-6 (IL-6), la proadrenomedullina (proADM) e la procalcitonina (PCT), nel monitoraggio e nella prognosi dei pazienti con shock settico.Inoltre, nello shock settico si osserva l'attivazione di differenti popolazioni leucocitarie, ciascuna con un ruolo specifico nella risposta immunitaria.Scopo del lavoro è quello di valutare la capacità di parametri ematochimici di prevedere il deterioramento del quadro clinico del paziente, anticipando eventuali variazioni delle funzioni d'organo. Scopo secondario è, inoltre, quello di arricchire le conoscenze relative ai marcatori biochimici rispetto ai parametri ematologici e formulare ipotesi sui possibili meccanismi coinvolti negli sviluppi della condizione clinica del paziente.La presente ricerca analizza le concentrazioni sieriche quotidiane di questi analiti e i dati relativi al quadro ematologico in pazienti ricoverati presso la terapia intensiva del polo neuromotorio dell'AOU-Careggi per un periodo di 4 settimane, fino a dimissione (exitus/passaggio a regime di minore intensità).Analisi preliminari hanno evidenziato che la ProADM anticipa di 24 ore la diagnosi di shock settico rispetto alla PCT, probabilmente legato a un deterioramento del danno d'organo del paziente; ulteriori analisi volte a capire il ruolo dell'IL-6 sono ancora in corso.Inoltre, fra le varie correlazioni con i parametri ematologici, particolarmente interessante risulta quella tra proADM ed il numero dei monociti, proADM e numero di granulociti immaturi, PCT ed il rapporto tra neutrofili/monociti e IL-6 e parametro di fluorescenza dei monociti (con relativa dispersione).Ulteriori analisi sono in corso, per poter delineare non solo l'interazione tra questi biomarcatori e le differenti popolazioni leucocitarie attivate nello shock settico, ma anche l'utilità dei parametri ematochimici nella gestione del paziente critico.

EP325

Evaluation of cardiovascular risk in patients affected by Wilson Disease

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Background: Wilson's disease is an autosomal recessive disease caused by homozygous or compound heterozygous mutations in the ATP7B gene, which encodes a transmembrane ATPase for copper transport. The defective function of ATP7B leads to copper overload in hepatocytes, with related liver symptoms. Excess copper is also responsible for secondary pathological accumulation in other tissues, particularly in the brain, which can cause neurological symptoms and psychiatric disorders. In the liver, copper overload causes mitochondrial damage, alteration of lipid metabolism and the development of steatosis, but the mechanisms underlying these changes are not yet fully understood.Methods: To evaluate the cardiovascular risk in Wilson patients, we recruited 20 patients, 10 pediatric and 10 adults, and we evaluated lipid profile (Total Cholesterol, LDL-cholesterol, HDL-Cholesterol and triglycerides) and specific biomarkers related to cardiovascular risk such as Lipoprotein(a) [Lp(a)], leptin and PCSK9 by ELISA assay.Results: Our results showed an increase in serum levels of Total-Cholesterol and LDL-Cholesterol in adults if compared to children (p-value<0.001); whereas HDL and triglycerides showed no statistically variations. Finally, PCSK9 levels are significantly increased in adults (p-value<0.01), but no changes have been observed in leptin and Lp(a) serum levels.Conclusions: The results of this study showed that the accumulation of copper in adults' patients could be responsible of alterations in lipid metabolism, exposing them to cardiovascular risk. Further studies are needed, but the continuous monitoring in children and adults affected by chronic disease such as Wilson Disease could represent a valuable tool to safeguard the health of these patients, protecting them from cardiovascular risks.

EP326

Parathyroid hormone-related protein (PTHrP), N-terminal type I collagen extension pro-peptide (P1NP) and tartrate-resistant acid phosphatase (TRAcP) assayed salivary matrix during tooth movement

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Orthodontic treatment is based on the application of a system of mechanical forces of tension and pressure on the teeth which lead to the controlled movement of the teeth. The evaluation of the concentrations of bone turnover markers (BTMs) on the salivary matrix represents a good indicator of the biological phenomena that occur during tooth movement. Parathyroid hormone-related protein (PTHrP) with a regulatory role on odontoblastic cells, N-terminal type I collagen extension pro-peptide (PINP), marker of bone formation and tartrate-resistant acid phosphatase (TRAcP) marker of bone remodelling have been evaluated in saliva of patients undergoing orthodontic treatment. These BTMs can be measured on salivary matrix because their concentrations are higher than the limit of detection (LoD) of the method used. Methods: The study was conducted at the Dental Clinic of Foggia in collaboration with the Clinical Pathology Unit of the Policlinico-Bari. 45 patients with class I dental and skeletal relationships, aged 15 to 18 years with upper and lower crowding rated between 2.1 and 4.0 mm. Salivary samples were collected 3 times: before bonding of the orthodontic appliance (T1), 25 (T2) and 45 (T3) days after the start of treatment. The collection of salivary samples was performed with the passive salivation method using Salivette® with cotton buds (Sarstedt). PTHrP assay was measured with the competitive enzyme immunoassay (ELISA) (LoD 0.033 ng/ml linear range 0.033–6000 ng/ml, Analytical Coefficient of Variation (CVA) 9%). PINP and TRAcP were performed with chemiluminescence assays using DSX ® TGSTA Dynex Technogenetics Instrumentation TRAcP isoform 5b (LoD 0.9 U/L, linear range of 0.9-14.0 U/ L, CVA 4.5%) and PINP (LoD 2 ng/mL, linear range of 2-230 ng/mL, CVA 5.2%).Results: PTHrP (ng/ml) concentration were: T1-0.65, T2-1.24, T3-1.61; P1NP concentrations (µg/l) were: T1-2.10, T2-2.90, T3-5.20; TRAcP concentrations (U/L) were: T1-1.50, T2-3.29, T3-3.90. MannWhitney U test, used to compare the salivary biomarkers in the group of subjects stratified by sampling period, confirmed a statistically significant difference in T1 compared to T2 and T3.Conclusion: Preliminary data highlighted that PTHrP, TRAcP and PINP show variations in concentrations. They can provide informations on the progress of orthodontic treatment even in the absence of evident clinical signs and could be used in monitoring biomechanical therapy.

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Telomere Length and Global DNA Methylation as Predictors of Pregnancy Outcome: Insights into Reproductive Medicine

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Spontaneous pregnancy loss is a severe pathological condition influenced by several inherited and acquired factors such as genetics, environment, and stress conditions that can affect embryo implantation and development. Epigenetics, by modifying gene transcription, genomic stability, and telomere length, may also have a key role. In addition, pregnancy maintenance is also regulated by a controlled inflammation. We analysed a cohort of 179 women who underwent to pregnancy interruption within the third gestational month (spontaneous EPL, n=92 and volunteer VPI, n=87). LINE-1 methylation level (%), as surrogate of global DNA methylation, and telomere length (T/S), as biological age assessment, were performed after bisulfite conversion plus pyrosequencing, and real-time qPCR respectively. Cytokines levels (IL-6, IL-10, IL-17A, IL-23) were quantified by Luminex technology and MILLIPLEX-Analyst Software.Telomere length is a valid indicator of biological age and tends to shorten with aging, we therefore compared the T/S values in the two subgroups. EPL had telomere length significantly shorter than the VPI subgroup (EPL 323.4 ± 134.72 vs VPI 425.8 ± 268.24; P=0.0014). Following correlation analysis, we found that T/S values were similar considering slopes comparison (Pslope=0.8958) and Y-axis intercept significantly differed (r2EPL=0.0001587 vs r2VPI =0.0005965; Pintercept=0.0019). Moreover, methylation assessment ascribed to EPL subgroup a significant lower mean value (EPL 82.04 ± 3.63 vs VPI 85.28 ± 3.44; P<0.0001). To test whether telomere length was somehow dependent on global DNA methylation, we correlated T/S with LINE-1 and found that the variation in telomere length based on global DNA methylation in the two subgroups had similar slopes (Pslope=0.6782) and significantly different Y-axis intercept (r2EPL=0.004759 vs r2VPI =0.001896; Pintercept<0.0001). Finally, higher mean cytokines levels were found in the EPS subgroup (P<0.0001). Our results suggest that shorter telomeres, hypomethylation and higher inflammation status, as well as their mutual relationships, may be considered as predisposing factors for miscarriage serving in turn as possible predictive prognostic biomarkers.

EP328

VALUTAZIONE DELL'INFEZIONE DA STRONGYLOIDES STERCORALIS DERIVATA DA DONATORE: DATI PRELIMINARI DEL LABORATORIO TRAPIANTI DI PISA

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Introduzione. L'infezione da Strongyloides Stercoralis è per lo più asintomatica negli ospiti immunocompetenti, nonostante l'infezione cronica. Al contrario, gli ospiti immunocompromessi, come i riceventi di trapianto di organi solidi, sono a rischio di sindrome da iperinfezione e/o malattia disseminata che, spesso, ha esito fatale. Sulla base di studi epidemiologici che hanno evidenziato tassi di sieropositività nel nostro Paese dell'8% nella popolazione italiana e del 17% nella popolazione straniera, da gennaio 2024 il Centro Nazionale Trapianti (CNT) ha raccomandato di effettuare, a carico della sede donativa ed entro 72h, a tutti i donatori multiorgano la sierologia (anticorpi IgG) per Strongyloides Stercoralis il cui risultato non è necessario ai fini dell'allocazione ma può consentire un trattamento farmacologico precoce dei riceventi. Questo studio riporta i dati preliminari della frequenza di questo agente infettivo nei donatori di organi la cui valutazione sieroinfettivologica è stata effettuata presso il Laboratorio Trapianti. Inoltre, poiché in letteratura sono pubblicati casi di co-infezione da Strongyloides, Citomegalovirus (CMV) E Toxoplasmosi (TOXO) in pazienti immunodepressi, abbiamo valutato una possibile correlazione fra queste infezioni.Materiali e Metodi. Da gennaio a maggio 2024 sono stati valutati 34 donatori multiorgano (17M/17F; 68±18 anni; 25.3±4.5 BMI). La determinazione quantitativa degli anticorpi IgG contro lo Strongyloides nel siero è stata effettuata mediante metodica ELISA (dati espressi come ratio), gli anticorpi IgG anti-CMV e anti-TOXO sono stati determinati con metodica ELFA (Enzyme-Linked Fluorescent Assay).Risultati. Alla data di invio dell'abstract nel nostro laboratorio non sono stati rilevati casi di positività allo Strongyloides fra i donatori multiorgano afferiti alla nostra struttura. Gli anticorpi IgG per Strongyloides sono risultati valutabili in tutti i donatori, con un valore mediano di index pari a 0.27 D.O. Nelle donne (0.37±0.13 D.O.) il valore per gli anticorpi anti-Strongyloides era significativamente più elevato rispetto agli uomini (0.26±0.15 D.O.) (p = 0.03, test-t di Student). Nei 33 donatori valutati non sono state evidenziate correlazioni fra Strongyloides, CMV e TOXO.Conclusione.Allo stato attuale, questi dati preliminari non confermano la percentuale di positività allo Strongyloides osservati nella popolazione italiana. Allo stesso tempo, gli studi di correlazione dovranno essere rivalutati aumentando la casistica, al fine di confermare o meno quanto riportato in letteratura.

EP329

Hyaluronan in the pathogenesis of lung fibrosis associated to autoimmune Pulmonary Alveolar Proteinosis (aPAP).

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Rationale.Pulmonary fibrosis is a rare but well-recognized event which may occurs in a minority of patients with aPAP. The accumulation of intra-alveolar material may alter local cytokine expression, promoting proinflammatory and profibrotic pathways however much remains to be known regarding the pathogenesis of this complication.Aim.We aimed to investigate serum biomarkers able to serve as prognostic factor for the development of lung fibrosis in aPAP patients (pts).Methods.We perfomed the ELF™ test (Siemens), a multivariate index assay providing a score of fibrosis based on quantitative measurements of hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1) from serum of 17 aPAP pts, collected at diagnosis.Results.We compared 7 aPAP pts who show lung fibrosis evidence on chest HRTC (PAPFIB) with 10 pts (PAP) who did not developed lung fibrosis within at least 9 years from aPAP diagnosis. We found that both PAPFIB and PAP show a positive association with the risk of fibrosis (mean ELF score 9.3+0.71 vs 8.5+0.48 respectively, positive score>7.7), with a significant increase in PAPFIB group (p=0.016). In particular, mean HA levels were found to be significantly elevated in PAPFIB compared to PAP (58.42+47.3 vs 22.53 +9.57 ng/mL, p=0.032, positive range> 34.6+ 8.8).Conclusion.Although within a limited sample size, our preliminary results seem to indicate that a robust HA production in the lungs and peripheral tissues contributes to the chronic inflammatory and micro injury to the alveolar epithelium and describe the utility of HA as a prognostic biomarker of fibrosis associated to aPAP.

EP330

SALIVARY ALPHA-AMYLASE: A RAPID MARKER OF RESPONSE TO STRESS WITH A TYPICAL CIRCADIAN PATTERN

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Background and aim. More recently, some authors have demonstrated the important role of neuroendocrine markers detectable in saliva in establishing bodily reactions to psychosocial, physical, and biological stress stimuli. Whereas salivary cortisol is a reliable measure for evaluating hypothalamic-pituitary-adrenocortical (HPA) axis activity, salivary alpha amylase (sAA) has been suggested as a marker for sympathetic adrenomedullary (SAM) systems activity. sAA may be a preferable marker of stress to cortisol due to limitations related to cortisol measurement, such as diurnal and seasonal rhythms or drugs interferences. To date, knowledge about factors influencing sAA levels is limited. The aim of this study was to assess whether sAA could be useful to evaluate the stress response, also verifying sources of variability.Methods. To identify any sources of variability, saliva samples were collected from six healthy subjects at four different times (8:00, 10:00, 12:00 and 16:00) over five consecutive days using two different collection devices, Salivette (SARSTEDT AG & Co, Nümbrecht, Germany) and Esaliva (ESAMED, Vigonza, Italy). Saliva samples were then collected from 35 obstetric residents before and after a simulated shoulder dystocia scenario, one of the most traumatic birth events in the professional life of gynecologists. Saliva samples from residents were also collected in a "non-simulation day". sAA levels were measured with two different assays (AMYL2 and Salimetrics).Results. Overall, sAA levels increased significantly from morning to afternoon. Levels varied between subjects, but no differences were found between days or sampling devices. sAA activity levels in samples from 35 residents were significantly higher than those obtained before the scenario took place (about ten minutes later). Any difference was measured in "non-simulation day" (at the same collection time). These results were confirmed by two different assays.Conclusions. Salivary alpha-amylase activity is a reliable, quick and efficient marker for stress response, then its measurement will be of potential interest in the field of stress-related disorders. However, it is important to consider the timing of sample collection before introducing sAA in a clinical setting.

EP331

SuPAR: a chronic inflammatory marker useful for predicting disease progression in myelofibrosis.

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Introduction: Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by a chronic inflammatory state that plays a relevant role in the disease pathogenesis (as proven by high levels of inflammatory cytokines with prognostic significance and by a persistent oxidative stress) and by extensive neoangiogenesis in bone marrow (BM) and spleen. SuPAR is the soluble form of urokinase Plasminogen Activator Receptor (uPAR). SuPAR has been recently reported as a promising biomarker for systemic chronic inflammation, since it is a protein minimally affected by acute changes and short-term influences, in contrast to many currently used markers of systemic inflammation (e.g., CRP, IL-6, and TNF-α). Aim: We investigated suPAR as serum infiammatory biomarker and as new biological marker predicting clinical evolution and patients survival associated with PMF. We enrolled 29 PMF patients and 20 controls (CTRLs). Methods: We performed the suPARnostic® TurbiLatex test (ViroGates), a quantitative diagnostic assay in vitro used to determine the soluble urokinase Plasminogen Activator Receptor (suPAR) level in human K2-EDTA and Lithium Heparin plasma samples on Siemens Advia Xpt (Munich, Germany). Mann Whitney test was used to evaluate differences in suPAR between the two groups. Results: We found that PMF patients had significantly higher (p=0.01) levels of suPAR (median 4.3 ng/ml ; range 2.7-8.8) than CTRLs (median 3.1 ng/ml; range 2.1-4.4). Conclusions: Routinely used prognostic scoring models are currently based on clinical symptoms, laboratoristic parameters, and genetic and molecular markers. This has allowed medical professionals to define risk categories that have become progressively more precise. The identification of new biomarkers of diseases predicting clinical progression and patients survival in a more refined prognostic models will offer the possibility to improve our capacity of patients stratification, resulting in more efficient therapeutic approaches. Further larger studies are necessary to confirm and validate these favorable results.

EP332

Il contenuto degli acidi grassi nelle membrane dei globuli rossi correla positivamente con i livelli di tranferrina desialata (CDT) nel siero di pazienti con sindrome metabolica

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Il dosaggio della CDT (Carbohydrate-deficient transferrin) o transferrina desialata nel siero è uno dei biomarcatori sierici per rilevare il consumo cronico di alcol. La CDT può anche essere utilizzata per identificare la steatosi epatica non alcolica (NAFLD). La NAFLD è considerata una manifestazione epatica della sindrome metabolica, caratterizzata da alterazioni patologiche nel metabolismo dei lipidi e dei carboidrati. Diversi studi hanno dimostrato l'associazione tra il diverso grado di NAFLD e la variazione degli acidi grassi saturi (SFA) nelle membrane degli eritrociti. La composizione degli acidi grassi di membrana è analizzata attraverso la lipidomica, tecnica che permette di studiare sulle membrane dei globuli rossi lo stato nutrizionale e lo stile di vita alimentare di un soggetto.L'obiettivo dello studio è quello di valutare la correlazione tra CDT sierica e variazione dei profili lipidomici di membrana in soggetti con NAFLD.64 pazienti (50 Maschi/14 Femmine) con NAFLD sono stati sottoposti a prelievo venoso. Un campione di sangue periferico, prelevato in provette con gel di silice, è stato impiegato per il dosaggio della CDT sierica con metodo CDT-IFCC (International Federation of Clinical Chemistry) utilizzando lo strumento CAPILLARYS 3 OCTA (Sebia), un altro campione dello stesso paziente, prelevato in provette di EDTA, è stato processato in un estrattore automatizzato di acidi grassi, in grado di selezionare i globuli rossi maturi con metodo di Folch. I fosfolipidi ottenuti sono stati transesterificati e iniettati in un gas-cromatografo con un detector FID. I profili lipidici ottenuti sono stati analizzati e quantificati utilizzando una miscela di 37 standard e il software Cromequest.In questo studio confermiamo che l'aumento dei SFA nelle membrane dei globuli rossi è associato al grado di NAFLD. Inoltre, suddividendo i soggetti in base ai range di CDT, sono stati trovati valori più alti di SFA nel gruppo con CDT patologico (>2.0) rispetto ai gruppi con CDT normale (≤1.7) e inconcludente (>1.7, ≤2.0). In conclusione i livelli di CDT nel siero sono correlati positivamente al contenuto dei SFA nella membrana dei globuli rossi in pazienti con NAFLD.

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EP333

Influence of physical activity on cardiovascular risk in childhood obesity

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Background: Childhood obesity (CO) is a problem of considerable social importance worldwide and in Italy it affects 1/4 children. CO is the result of different causes, more or less evident, which interact with each other. Main causes are an inadequate diet, linked to a reduced physical activity and a genetic predisposition. During CO, there is an increase in adipose tissue that occurs with weight-gain, and consequently, persistent inflammatory state is created. The release and the increase of inflammatory markers affect insulin sensitivity, glucose metabolism and atherosclerosis, eventually leading to a higher cardiovascular risk. Methods: To evaluate the impact of physical exercise on cardiovascular risk, we evaluated lipid profile and specific biomarkers related to cardiovascular risk such as Lipoprotein(a) [Lp(a)], adiponectin and PCSK9 by ELISA assay. Moreover, molecular screening tests were performed to evaluate three putative mutations (S127R (c.381T>A, p.Ser127Arg); F216L (c.646T>C, p.Phe216Leu) and D374Y (c.1120G>T, p.Asp374Tyr)) in PCSK9 known to be associated with cardiovascular disease (CVD) risk. For this study, we recruited two population: 45 sedentary obese children (OSe), age=11±3.3, weight=70±23.3 kg, height=1±0.27 m, BMI=3 1±6.9 and 31 obese children who practice sports (OSp), age=10±2.5, weight=61±17.3 kg, height=1±0.13 m, BMI=28±4.4. Results: Our results showed a decrease in serum level of Total- Cholesterol, LDL- Cholesterol and Triglycerides in OSp population in comparison to OSe; on the other hand, HDL-Cholesterol levels in OSp group are increased respect to OSe. Moreover, Lp(a), adiponectin and PCSK9 levels are significantly reduced in OSp if compared to OSe. Finally, no one of the analyzed individuals was found to carry the genetic variants thus suggesting that other molecular mechanisms are likely to underlie PCSK9's expression variation. Conclusions: Physical activity can be considered a non-pharmacological therapy useful for the reduction of cardiovascular risk in obesity and related diseases. Introduce sport as integral part of anti-obesity strategies in children and adolescents could be a valuable tool to safeguard the health of obese children, protecting them from obesity-related risks.

EP334

CIRCULATING SERUM MICRO-RNA AS NON-INVASIVE DIAGNOSTIC BIOMARKERS OF ENDOMETRIOSIS

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Background: Endometriosis (END) is a debilitating gynecological disorder characterized by the presence of endometrial-like cells in ectopic places outside the uterine cavity. At present, only laparoscopy can provide a final diagnosis of END and the discovery of non-invasive biomarkers in liquid biopsy is warranted to anticipate it. MicroRNAs (miRNAs), a family of small non-coding RNAs involved in the modulation of gene expression, could be helpful non-invasive biomarkers of END.
The purpose of this study was to perform a serum miRNA profile in a cohort of patients with and without END to identify a set of miRNAs related to this pathology.

Methods: This study was performed on 67 patients with END and 60 women with other benign gynecological diseases (control group). Total RNA was isolated from 400 ul of serum and the expression profile of a panel of 754 miRNAs was performed with RT-qPCR carried out on a QuantStudio 12K Flex (Applied Biosystems) using the TaqMan OpenArray miRNA panel. RefFinder algorithm and the Two-One-Sided Test (TOST) were used for identification of optimal reference miRNAs. MiRNA expression was determined using the 2-ΔΔCt relative quantification method. Censored Regression Model was used for miRNA differential expression analysis. Finally, an enrichment analysis by hypergeometric test using different pathway annotations (KEGG, GO, REACTOME) was performed.

Results: One hundred and thirty miRNAs were detected in at least 75% of samples belonging to END or control group and were considered for subsequent analyses. Values were normalized by the arithmetic mean of Ct values of the

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Tissue Transglutaminase 2 expression in peripheral blood mononuclear cells in patients with Radiologically Isolated Syndrome: A Preliminary Study

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Introduction: Radiologically Isolated Syndrome (RIS) is a presymptomatic stage of Multiple Sclerosis (MS) and although there are many missing informations on pathological side, RIS patients (pwRIS) and MS patients (pwMS) seem to share the same radiological aspects. Several potential MS biomarkers have been investigated for RIS prognosis, to evaluate the presence of potential inflammatory signals in peripheral blood mononuclear cells (PBMCs) of pwRIS. Transglutaminase 2 (E.C. 2.3.2.13; TG2) has been shown to be directly involved in the neuroinflammatory process. Several findings emphasize the possible role of the TG2/NF-kB activation pathway in neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease; moreover, the expression of TG2 has been associated with disease progression (evaluated through EDSS, MRI & clinical parameters) in relapsing (RRMS) and progressive (PMS) MS. The present study aimed to assess the levels of TG2 in pwRIS compared to pwRRMS and HCs. Methods: We analyzed PBMC-derived TG2-mRNA levels of 10 HCs (4M, 6F), 10 naïve pwRRMS (2M, 4F) and 7 pwRIS (1M, 6F) from our MS Center. Patients and HCs were recruited with a wide age range (24-53 years) based on a previous study showing that TG2 mRNA level expression is independent from age. PBMCs collected from peripheral blood were plated to isolate monocytes. We then proceeded with RNA extraction and Real-Time PCR analysis. Gene expression was quantified by calculating Fold Change (2-ΔΔCt), using Actin as an endogenous housekeeping gene. Results: Preliminary data analysis of PBMC-derived TG2-mRNA levels show that TG2 mRNA expression levels in pwRIS are similar to those of HCs ($p=0.99$). Conversely, pwRRMS show higher levels of TG2 mRNA expression compared to HC ($p<0.0001$) and pwRIS ($p<0.0001$). Conclusion: Our preliminary data show that pwRIS have low levels of TG2-mRNA expression compared to pwRRMS; moreover, there are no differences in TG2 mRNA expression between pwRIS and HCs. This could suggest that pwRIS have a low grade of neuroinflammation compared to pwRRMS. Future investigations may include: i) data expansion and replication of results and ii) longitudinal assessment of the potential role of TG2-mRNA blood levels in predicting/monitoring the evolution from pwRIS to MS.

EP336

Anticancer activity of Orobanche crenata Forssk. leaves extracts against different human cancer cell lines

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Cancer is the second cause of death worldwide and it was estimated to become the leading in 2060. Cancer treatment is mainly based on chemotherapy, which is associated with a large variety of side effects. The use of natural adjuvants endowed with anticancer properties is considered a good strategy to reduce the chemotherapy dose, thus increasing its tolerability. In our previous work, we proved the anticancer activity of O. crenata leaves acetonic extract (OCLAE) against human breast cancer cell line MCF-7. The Human Foreskin Fibroblasts (HFF-1) cell line was used as the control cell line. The cytotoxic activity of the extract was compared to the standard drug Doxorubicin. MCF-7 cells were treated with increasing concentrations of OCLAE (75-1200mg/mL) for 24h. The cytotoxic effect of the extract was evaluated by MTT and LDH assays. The extract induced a significant reduction of MCF-7 cell viability and a significant increase in LDH release. These effects were correlated to the antioxidant properties of the extract. The obtained results led us to further explore the anticancer properties of O. crenata. In the present study, we evaluated the anticancer activity of O. crenata leaves aqueous extract (OCLAqE) against human colorectal cancer cell lines Caco-2 and HCT-116. Cisplatin, a potent chemotherapeutic agent, was used as the standard drug. The effect of both extract and Cisplatin was also tested on non-cancerous Human Dermal Fibroblast (HDF). Caco-2 and HCT-116 cell lines were exposed to increasing amounts of OCLAqE (10-160mg/mL) or Cisplatin (0.1 to 100 μM) for 24h, 48h, and 72h. The anticancer activity was evaluated by MTT assay. The potential synergistic effect between the two agents was assessed through MTT and Annexin V/ Propidium Iodide assays. The effect of the extract on ROS levels was revealed using 2,7-dichlorodihydrofluorescein diacetate. Finally, by UPLC-Ms/Ms the chemical profile of OCLAqE was obtained. The extract affected Caco-2 and HCT-116 cell viability at all time points. However, it dose-dependently reduced Caco-2 cell viability, starting from 40mg/mL. The treatment of HDF with the extract induced a significant reduction of cell viability only at the highest tested concentration (160mg/mL). Co-treatment of extract (80μg/ml) with a subtoxic concentration of cisplatin (1 μM) potentiated the drug effect. The extract was also able to modulate ROS production. Finally, the chemical analysis detected different polyphenolic compounds that could mediate the observed effects. These findings highlighted the anticancer properties of OCLAqE, thus suggesting its potential value as a promising therapeutic adjuvant.

EP337

Health and nutritional biomarkers in honeybees: opportunities and challenges under field conditions

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The decline of honeybee (Apis mellifera) populations has negative consequences not only for agriculture and beekeeping, but also for ecosystems. In human and veterinary medicine, proteomics and metabolomics provide valuable biomarkers to assess the health and nutritional status of organisms. In honeybees, the application of these techniques is still in its infancy and remains underexplored from a clinical perspective. This study aims to investigate the most abundant proteins of honeybee hemolymph as potential biomarkers of health and nutritional status at the colony level. In addition, an untargeted metabolomics-based approach was applied to honeybee extracts.

Samples of hemolymph were collected from honeybees in different apiaries in the province of Bologna in different periods of the year, from April to November. Hemolymph proteins were separated and quantified by 1D SDS-PAGE. Honeybee cytosolic extracts were fractionated using size exclusion chromatography (SEC) and metabolites were analyzed in fractions using mass spectrometry (Orbitrap Exploris 480, Thermo Fisher).

The five most abundant hemolymph proteins, namely vitellogenin, apolipophorin I and II, transferrin, and hexamerin 70a, represent a panel of biomarkers related to key metabolic processes. These proteins are subject to interesting variations depending on many physiological and environmental factors, including the honeybees' age (nurse bees had the highest vitellogenin concentration compared to the other two sub-castes), the season (in November, a peak of vitellogenin and transferrin concentration was observed in winter bees), and the presence of parasites (in bees parasitized by Varroa, a decrease of vitellogenin, apolipophorin II, transferrin, and hexamerin 70a was detected). One hundred and ninety-eight different pathways and more than 2000 metabolites were identified. The most abundant metabolites belonged to the flavone pathway, followed by the lipoxygenase pathway. Many metabolites were of plant origin and may be related to the environmental availability of nectar and pollen, which in turn are essential for honeybee nutrition, suggesting a possible role as biomarkers of nutritional status.

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EP338

The accuracy of GFAP, UCH-L1 and S100 for excluding mild traumatic brain injury. A prospective study

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Background:Glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1) and S100 protein are been studied for their potential ability to exclude a brain damage in patients with mild traumatic brain injury (mTB). We evaluate the prognostic accuracy of these biomarkers in a cohort of admitted in the emergency department (ED) of the Modena Hospital.Methods:We enrolled consecutive adults patients presenting in ED with suspected mTBI within 3h of traumatic event and with a Glasgow Coma Scale score of 14-15/15. Patients were eligible if they had undergone head CT as part of standard emergency care. Serum GFAP and UCH-L1 were measured on Alinity I (Abbott) upon arrival at the ER (T0) and then after 3 hours (T1) using prespecified cut-off values of 35 pg/mL and 400 pg/mL, respectively. Their assay results were combined into a single qualitative test result (positive or negative). Serum S100 was determined on Maglumi X8 (SNIBE) at T0 using the cut-off value of 0.15 ng/ml. The sensitivity (SE), specificity (SP), positive and negative predictive value (PPV and NPV) were calculated. Results:We included 59 patients (57.6% men, mean age 73 [22-92] years). In ten (17%) patients, the CT scan was positive. GFAP and UCH-L1 were detectable upon arrival in ED for all patients (mean ± SD were 230.8±573.7 pg/mL and 678.5±586 pg/mL, respectively). After 3 hours the serum concentration of GFAP was higher (mean ± SD 270.8±469.4 pg/mL, p=0.7), while the concentration of UCH-L1 was statistically lower (mean ± SD 472.8±381.8 pg/mL, p=0.025). At T0, 53 (93%) and 34 (58%) of patients had a positive GFAP and UCH-L1 results, respectively. After 3 hours, 50 (85%) and 29 (49%) patients had a positive GFAP and UCH-L1 results. When GFAP and UCH-L1 tests were used in combination, the SE was 100% (95% CI 69-100), SP 12.2% (4.6-24.7), PPV 18.9% (17.3-20.5), and NPV 100% (54-100). Therefore, the overall mean concentration of S100 was 0.5±0.9 ng/ml, 40 patients (67.8%) had a positive result. The SE was 100% (95% CI 69-100), SP 38.8% (25-54), PPV 25% (21-29.4), and NPV 100% (82.3-100).Conclusions:Our results showed high SE and NPV for all tests. This supports the hypothesis about their potential clinical role for ruling out a brain injury among patients with suspected mTBI.

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VERIFICATION OF THE ANALYTICAL PERFORMANCE OF THE KING SYSTEM

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Introduction:

The kidney maintains the electrolyte components of the organism within a fairly narrow range by quickly adapting to small changes in the hemodynamic equilibrium and acid-base balance. When it comes to the care of critically ill patients, constant monitoring of the previously described components is crucial.Recently, a new monitoring system of kidney function has been developed, the Kidney Instant Monitoring (KING). The KING instrument is directly connected to the patient by an indwelling urinary catheter and allows the continuous measurement of urinary sodium, potassium, chloride, ammonia, pH, and volume.The introduction of a new method or system into the laboratory requires the verification of its performance. The scope of the study was to verify the analytical performance of the new monitoring device and warrant its use for clinical purposes.

Materials and Methods:

Analytical verification was carried out according to Clinical and Laboratory Standards Institute guidelines (CLSI-EP5, EP6, EP10) with the evaluation of linearity, precision, and carry-over. Sixty urine samples were also analyzed with KING for method comparison, and the results were correlated to those obtained on the same samples with the corresponding reference methods (ISE CobasPro, c503 CobasPro, Roche Diagnostics, Mannheim, Germany; pHmeter, Mettler Toledo, Columbus, Ohio, USA). Correlation was analyzed by Passing-Bablok.

Results:

Linearity and respective regression indeces were the following for each analyte: Cl- 76-442 mEq/L (R2 0,99); K + 23,8-168,2 mEq/L (R2 0,99); Na+ 94,6-294,1 mEq/L (R2 0,99); NH4+ 10 a 132 mEq/L (R2 0,99); pH 2,58-8,05 (R2 0,99).The within run precision (CV%) obtained for each was < 3% and for the within laboratory was < 6%. There was a good correlation, with a constant significant difference of all the analytes between the KING system and the corresponding reference methods.

Conclusions:

Laboratory verification of the KING's analytical performance confirmed that it is a valid monitoring system for kidney function in the critical patient.

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EP340

Valore predittivo positivo del saggio di radioimmunoprecipitazione per gli anticorpi anti recettore dell'acetilcolina nella diagnostica della Miastenia grave

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Introduzione e obiettivo: gli anticorpi anti-recettore per l'acetilcolina (AchR-IgG) confermano la diagnosi di Miastenia grave (MG) quando rilevati nel siero di pazienti con fenotipi clinici compatibili. Il test di Radioimmunoprecipitazione (RIPA) rappresenta il gold standard per il rilevamento degli AchR-IgG con una specificità di circa il 99%. La sua accuratezza diagnostica, tuttavia, in popolazioni ampie e non selezionate non è stata ancora completamente chiarita. Abbiamo determinato il valore predittivo positivo (VPP) e il rischio di falsa positività per AchR-IgG in un contesto di vita reale.Materiali e metodi: presso la Neurologia dell'AOU di Sassari sono stati identificati retrospettivamente i pazienti consecutivamente testati per AChR-IgG e le cartelle cliniche di quelli che presentavano un titolo anticorpale ≥ 0.5 nml/L sono state revisionate in modo indipendente da due neurologi per determinare la vera o falsa positività anticorpale sulla base del fenotipo clinico e/o della presenza di chiare diagnosi alternative all'ultimo follow-up, escludendo i pazienti AChR positivi con informazioni cliniche insufficienti (n=84). La prestazione diagnostica è stata riassunta con specificità puntuale, valori predittivi positivi e relativi intervalli di confidenza (IC) al 95%.Risultati: dei 362 pazienti positivi per AchR-IgG inclusi nello studio, 50 (13,8%) sono stati designati come falsi positivi. La specificità e il VPP erano rispettivamente del 98,9%) e 86,2%. Diagnosi alternative nei pazienti con falsa positività per AchR-IgG includevano malattie altre malattie neurologiche. Rispetto ai pazienti con vera positività per AchR-IgG, i pazienti falsi positivi erano più giovani, più frequentemente donne e avevano un titolo anticorpale più basso. Dopo la stratificazione con titoli AchR-IgG ≥ 1 nmol/L, la specificità e il VPP sono aumentati rispettivamente al 99.8% e al 96.6%.Discussione e conclusioni: nonostante l'elevata specificità del test AchR-IgG mediante RIPA, il rischio di falsa positività anticorpale non è trascurabile nella pratica clinica (14% in questo studio). È necessaria cautela quando si rileva positività per AchR-IgG a basso titolo (0,5-0,9 nmo/L) in pazienti con sintomi non specifici per la MG o spiegabili con eziologie alternative più comuni.

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Enhancing Sustainability in Clinical Laboratories: A Multidisciplinary Approach to Waste Management and Energy Conservation

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Objective: The environmental impact of healthcare is significant yet often neglected. Clinical laboratories are particularly notable for their extensive energy use, primarily driven by ventilation systems that account for 50 to 80% of energy consumption. Factors such as specialized equipment, continuous operational hours, and stringent humidity and temperature controls significantly contribute to this. Moreover, laboratories also heavily utilize water and generate substantial waste, particularly from chemicals. It is essential for these facilities to proactively enhance their environmental practices beyond current regulations by adopting comprehensive waste management and energy-saving strategies.

Methods: The study encompasses detailed waste categorization, an energy audit of refrigeration systems, and an evaluation of plastic recycling technologies.

Results: A systematic re-evaluation of waste and energy management was initiated, focusing on plastic recycling and energy efficiency in lab equipments. Innovations, such as the potential use of autoclaves for medical waste and improved recycling protocols for both mono and multi-polymer plastics, may significantly reduce environmental impacts. An example is the separation from the non-infectious waste stream of multipolymeric high-density polyethylene (HDPE) plastics, which could allow recycling of up to 1,068 kg/year. Energy audits of refrigeration units highlighted the high consumption by combined refrigerator-freezers (2,863 kWh) compared to +4°C refrigerators (922 kWh) and -20°C freezers (1,158 kWh). The study suggests consolidating smaller, outdated freezers and replacing them to reduce energy use. Further energy reductions were achieved through optimizing the management of ultra-low temperature freezers and improving refrigerator maintenance.

Conclusions: Through interdisciplinary collaboration, notable progress has been made in implementing sustainable practices in clinical laboratories. By combining engineering solutions, environmental science, and laboratory medicine, effective strategies for reducing energy and waste were demonstrated, establishing a model for future sustainable efforts in healthcare settings.

EP342

Novel Predictive Biochemical Biomarkers for frailty: th BioGERT experience

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Background: Frailty is considered a clinical condition characterized by an individual greater vulnerability to endogenous and/or exogenous stress factors, which favour the development of negative outcomes. Frailty can represent a reversible condition, bridging between successful aging and disability. Various tools have been proposed for the objective assessment of frailty over the years; however, to date, there is no univocal and objective assessment to identify pre-frail subjects, avoiding the progression to disability. Therefore, the aim of this study is to identify biomarkers which, in association to a holistic evaluation of the patient, will be able to recognize the pre-frail subject to promote successful aging instead.

Materials and Methods: To achieve the proposed objective, starting from October 2022, the first geriatrics biobank of the Policlinico Foundation Tor Vergata, the BioGert, was established. All enrolled patients, after signing an informed consent, underwent a multidimensional assessment (VMDG), to define the medical, socioeconomic, and environmental condition, functional (handgrip test), physical (PASE scale), nutritional (MNA test), and mental status (MMSE, IADL scale and GDS scales) of elderly subjects. Moreover, serum levels of SIRT1 and Kloth, were measured by using an enzyme immunoassay (ELISA) kit.

Results: Firstly, we evaluated the associations between two relevant biochemical parameters: albumin and D-Dimer with the most important predictor of frailty: the handgrip test. Our data demonstrated a direct and significant relationship between sera albumin levels and handgrip test ($p=0.0091$). Conversely, the correlation between sera D-Dimer levels and handgrip test ($p=0.04$) was negative: reduced handgrip test values were associated with higher D-Dimer levels. We, then, compared the handgrip test score to different VMDG scales. Our results demonstrated a significant positive linear relationship with MNA ($p=0.0012$), IADL ($p=0.0001$), GDS ($p=0.0222$) and PASE ($p<0.0001$), highlighting that patient with isometric strength, exerted by forearm muscles, achieved higher scores on scales related to their physical and mental health. Subsequently, we evaluated the correlation between SIRT1 and several VMDG scales. In particular, a reduction in the AGILE scale score was associated with significant increase in the expression of SIRT1 ($p=0.04$), indicated that higher SIRT1 levels were associated with lower severity of frailty. Similarly, although not statistically significant, the association with the handgrip test suggests that increased expression of SIRT1 correlates with greater muscle strength. Inverse (but not significant) correlations were observed between SIRT1 levels and the GDS and the MNA scales: as SIRT1levels increased, lower scores on these scales were observed. In the and, we also measured Klotho levels, and studied their associated with multidimensional assessment scales. Although statistical significance was not been reached, high Klotho expression levels tended to correlate with higher scores on the MNA test and the IADL scale, indicated an inverse correlation between these enzyme and frailty.

Conclusions: The data obtained in our study, although preliminary, show how it is possible to determine a state of frailty by relating the different scales of the VMDG. Moreover, we also correlate the most important parameter for predicting frailty, the handgrip test, with sera albumin and D-Dimer levels. Additionally, for the first time, we reported a direct association between SIRT1 and frailty measured through the AGILE scale. In conclusion, the reported results have important implications for clinical practice, the use of the VMDG scales and biochemical parameters, together with a future use of SIRT1, can help healthcare professionals in the early identification of patients at risk of developing a depressive state and cognitive decline that negatively impact the aging process. This allows for preventive and timely action, promoting the reversal of this pre-frailty condition towards a state of robustness and successful aging.

EP343

Targeted metabolomic and lipidomic profiling combined to chemometric analysis to discover new biomarkers and diagnostic approaches: application on Down's Syndrome patients

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Evaluation of a wide panel of metabolites in biological samples could be used as a useful diagnostic approach. Most pathologies brought to an alteration of specific biological pathways. When a several number of parameters is measured, chemometric statistical analysis is an essential tool for specific biomarkers determination.Intellectual disability can be mainly caused by Down's syndrome (DS), a genetic alteration leading to common phenotypical expressions as craniofacial dysmorphism, cardiovascular defects and increased comorbidity.The aim of our study was to determinate some characteristics alteration in metabolic profile in DS subject if compared to healthy subject. Twenty-four DS subject and twenty-two healthy controls were enrolled for this purpose. Serum metabolome evaluation was performed on a panel of 98 small molecules and 524 lipids, belonging to 26 different biochemical classes. The analysis was performed on an Ultra Performance Liquid Chromatography–Tandem Mass Spectrometry (UPLC-MS/MS) platform consisting in an ExionLC AD and a Qtrap 6500+ (ABSciex, Framingham, USA) equipped with electrospray ion source, operating both in positive and negative mode. The measures were conducted both in Liquid Chromatography and Flow Injection mode using MxP Quant 500 Kit (Biocrates life science, AU). After analysis, metabolites concentrations were used to build Partial Least Squared Discriminant Analysis (PLS-DA) classification models. In PLS-DA model obtained using LC-MS/MS data, the algorithm allowed correct classification with 96.4% of accuracy. A wide expressed alteration in polyamine metabolism, oxidative stress markers and neurotransmitters is characterizing the DS population when compared to healthy subject. When FIA-MS/MS data were used to build PLS-DA models, the amount of correct classification obtained was 92.2%. In DS subjects greater levels of circulating triglycerides and characterizing ceramides profiles were reported.In conclusion we can state that DS could lead to specific metabolome alterations, that may provide a new input, to better understand the pathophysiology of the syndrome. This pilot study can be considered a beginning for more accurate investigations, that could involve a higher number of subjects and data fusion multivariate analysis.

EP344

The role of the laboratory medicine in clinical research

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Background: The clinical research includes human subjects and had the aim to evaluate the efficacy, effectiveness and safety of new drugs, treatment or diagnostic pathway. Likewise, laboratory research assesses the accuracy of diagnostic tests contributing significantly to medical research, without the direct human involvement. Laboratory medicine is a key component of the medical research that is usually carried out by clinicians who have also the role of principal investigator. We investigate the contribution of the clinical laboratory in the clinical research analysing the scientific literature.

Methods: We performed a literature search on Medline to identify all references satisfied the following criteria: (1) categorized as "clinical trial"; (2) at least one author with affiliation "laboratory medicine" or "pathology". In the next step, we selected the references in which the first author's affiliation referred to laboratory medicine.

Results: The literature search conducted in June 2024, identified 1.029.161 reference in which at least one author has an affiliation refereed to laboratory medicine. Of these references, 17660 (1.7%) are clinical trials and 13814 (78%) have been published since 2014. We observed a significant increase in publications relating to laboratory medicine starting from 2014, with an increment of 149% from 2013 to 2014. However, among clinical studies published in the last 10 years (2014-2024) we have identified that only 8% of publications have as first authors a laboratory professional. Most publications focus on clinical pathology (32%), followed by molecular biology and genetics (23%) and biochemistry (8%). Only 9% of publications are about immunology and immunotherapy, the 6% about microbiology, and only the 3% of references were studies on biomarkers.

Conclusions: The laboratory medicine is essential in majority of clinical trials, and the close cooperation between laboratory professionals, who critically interpret laboratory data, and clinicians who elaborate the medical information, is pivotal to improve clinical outcome, to increase the diagnostic test development and the knowledge about disease pathophysiology and treatment. In this contest is important to emphasize the role of laboratory medicine in clinical research supporting the innovation in the new diagnostic tests and encouraging multi-professional collaboration.

EP345

The microbioma dysbiosis in patients at different stages of the transformation from liver HCV alterations to hepatocellular carcinoma

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Recently, mounting evidence has suggested that the gut microbiota plays a significant role in chronic liver disease and in the development of its complications, such as infections, gastrointestinal bleeding, hepatic encephalopathy, and hepatocellular carcinoma (HCC), which is the leading cause of death among patients with advanced liver disease (1). However, little is known regarding the composition of gut Mucosal-Associated-Microbiota (MAM) in individuals with hepatitis C virus (HCV) and in the subsequent 2 different stages of disease that lead to HCC (2, 3). To address this gap, we characterised MAM taken from terminal ileum and sigmoid mucosal tissues in patients at various stages of HCV-related liver disease and we compared it to healthy controls. We included in the study 13 healthy controls, 6 chronic HCV hepatitis, 9 liver cirrhosis (LC), and 14 HCC patients. We performed 16s rRNA sequencing of mucosal samples obtained from biopsies of the terminal ileum and sigma of patients with HCV-related liver diseases and healthy controls. Analysis of sigma specimens from HCC patients showed a substantial decrease in α-diversity compared to controls and LC, with a modest rise ($p= 0.047$) according to the Shannon index. The β-diversity measure weighted UniFrac revealed substantial grouping between the MAM of the control and HCV groups ($p\leq 0.05$). The analysis revealed abundance of certain taxa at phylus-, family- and genus- levels both in ileum and sigma tissues. We observed a marked dysbiosis from healthy subjects to patients with LC, while a slight increase of some taxa from LC to late-stage of HCC. The aim of the study is to investigate the microbioma in the progression of liver disease through the different stages of the pathology, using mucosal tissue samples, instead of the more common stool samples.

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EP346

Urinary steroids analysis by dilute & shoot LC-MS/MS: the case of Pregnanediol Glucuronide and future perspectives

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The dilute & shoot (DS) analysis is a simple procedure where the sample is directly diluted with an appropriate solvent before the injection or “shooting” into the LC-MS/MS system. This approach, which allows the use of limited sample volume, is thus quick, easy and cost saving. Furthermore, the absence of pre-analytical sample extraction step could help increase lab productivity making the method more attractive to clinical biochemistry laboratories thanks to its adaptability to automation.

In our work a first DS LC-MS/MS method was developed for measuring Pregnanediol-3-Glucuronide (PDG) urinary concentration from 7μL of spot urine samples for the detection of ovulation. The analysis was performed employing a fully-porous C18 analytical column and adding 2mM ammonium fluoride to aqueous mobile phase to reach the high sensitivity level needed, for a total chromatographic run time of a 6 min. The method was validated in accordance with ISO 17025 requirements for quantitative methods and it was applied to two sets of real samples. Firstly, a series of 24 daily samples collected from a female individual across a menstrual cycle were analyzed, then a population of 7 healthy female volunteers were recruited and a set of 4 spot urine samples (1 per week) was collected from each individual and analyzed.

The optimized analytical method allowed to efficiently separate PDG from other structurally similar glucuro-conjugated steroid metabolites, guaranteeing a sufficient sensitivity for detecting target analyte down to 0.01 μg/mL. The performed validation protocol, including the assessment of selectivity, quantitative performance, carry-over, sample stability and robustness gave satisfactory results. The application to real spot urine samples allowed proving method’s ability to measure PDG throughout an entire menstrual cycle, highlighting the ovulation period also when only one sample per week was collected, and opening the way for the implementation of this DS analysis in routine clinical laboratories.

In the future, it would be possible to extend the number of steroidal analytes monitored by DS analysis, starting with additional ovulation markers such as Estrone Glucuronide, which is another ovarian steroid whose monitoring, combined with the determination of PDG concentration, could help in determining more efficiently whether ovulation has occurred. Moreover, research efforts are currently focused on developing a novel DS method for the measurement of up to 50 urinary steroid hormones as well as selected glucuro- and sulpho-conjugated phase II metabolites. Following its analytical validation, such extended steroid panel will be applied to various clinical settings with the aim of characterizing the steroidal asset in physiological conditions as well as in rare and chronic non-communicable endocrine diseases.

EP347

T-cell functional analysis to evaluate the cross-reactivity between SARS-CoV-2 antigens and type 1 diabetes autoantigens

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Pre-existing immunity to SARS-CoV-2 is primarily attributed to sequence homology between it and closely related common cold coronaviruses, suggesting that molecular mimicry plays a crucial role in cross-reactivity. This protective pre-existing immunity may explain the varying susceptibility to SARS-CoV-2 infection and the differences in the clinical course of COVID-19 observed among individuals.

The goal of this study was to assess pre-existing immunity to SARS-CoV-2 antigens in individuals with type 1 diabetes (T1D) who have not been previously exposed to the virus. To achieve this, peripheral blood mononuclear cells (PBMCs) were collected from SARS-CoV-2 unexposed subjects with T1D and healthy controls. SARS-CoV-2 specific T cells were identified in PBMCs by ex-vivo interferon (IFN)γ-ELISpot and flow cytometric activation-induced marker assay (AIM assay), following T cell stimulation with peptide pools derived from SARS-CoV-2 antigens (Spike glycoprotein, ORF7a, ORF7b, ORF3a, Membrane, Nucleoprotein) and T1D autoantigens (ZnT-8, INS, G6PC2, GAD65). The epitope specificity of T cells in T1D was inferred through T Cell Receptor (TCR) sequencing and GLIPH2 clustering analysis.

Using these methods, we found that T1D patients unexposed to SARS-CoV-2 exhibited higher rates of virus-specific T cells than controls. The T cells primarily responded to peptides from the ORF7/8, ORF3a, and nucleocapsid proteins. Nucleocapsid peptides predominantly indicated a CD4+ response, whereas ORF3a and ORF7/8 peptides elicited both CD4+ and CD8+ responses. The GLIPH2 clustering analysis of TCRβ sequences suggested that TCRβ clusters associated with the autoantigen proinsulin and ZnT-8 might share specificity toward ORF7b and ORF3a viral epitopes. Notably, PBMCs from three T1D patients exhibited T cell reactivity against both ORF7b/ORF3a viral epitopes and proinsulin/ZnT-8 autoantigens.

By using T cell functional and cytofluorimetric AIM assays, we demonstrated the shared reactivity of T1D T cells against both SARS-CoV-2 and autoantigens, unveiling a possible mechanism that accounts for the varying susceptibility to SARS-CoV-2 infection and clinical course of COVID-19 observed in T1D individuals.

EP348

Use of Total Attenuated Reflection - Fourier Transforms Infrared Spectroscopy (ATR-FTIR) in Muscle Diseases

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Introduction: Inherited muscle diseases are a heterogeneous group of clinical conditions, characterized by histological and functional abnormalities of skeletal muscle. Infrared spectroscopy is an analytical technique used in many clinical areas being sensitive, specific and robust. Attenuated Total Reflectance (ATR) is one of the sampling technologies used for infrared spectroscopy and, as a rapid and non-destructive technique. The aim of this study was to distinguish patients with late-onset Pompe disease (LOPD), Becker muscular dystrophy (BMD), limb girdle muscular dystrophies (LGMD) and healthy subjects (HS) by evaluating the profile biochemical determined by ATR analysis. Methods: 40 patients were included in the study: 11 LOPD, 10 BMD, 10 LGMD, 9 HS. For spectral analysis, the muscle samples were cut at 10 μm in the cross section and placed on ATR. The statistical analysis of clinical data was conducted with a chemometric approach. Results: The results obtained show that the spectroscopic fingerprint embeds sufficient information to allow a correct classification of the majority of participants in three groups: dystrophic (BMD and LGMD) and metabolic (LOPD) myopathies, healthy subjects (accuracy 88.4±7.1%). The ATR-FTIR analysis was also effective in classification rates using a two-class model: LOPD vs LGMD (accuracy 95.7±3.2%), LOPD vs BMD (accuracy 82.9±4.6%) and LOPD vs BMD+LGMD (accuracy 93.4±3.0%). Discussion: Our results suggest that ATR profile is a reliable diagnostic biomarker for a classification of muscle disease (LOPD, BMD and LGMD). Future perspectives will include evaluating its role as a prognostic biomarker in these genetic diseases, also analyzing biofluids, and the ability of this technique to shed light on the underlying pathogenic mechanisms.

EP349

An LC-MS/MS method for the simultaneous quantification of all 5 isomers of the semi-synthetic antibiotic Dalbavancin in human plasma suitable for therapeutic drug monitoring

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Background: Dalbavancin (DBV) is a semi-synthetic lipoglycopeptide antibiotic characterized by a long elimination half-life used to treat acute skin, skin structure, osteoarticular and prosthetic infections in adults caused by susceptible Gram-positive bacteria. It is administered as an infusion at a dose of 1,500 mg. According to literature, the optimal dosage is achieved when the plasma DBV concentration is above 8 mg/L; therefore, it is essential to evaluate the plasma concentration of the drug before administering a new dose. The pharmaceutical formulation of DBV is composed by 5 isomers (A0, A1, B0, B1 and B2), of which the most abundant form is B0 (75%); Consequently, pharmacokinetic (PK) studies and therapeutic drug monitoring (TDM) work rely on the quantification of B0 in plasma. The impact of other isomers on DBV pharmacokinetic behavior and dosage is still unclear. Our work has been focused on the development of a fast and reliable LC-MS/MS method to simultaneously assess the plasmatic levels of all five DBV isomers in human plasma samples.
Methods: Samples were analyzed on an Acquity I Class Plus UHPLC-MS/MS apparatus coupled with a Xevo TQ-S Micro ESI-Q-q-Q mass spectrometer (Waters), using an Acquity C18 column for the separation. Plasma samples were processed using methanol and 1% formic acid for protein precipitation. Individual reaction monitoring transitions were set up to detect each isomer, using different retention times to identify individual isomers.
Results: The method was validated according to the most recent international guidelines (ICH) and will be used to evaluate plasma DBV levels in treated patients. The preliminary data met all validation and analytical reproducibility requirements on plasma samples.
Conclusion: The use of DBV combined with TDM may reduce the frequency of administration, resulting in a lower likelihood of developing resistance, reduced hospitalization days, and overall decreased hospitalization costs. The methodology applied in this study may provide information about the contribution of all the isomers present in the formulation and their impact during therapy.

EP350

Predictive value of S100 calcium-binding protein B and neuron specific enolase by a set of clinical criteria, in patients with mild traumatic brain injury

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Background: During recent years several neurobiomarkers have been studied in the diagnosis of mild traumatic brain injury (mTBI). The primary objective of this investigation was to determine if a set of preclinical and clinical parameters combined with S100B and NSE serum concentrations can effectively be used for accurate diagnosis.
Methods: The authors conducted a retrospective clinical study from May to September 2023, that involved a cohort of head trauma patients with Glasgow Coma Scale scores of 13-15. Blood samples and cranial CT studies were collected within 3 hours after the traumatic event. Moreover, we analyzed 15 sera from healthy subjects as negative control.
Results: 59 adult patients samples were analysed on Liaison XL (DiaSorinS.p.A., Saluggia,Italy) for S100B and NSE, whose manufacturer's cut-off were respectively: 0.15μg/L, 17μg/L. The average for S100B was 0,56μg/L±1μg/L and for NSE was 19,9μg/L±12μg/L. Among all patients 12% CT positive, patients withsubdural and subarachnoid hemorrhage NSE was 18,9μg/L± 8,3μg/L and S100B on Liaison XL 1,5 μg/L± 1,7μg/L. For patients with PTA (post-traumatic amnesia), neurological deficits and changes in mental state (confusion, disorientation, slowdown) NSE was 21,3μg/L± 14,1μg/L and for S100B 0,6μg/L± 1μg/L. In our study, 37 patients was being treated with antiaggregant and anticoagulant drugs, NSE was 18,1μg/L± 5,2 μg/L and for S100B 0,6μg/L± 0,6μg/L. Ultimately, in our cohort S100B seem to be more accurate (AUC 0.84) than NSE (AUC 0.52).
Conclusions: the integration of the neuromarker panel as part of a diagnostic rule including the high risk factors of nausea, vomiting, amnesia, hemorrhage and use of anticoagulants is safe and reliable in determining a diagnosis, pending the availability of more brain-specific neuromarkers. Nevertheless, we believe it is necessary to increase the sample size of our study to validate our preliminary results

EP351

A novel LC-MS/MS method for the simultaneous quantification of thirty-nine biliary acids in human samples as predictive markers of hepatic and extra-hepatic pathologies.

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Background: Bile acids (BAs) synthesis, modification and metabolism are intrinsically related to a wide range of physiological processes. BAs are recently gaining much attention in clinical chemistry since several physiopathological conditions, including non-alcoholic fatty liver disease, obesity, inflammatory bowel disease and diabetes, affect their levels and structure. Growing evidence is emerging that these molecules, in addition to their emulsifying action to improve absorption of fats, lipid-soluble vitamins and steroids, may act as hormone-like signaling molecules whose homeostasis is crucial to avoid the onset and development of several diseases. In this work we present a novel method to simultaneously quantify 39 BAs suitable for feces and tissue samples.
Methods: Biliary acid samples were analyzed on an Acquity I Class Plus UHPLC-MS/MS apparatus coupled with a Xevo TQ-S Micro ESI-Q-q-Q mass spectrometer (Waters), using a Luna Omega 1.6 μm Polar (C18, 100 Å, 50 × 2.1 mm; Phenomenex) at 40°C, and at a flow rate of 400 μl/min. Mobile phases consist in H2O, 5 mM Ammonium acetate (AmAc), 0.01% formic acid (FA) and MeOH/ACN (80:20), 5 mM AmAc, 0.01% FA for phase A and B respectively. Mass spectrometer was operated in negative MRM scanning mode, using a low collision energy to avoid BAs fragmentation.
Results: Using different retention times and specific transitions, the method can effectively differentiate 39 BAs, including isomers, extracted from feces or tissue samples. Total run time is 22 min. The calibration curves obtained from the analytical standards all had a satisfactory R2 values (≥ 0.99), and the lower limits of detection (LOD) for all the analytes were at least of 25 nM.
Discussion: We have developed and implemented a novel method that simultaneously quantifies 39 BAs in a reasonable run time, which is appropriate for clinical routine. Aberrant circulating BA profiles are a promising predictor of the onset and progression of various gastric and extra-gastric disorders, such as neurological diseases. Characterization of BA profiles may provide a basis for the development of novel biomarkers for the diagnosis and prevention of a wide range of pathologies.

EP352

ROLE OF VITAMIN D STATUS AND ALTERATIONS OF GUT MICROBIOTA METABOLISM IN CHRONIC INFLAMMATORY PAIN

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Aim: Fibromyalgia (FM) is a central sensitization syndrome characterized by widespread intense musculoskeletal pain and extra-skeletal disorders. Several studies suggest gut microbiota metabolites as important immuno-modulators in inflammatory pain(1). We aimed to investigate the relationship between vitamin D status and gut dysbiosis markers in FM-associated chronic inflammation.
Methodology: Blood samples were collected from fifty-one female FM patients (50.3 ± 11.5 years), after release of informed consent. Pain intensity was assessed by fibromyalgia impact questionnaire revisited (FIQR). Serum levels of pro-inflammatory cytokines IFN-γ, IL-6, IL-17, TNF-α as well as those of vitamin D (25(OH)D3) and kynurenine/tryptophan ratio (Kyn/Trp) were determined by ELISA and HPLC, respectively. Plasma levels of short-chain fatty acids (SCFAs) acetate, butyrate, and propionate were detected by GC-MS.
Results and Discussion: FIQR scores and mean levels of all cytokines, but IL-6, were higher than normal reference values in FM cohort. The highest concentrations of pro-inflammatory cytokines were observed in patients showing the highest FIQR scores and lowest 25(OH)D3 levels, suggesting the involvement of vitamin D in the attenuation of FM inflammatory pain. Deficient levels of acetate and Trp, paralleled by an increase in Kyn/Trp, were found, indicating a state of gut dysbiosis(2). The highest SCFAs concentrations were detected in patients with the lowest FIQR scores. Significantly negative correlations were found between 25(OH)D3 concentrations and FIQR score ($p=0.014$) as well as IL-17 ($p=0.043$), and between acetate and TNF-α ($p=0.019$) as well as IL-17 ($p=0.027$), while significantly positive correlations were observed between Kyn/Trp and IL-17 ($p=0.021$) as well as IFN-γ ($p<0.001$). These results highlight the role of SCFAs in immune tolerance(3).
Conclusions: Our preliminary data suggest that vitamin D status along with altered gut microbiota metabolisms play a major role in FM-related inflammatory pain. Replication of these findings in a larger cohort, using a case-control method, is required to provide additional insights.

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2. Hou Y, Li J, Ying S, Metabolites 2023
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EP353

Confronto tra metodiche RIA (Radio Immuno Assay) vs LC-MS/MS (Cromatografia liquida abbinata alla spettrometria di massa in tandem) per la misurazione del cortisolo libero urinario.

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Introduzione Il cortisolo è uno dei principali ormoni steroidei prodotti dal nostro organismo a partire dalle ghiandole surrenali ed appartenente alla categoria dei glucocorticoidi. La sintesi è regolata direttamente dall'ipofisi-ipotalamo. Le principali funzioni riguardano la gluconeogenesi epatica, regolazione della pressione arteriosa, azione anti-infiammatoria. Un'aumentata produzione di cortisolo (Morbo di Cushing), può avvenire a seguito di somministrazione prolungata glucocorticosteroidi, tumori secernenti ACTH, aumentata sintesi a livello surrenalico.Una diminuita produzione si verifica a seguito di insufficienza surrenalica secondaria o insufficienza surrenalica primaria (morbo di Addison). La diagnosi ed il monitoraggio di queste sindromi si effettua tramite il dosaggio della frazione libera, biologicamente attiva, di cortisolo. Materiali e metodi Lo studio in questione ha preso come riferimento un totale di 88 campioni di urina raccolta nelle 24 ore esaminati contemporaneamente sia con il metodo in uso RIA che con il metodo in prova LC-MS/MS. È stata verificata la ripetibilità del metodo in prova con schema 3x5 utilizzando il verification value. In seguito sono stati analizzati i valori ottenuti con i due metodi attraverso il grafico della regressione di Passing-Bablok, delle differenze di Bland-Altman. Risultati CV e DS del metodo in prova risultano accettabili. Non risultano outliers applicando il test di Grubbs. Il grafico di Passing-Bablok mette in luce la presenza di un errore sistematico indipendente dalla concentrazione. Il grafico delle differenze di Bland-Altman dimostra come il 63,6% delle differenze siano esterne all'intervallo 0±1.96*CV combinata. Conclusioni La presenza di un "bias" significativo e costante è dovuta principalmente agli evidenti limiti che si accompagnano ai dosaggi radioimmunologici, soprattutto per quanto riguarda la scarsa specificità rispetto alla LC-MS/MS che rende complesso discriminare il cortisolo da altre molecole strutturalmente simili. Ne consegue, dunque, valutando anche ulteriori aspetti nel confronto, che in generale sia molto più efficace ed affidabile ricorrere all'utilizzo della LC-MS/MS per quantificare in ambito clinico i livelli di cortisolo libero nei campioni urinari.

EP354

Therapeutic drug monitoring and pharmacokinetics of tacrolimus in pediatric recipients of allogeneic hematopoietic stem cell transplant

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the standard treatment for several malignant (i.e., acute leukemia, myelodysplastic syndromes) and non-malignant hematologic diseases. However, it is often complicated by the occurrence of graft-versus-host disease (GVHD), for which tacrolimus (TAC) is a common therapy. The drug is characterized by a narrow therapeutic window and high interindividual variability, especially in pediatric patients, hence a therapeutic drug monitoring (TDM) is mandatory.

The present retrospective study was aimed at investigating a) the pharmacokinetics (PK) of early post-HSCT TAC (administered at the dose of 0.03 mg/kg/day as continuous i.v. infusion) in children admitted to the IRCCS "Burlo Garofolo", Trieste, Italy, and b) any possible relationship between PK and transplant-related events. Blood concentrations of TAC were daily measured by an immunoassay to achieve stable concentrations in the range 15-20 ng/ml during the first month after transplant, while those values collected in the first 14 days after allo-HSCT were subjected to a population PK (POP/PK) modeling to obtain the main PK parameters (i.e., area under the time-concentration curve, AUC).

One hundred and twenty-five children (78 males and 47 females, with a median age of 8 years (IQR, 5-13 years) were enrolled, and 1897 tacrolimus blood concentrations (median number of values per patient, 16; range 11-16) were available for POP/PK analysis. Notably, mean (± SD) and median values of TAC daily doses were 0.032 (± 0.010) and 0.030 mg/kg, respectively, being the interindividual variability in daily doses largest during the first two days of treatment (CV% >140%) and progressively diminished from the third day onward (CV%, 38.1-57.8%). The final PK model was a 1-compartment model with body weight exerting a significant effect on both TAC clearance and volume of distribution.

High TAC concentrations (>12-15 ng/ml) in the initial weeks post-HSCT were associated with reduced post-transplant infections ($p<0.0001$) and a lower incidence of acute GVHD ($p<0.05$ on day 0). However, high TAC exposure (expressed as AUC values) was significantly related to an increased risk of chronic GVHD ($p<0.0001$) and reduced overall survival ($p<0.01$).

In conclusion, TDM helps the individual optimization of prophylactic TAC in pediatric recipients of allo-HSCT thus increasing the therapeutic benefit and significantly decreasing the risk of early and late complications of transplantation. The prompt PK analysis of TAC in those patients may further improve the efficacy and safety of the drug.

EP355

Rapidity in Parathyroid Surgery: Laboratory Monitoring for Successful Intervention

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Introduction: Intraoperative monitoring of parathyroid hormone (PTH) is an highly accurate tool used to determine the extent of necessary intervention in cases of primary hyperparathyroidism. Accurate interpretation of changes in PTH level is crucial and it is fundamental to choose an appropriate approach to ensure surgical success. Methods: The study population comprised 47 patients diagnosed with primary hyperparathyroidism, including 77.6% females and 22.4% males, with a mean age of 59 years. The instrument used for intraoperative PTH monitoring the NBCL device (PANTEC, Italy), which performs analysis on whole blood with a time response of 5 min. This study follows the Irvin standard, which defines a successful surgery with a PTH reduction of 50%. PTH testing was performed at two time-points: at baseline and 10 minutes after removal of the hyperfunctioning tissue. Results: The first baseline PTH measurement averaged 182 pg/mL (SD: 201), while the second measurement recorded a mean value of 80 pg/mL (SD: 115). Twenty-eight patients underwent a third measurement, averaging 53 pg/mL (SD: 34.5), four patients undergoing a fourth measurement with a mean of 27.9 pg/mL (SD: 16). The percentage reduction in PTH between the first and second measurements was 45%, and between the second and third measurements, it was 43%. Using the 50% standard, twenty-six patients achieved at least a 50% reduction in PTH values, and seven patients underwent a third measurement. Overall, 19 patients underwent a third measurement due to insufficient decrease in PTH levels. Conclusion: The 50% PTH reduction as a standard for successful surgery, although not always achieved by all patients, provided a useful guide for surgical decision-making. The requirement for multiple sampling in some patients underline the importance of continuous monitoring to optimise surgical outcomes and ensure complete removal of hyperfunctioning tissue. The multidisciplinary collaboration between laboratorists and surgical team could help to define interpretation criteria in each clinical context, according to differences in surgical techniques, PTH rapid assays and organizative models.

EP356

Diagnostic appropriateness of hemoglobin monitoring in patients with sickle cell disease treated with oncocarbide

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Introduction

The state of normality or the presence of hemoglobin variants, already present at birth, will no longer change during life, but there are conditions linked to age, pregnancy and pharmacological therapies for which monitoring is necessary during the course of treatment . In the case of patients suffering from sickle cell anemia (SCA) undergoing pharmacological treatment with oncocarbide, monitoring the percentages of HbF and HbS is essential to optimize therapy, improve clinical results and guarantee the long-term safety of the treatment. In addition to this, it is also to emphasize the need to have available clinical-diagnostic questions and/or anamnestic information useful for an initial evaluation of possible hemoglobin defects and more

Presentation of the case

A 59-year-old male patient arrives with a request for a complete blood count, reticulocytes, dosage of folate, vitamin B12 and for HbA2, Fetal Hb (HbF) and abnormal Hb.

Detected: RBC 2.94*106/mmc; Hb 11.9g/dl; HCT 33.3%; MCV 113.3fl; PLT 519/mmc, RET 2.32%; total bilirubin 0.7mg/dl; folate 7.3ng/ml; vitamin B12 321pg/ml; HbA2 3.92%, HbF 47.38% and Hb S 36.07%. The hemoglobin data is also confirmed by another laboratory. In the absence of anamnestic data, the patient is contacted, who informs us of his condition as a patient suffering from SCD being treated with oncocarbide, and about to change the dosage of the drug. Analysis of the clinical context based on relevant research and studies CSA is a genetic disease characterized by the presence of hemoglobin S (HbS), which leads to deformations of red blood cells, causing painful vaso-occlusive crises and other complications. HbF has a protective effect against sickling, and the use of oncocarbide can increase its levels, improving clinical symptoms.

Evidence

1.Reduction of Painful Crises:

-The increase in HbF is correlated with a reduction in painful crises in patients with sickle cell anemia (1).

-Studies have shown that higher levels of HbF reduce the polymerization of HbS, decreasing erythrocyte deformation and improving blood flow (2).

2.Effects of oncocarbide:

- Oncocarbide is a ribonucleotide reductase inhibitor, which increases the concentration of HbF in patients with sickle cell anemia. In addition to this, the number of HbF-containing erythrocytes also increases. It reduces the number of circulating reticulocytes and leukocytes, increases the MCV of red blood cells, reduces the deformability of red blood cells, improves blood flow through the capillaries and alters the expression of adhesion molecules, preventing vaso-occlusive crises. (3)

- Hydrocarbamide therapy has been shown to reduce complications (4), and the need for transfusions, as well as improve survival

3. Screening for banormal hemoglobins:

-Test for abnormal hemoglobins is crucial to monitor and manage patients with SCA, especially to detect the percentage of HbF, which can influence the course of the disease

Conclusion

The laboratory that carries out first-level tests for hemoglobinopathies, inserts comments on the result, or recommends second-level diagnostic investigations for correct genotypic characterization. Sometimes, due to a lack of clinical-anamnestic information or the diagnostic question, only numerical results are released and a report is issued without comments. In this specific case, the absence of such information led to a delay in reporting linked to the need to confirm, even with other instruments, the presence of such anomalous hemoglobins in significantly high percentages and not attributable to known or previously characterized hemoglobin levels. Monitoring hemoglobin S in patients with sickle cell disease treated with hydroxyurea is crucial to optimize treatment, improve clinical outcomes, and ensure long-term safety of treatment.

EP357

Predictive prognostic value of gene methylation hotspots of IL6-IL6R inflammatory pathway in cancer

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In the tumor microenvironment, the IL-6 signaling pathway is dysregulated and affects various aspects of cancer (e.g proliferation, invasiveness and metastasis, cancer stem cells self-renew and immune response). Generally, this signaling pathway is hyperactivated and associated with a poor prognosis but the epigenetic modulations of IL-6/IL6R in cancer has not been comprehensively examined.We performed a bioinformatic analysis on the Cancer Genome Atlas (TCGA) datasets aimed to investigate the epigenetic control by DNA methylation in the regulation of IL-6 pathways in cancer.Our analysis mainly reveals that the DNA methylation levels (Methylation450k) of some gene probesets (promoter and body regions) were negatively correlated ($r < -0.3$, $p < 0.05$) either with IL6 or IL6R expression in a large number of tumors. Particularly, the clinical significance of DNA methylation of IL6 and IL6R genes was evaluated by the OS and PFI analysis stratifying the TCGA tumor samples according to the methylation levels of the highly representative cg13104385 and cg05265849 probesets of IL6 and cg09257526, cg04437762, and cg05756780 relative to IL6R. As for IL6, its probesets were widely hypermethylated in all tumors and were generally associated with a favorable prognosis, except for the melanoma in which the methylation of cg13104385 probeset was a negative prognostic factor (log rank: 5.75). IL6R gene expression was affected by intragenic hypermethylation in most TCGA tumors, suggesting a novel regulatory region near the IL6R promoter. The methylation levels of selected IL6R CG probesets negatively affected the patient survival. However, the methylation status of these probesets was a favorable prognostic factor in some cancers such as breast cancer, glioblastoma, lower grade glioma, melanoma, and thymoma.Overall, our results highlight the specific role of IL6R expression and its epigenetic regulation in tumor progression. Although IL6R methylation deserves further validations studies, our results pave the way to identify novel epigenetic biomarkers that are useful for predicting both clinical outcome/survival of cancer patients and the therapeutic response to biologic drugs direct towards IL6/ STAT3 axis.

EP358

Optimization of sodium MR imaging on a clinical 3 T scanner: phantom validation and early human translation

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Introduction:
Sodium ions (Na+) play a crucial role in several physiological processes, and 23Na MR imaging represents an advanced and non-invasive in vivo technique to assess sodium concentration in tissues such as brain, cartilage or skeletal muscle [Gast LV et al., 2023]. This study aimed to develop and validate a protocol for 23Na MR imaging using a dual-receiver birdcage coil (23Na/1H) on a high field 3-T scanner. To ensure reproducibility of sodium signal measurements, quality control procedures were standardized in vitro on phantoms and in vivo on a sample of healthy controls. Preliminary data from patients with hereditary spastic paraplegia type 4 (HSP SPG4) are presented.
Materials and methods:
A custom phantom holder was constructed to hold seven vials in the receiver coil. Vials contained a compound with known sodium concentrations ranging from 20 to 140 mM, reflecting human brain condition in vivo. During post-processing, selected regions of interest were normalized to a linear calibration curve derived from two sodium vials, for estimation of [sodium] and signal-to-noise ratio.Sequence parameters were optimised in vivo based on data from 4 healthy controls (HC). Subsequently, 15 patients with HSP SPG4 (8F/7M, 52±12 yo) and 11 HC (7F/4M, 53±8 yo) were recruited. Morphological images were segmented using FreeSurfer and microstructural diffusion data were used to reconstruct the corticospinal tract (CST). This approach allowed for the extraction of mean total [sodium] values, including both intra- and extra-cellular components, in the regions of interest.
Results:
The measured [23Na] concentrations in the phantom vials demonstrated stability over time: 41.0±2.9 mM for the nominal 40 mM vial and 99.5±1.2 mM for the nominal 100 mM vial.An increase of total sodium concentration in the CST of patients (41±12 mM) was detected compared to HC (32±4 mM) with a corrected p-value of 0.014.
Conclusion:
The optimized 23Na MR sequence demonstrated stability and reproducibility in phantom studies. This metabolic technique revealed alteration of sodium concentration in a clinically relevant target structure of hereditary spastic paraplegia type 4 highlighting the potential role of 23Na measurement as a in vivo biomarker of early neurodegeneration [Müller HP et al., 2022].

EP359

The Pavia Amyloidosis Research and Treatment Center's experience in clonal immunoglobulin gene sequencing through SMaRT M-Seq

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In monoclonal gammopathies (MGs), patients (pts) are characterized by a unique M-protein, produced by a B-cell/plasma-cell clone, that can be used as a specific tumor biomarker and can be involved in organ damage pathogenesis. In our laboratory, was recently established an NGS-based technique, named SMaRT M-Seq, enabling to unbiasedly sequence the full-length variable region of the expressed M proteins. SMaRT M-Seq underwent technical validation to assess sensitivity, reproducibility and accuracy. Since its publication in 2022, the cohort of sequenced pts was extensively expanded and actually account to 432 MGs pts (n=260 AL amyloidosis, n=148 multiple myeloma MM, and n=24 pts with other MGs including MGUS, IgM-related neuropathy, Waldenström's macroglobulinemia), sequencing n=335 bone marrow (BM), n=9 peripheral blood (PB), and n=88 matched BM and PB samples. Initially designed for light chain (LC) sequencing, SMaRT M-Seq was later employed to also study heavy chain, obtaining a total of 463 sequences (n=234 λ and n=195 κ LCs, and n=23 γ, n=8 μ, and n=3 α heavy chains).Validation of the sequence accuracy was performed on 230 pts, integrating SMaRT M-Seq data with Sanger sequencing, biochemical and mass spectrometry (MS) analyses on biological samples from MGs pts, including BM-MNC, fat biopsies, urines and sera. Moreover, the germline gene usage identified in our AL and MM LCs resulted in line with the published literature, reflecting differential gene usage in the two different diseases.In 99% a unique clearly dominant LC sequence was identified with a median molecular clonal size of 90% (IQR: 80,5 – 93,9%) in BM of AL pts and 94,3% (IQR: 90,3 – 95,6%) in BM of MM pts. SMaRT M-seq correctly identified a dominant LC sequence even in pts with a low tumor burden (AL and MGUS), as well as in cases with negative M protein studies. SMaRT M-Seq in PB, eventually combined with MS-based analysis of the urinary proteome, allows the identification of the full-length clonal LC sequence in 98% of pts with MGs without the need of BM studies.Knowledge of the M-protein sequence may allow studies on mechanisms of M-protein related clinical manifestations, as well as personalized medicine approaches for the sensitive detection of the pt-specific monoclonal component.

EP360

Radiotherapy-induced upregulation of PD-1 expression in circulating lymphocytes and increase in soluble PD-L1 levels in Mesothelioma patients: potential biomarkers for immunotherapy eligibility?

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The identification of valuable biomarkers for immunotherapy eligibility is a current challenge in cancer treatment, especially for malignancies managed with multimodal treatments such as Malignant Pleural Mesothelioma (MPM), which is eligible for immunotherapy only under defined circumstances. With the advent of high-throughput multiplexed analytical technologies, peripheral blood is suitable for a deep immune profiling at this purpose. Several blood-based immune biomarkers, as high baseline PD-1+ CD8 T cells and the increase in Ki-67+ T cells, have been recently defined as predictors of better clinical outcome to immunotherapy. In order to identify potential biomarkers of immunotherapy eligibility, we performed the immunomonitoring of MPM patients (n=35) undergoing radical radiotherapy (RT) on the chest wall, a potential immunogenic treatment, by characterizing several immune biomarkers in the peripheral blood. Blood count performed before RT revealed that a higher Lymphocyte-to-Monocyte Ratio correlated with a better overall survival ($p=0.02$), thus suggesting a role of lymphocytes in the prognosis of MPM patients treated with RT. A deeper analysis of T lymphocytes through flow cytometry, revealed that the amount of proliferating cells (expressing the Ki-67 marker) significantly increased at the end of RT ($p<0.01$). Intriguingly, the percentage of CD3, CD4, and CD8 T cells expressing PD-1 raised at the end of RT and even more 1 month after treatment ($p<0.02$). The characterization of the T-Cell Receptor (TCR) repertoire through Next Generation Sequencing showed a higher prevalence of expanded T-cell clones compared to the contracted ones after RT. Moreover, the TCR clonality after RT correlated with the percentage of Ki-67+ CD8 T cells (Spearman rho=0.685, $p=0.02$).Finally, the levels of soluble PD-L1 monitored through ELISA assay significantly increased after RT ($p<0.02$).The immunomonitoring of MPM patients undergoing radical RT revealed that this treatment induced several immunomodulating effects, which have been associated with a better response to immunotherapy, including the increase in PD-1+ CD8 T cells. These data support the pivotal role of circulating T cell monitoring in cancer patients, who could potentially benefit from an immunotherapeutic approach.

EP361

The Pavia Amyloidosis Research and Treatment Center's 25-year experience in molecular diagnostics for hereditary amyloidosis

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Hereditary amyloidoses are caused by destabilizing, toxic, gain-of-function mutations leading to protein misfolding and amyloid deposition. Hereditary amyloidoses can mimic other, more prevalent, acquired forms of amyloidosis, can occur in the presence of an unrelated monoclonal gammopathy or chronic inflammatory disease and/or can lack a clear family history. Therefore, molecular diagnostics is not only essential to diagnose hereditary amyloidoses and identify asymptomatic carriers within affected families, but it also plays a central role in the differential diagnosis of transthyretin (ATTR), light chain (AL), and AA amyloidosis.We revised electronic records of molecular diagnostics for hereditary amyloidoses at the Pavia Referral Center for Systemic Amyloidosis between May-1998 and May-2024.A total of 14085 laboratory-developed molecular tests in 7258 subjects covering 15 genes were performed. A median of 2 genes were analyzed per subject (range: 1-10; IQR: 1-2).The total number of performed tests, evaluated subjects and tested genes showed a progressive increase over time, with a first increment between 2004-2005 related to the discovery of an endemic focus of AApoAI caused by the p.Leu99Pro variant in Northern Italy, and a second increment after 2020, due to increased referral of patients with suspected ATTR/AL cardiac amyloidosis. Also, the proportion of presymptomatic tests for hereditary ATTR has grown over the last few years reflecting increasing availability of effective therapies. In total, we identified 79 different variants (incl. 9 unreported variants) in 1062 different individuals, with TTR and APOA1 accounting for 50.9% and 40.3% of cases, respectively. The number of different variants of clinical significance identified varied from 49 for TTR to 1 for APOA2. One or few variants accounted for the majority of positive tests for TTR/APOA1/APOC2/LYZ, reflecting the presence of geographical clusters of hereditary amyloidoses caused by specific variants across the country.Molecular diagnostics panels for hereditary amyloidoses need to continuously adapt to increasing demand and growing complexity, reflecting improved disease awareness, expanding number of pathogenic genes, potentially overlapping clinical phenotypes and geographic peculiarities.

EP362

An N-glycosylation hotspot in immunoglobulin κ light chains as a risk factor for AL amyloidosis: a validation study

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AL amyloidosis is caused by a B cell/plasma cell clone secreting a patient-unique, amyloid-forming immunoglobulin light chain (LC). Although the amyloidogenic properties of these LCs are encoded in their sequences, the specific molecular determinants of their pathogenicity remain elusive. Over the years, several studies reported a higher rate of N-glycosylation in clonal κ LCs from patients (pts) with AL amyloidosis, compared to other monoclonal gammopathies, suggesting a pathophysiologic role for this post-translational modification.Recently, analyzing a cohort of 220 new and 835 published sequences from pts with AL amyloidosis and with other plasma cell dyscrasias, we reported a significant association between N-glycosylation of κ clonal LCs and AL amyloidosis, identifying a particular hotspot within the DE loop of framework region 3 (FR3DE). Here, we present a validation of our initial observations extending the in silico and biochemical analyses performed on a larger and independent cohort of pts, consisting of 594 published LC sequences from the CoMMpass study (9 AL pts and 585 non-AL pts), and 207 LC sequences we obtained through SMaRT M-Seq from 141 AL (54 κ and 87 λ) and 63 non-AL (49 κ and 17 λ – incl. biclonal pts) evaluated at our Institution. N-glycosylation predictions were performed using GlycoEP, based on superior performance after benchmarking of available N-glycosylation prediction tools. Notably, 44% (25/57) of the κ AL LCs were predicted as N-glycosylated according to GlycoEP, and in 20/25 (80%) the putative N-glycosylation site laid within the FR3DE region, showing the NFT sequon in 45% of these cases. Only 15% (57/380) of the non-AL κ LCs investigated were predicted as N-glycosylated, with 23 κ LCs (40%) located in the FR3DE sequon.For a subset of 101 pts, a biochemical verification of the N-glycosylation status was obtained through Western blotting on urine samples and in 97% of cases the N-glycosylation prediction performed by GlycoEP was confirmed, with only 3 discordant cases (1 false positive and 2 false negative). These data provide an independent validation of the results previously obtained by our group, further supporting the potential role of N-glycosylation in determining the pathogenic behavior of a subset of amyloidogenic LCs.

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Investigating cerebellar proton MR spectroscopy for differential diagnosis: amyotrophic lateral sclerosis versus hereditary spastic paraplegia type 4

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting both upper and lower motor neurons. Early diagnosis of ALS can be challenging as it needs to be distinguished from other motor neuron disorders like hereditary spastic paraplegia (HSP) [Musacchio et al., 2017; Donatelli et al. 2024]. Cerebellar involvement in ALS has been reported in imaging and post-mortem studies [Bede et al., 2021], while in vivo cerebellar metabolism through proton MR spectroscopy (^{1}H-MRS) remains not explorated.

Materials & Methods

We enrolled 29 ALS patients (11F, 66.8±7.3 yo) and 23 HSP patients (13F, 51.3±12.1 yo) with SPG4 gene mutation, without signs of cerebellar atrophy, and 31 healthy controls (HC) (17F, 49.8±16.1 yo). The 3T MR protocol included T1-w 3D morphological images and single-voxel (6 ml) cerebellar ^{1}H-MRS.

Values for N-Acetyl-aspartate (NAA), choline (Cho), and glutathione (GSH) absolute concentrations and relative to creatine (Cr) were extracted using LCmodel v.6.3.

The statistical analyses were conducted using ANCOVA for normally-distributed samples and linear regression with Mann-Whitney test otherwise, including age, sex, and tissue fraction as covariates.

Results

All the spectra were of good quality. Relative concentration of Cho was increased in ALS vs HSP [corrected mean: 0.79±0.10 vs 0.62±0.54, $p<0.0001$]. Only ALS patients showed a reduction of GSH/Cr compared to HC [corrected mean: 0.24±0.06 vs 0.26±0.09, $p=0.002$]. Using [Cho/Cr] as biomarker to classify the two diseases, the ROC curve obtained for corrected values resulted to have AUC=0.83, sensitivity=93% and specificity=78%; for ALS vs HC instead the values using [GSH/Cr] are AUC=0.56, sensitivity=52% and specificity=68%.

Summary

Our prospective ^{1}H-MRS study identified cerebellar biochemical alterations in patients with amyotrophic lateral sclerosis and hereditary spastic paraplegia, providing further insight into the multifocal brain involvement. We illustrated the feasibility (very short time duration of MR scan and spectra analysis: 2min 18s and < 1 min) of MRS-derived markers in a clinical setting to differentiate ALS from potential 'mimics'.

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Bone marrow-free sequencing of M protein genes: a liquid biopsy approach in monoclonal gammopathies

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In patients with monoclonal gammopathies, M proteins are patient-unique, can cause potentially fatal organ damage and be used for personalized tumor tracking. The presence of circulating tumor cells and the possibility to analyze the antibody repertoire through next generation sequencing (NGS) and proteomics approaches may provide a window of opportunity to sequence patients' specific M protein genes in the peripheral blood (PB), without invasive bone marrow (BM) investigations.For this purpose, we have designed an integrated NGS and proteomics pipeline to identify the full- length variable region of the clonal light chain sequences in the PB of patients with monoclonal gammopathies, exploiting SMaRT M-Seq, a novel NGS method recently established and validated in our laboratory.Mononuclear cells (MNC) from PB were subjected to SMaRT M-Seq to obtain the circulating repertoire of the involved light chain isotype in a cohort of patients with MGUS (n=2), AL amyloidosis (n=37), and MM (n=41). Mass spectrometry (MS) analysis of urinary peptides and peptide mapping against the human proteome augmented with the patient's specific circulating repertoire was performed to identify or confirm the clonal light chain sequence. SMaRT M-Seq on matched BM samples to identify the bona fide clonal light chain sequence was performed for confirmatory purposes.Reads with 100% identity to the bona fide clonal light chain were identified in the PB of 78 patients (98%) and were the dominant sequence in 67 cases (86%). In all cases where the most abundant, discrete clonal sequence represented >15% of all reads detected within the PB (n=53, 66%), such sequence invariably coincided with the bona fide clonal light chain sequence. In the remaining 27 patients, mass spectrometry-based analysis of the urinary proteome and mapping of tryptic digestion peptides against each patient's circulating repertoire, identified the bona fide clonal light chain sequence in all but 4 cases. In conclusion, our SMaRT M-Seq liquid biopsy approach enables the identification of the full-length clonal light chain sequence in virtually all patients with monoclonal gammopathies, paving the way for NGS and/or MS-based personalized tumor tracking and minimal residual disease assessment.