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Riassunti Sessioni Scientifiche

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CC	Casi Clinici

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

SP01 - Sfide ed opportunità per la creazione del valore

SP01 – 01

LABORATORY MEDICINE: NOT ONLY RESILIENCE

G. Da Rin

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The COVID 19 outbreak has spread in 2020 to become the most severe pandemic in the last one hundred years. Starting as a public health crisis, it has soon become an economic crisis as well, which will have serious consequences on both individual and societal well-being, now and in the future. COVID 19 has also revealed latent health system fragilities that existed before the outbreak, highlighting the need to consider the resilience of health systems as an equally important dimension of health system performance.

Laboratory Medicine has had and continues to play a crucial role in diagnosing the Covid-19 at an early stage, recognitioning patients that need hospital care, monitoring hospitalized patients, following up patients with long-term COVID-19, and in epidemiologic surveillance.

During the Covid-19 pandemic, Laboratory Medicine is once more demonstrating its inherent and well-known resilience, that means the ability to maintain the laboratory's core mission and operations despite facing a significant crisis.

In our opinion, Laboratory professionals should however embrace a holistic approach, looking beyond developing the ability to recover or adjust to change, but rather adopting an antifragility mindset.

Antifragility is beyond resilience or robustness. The resilient resists shocks and stays the same; the antifragile evolves and gets better.

The road to becoming antifragile is fostering innovation. Innovation is about new ways of create value.

Innovation is crucial, but must be responsible.

Responsible innovation means taking care of the future through collective stewardship of innovation in the present.

Responsible innovation has always been at the heart of laboratory medicine, where we have identified three main topics areas for implementation.

The first refers to the promotion of emerging technologies; new technologies are positively associated with greater efficiency, reduced errors, and improved quality in service delivery and patient outcomes.

The second refers to the enhancement of operational excellence; operational efficiency relates to the ability of a laboratory test to provide fast and efficient results, which may improve the management of the patient with an impact in hospital efficiencies.

The third refers to the improvement of relationships at the clinical interface; laboratory professionals must interact with clinicians throughout the whole diagnostic process as part of a multidisciplinary team aiming to optimize clinical outcomes.

In fact, the key objective of laboratory medicine is to contribute in the decision-making process that ensures the best health outcome for the individual patient.

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SP02 - 01

PERFORMANCE SPECIFICATIONS IN THE IVDR ERA

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In recent decades, the awareness of the important role of laboratory Medicine for the management of status of health of patient has become increasingly prevalent and numerous scientific evidences support this thesis. It is recognized that Laboratory Medicine is a strategic centre of diagnostic medicine and the Institute of Medicine has included Laboratory Medicine services among the ten categories of essential services in the healthcare system of the United States. The reliability of the laboratory results is therefore an essential element to ensure the best management of the patient's healthcare and the evaluation and monitoring of the laboratory performances are important tools to guarantee patient safety. The new European In Vitro Diagnostic Medical Device Regulation, IVDR (EU) 2017/746, requires manufacturers of in vitro diagnostic systems (IVDs) to demonstrate, with scientific evidences, the compliance of their IVDs with the intended purpose, by developing an evaluation plan that test the scientific validity, analytical performance and clinical performance.

In this context, as defined in the Milan Strategic Conference, it is strategic the identification of suitable specifications that define the level of performance to be achieved in relation to desired outcome and comply with practicable (test and economical) requirements and can be updated with regard to the continuous innovation of Laboratory Medicine.

The implementation of a stringent evaluation process of IVDs by manufacturers and the availability of detailed information in technical data sheet, in addition to facilitate the verification of examination procedure according to the ISO 15189 requirements by laboratories, could promote a higher level of quality of laboratory results through the use and spread of suitable performance specifications.

The need of laboratory performance at more and more higher quality levels, is a requirement that cannot be ignored by laboratory professionals that, in collaboration

with the stakeholder of diagnostic process, have to define suitable performance specifications to achieve the desired outcome or reference standards, as a tool to guarantee the patient safety.

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SP01-CO01

EVALUATION OF THE K-INDEX IN INFLAMMATORY DISEASES OF THE CENTRAL NERVOUS SYSTEM IN THE PEDIATRIC PATIENT

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INTRODUCTION: the analysis of cerebrospinal fluid (CSF) and the determination of free light chains (FLC) are routinely used in the laboratory. We investigated the utility of Kappa INDEX as a specific biomarker to identify beyond pediatric multiple sclerosis (MS) also the inflammatory neurological disorders (NID), non-infiammatory neurological diseases (NNID) and other congenital neurological conditions in a pediatric population. METHOD: among 978 patients enrolled in the Clinical Biochemistry Laboratory of Turin (AOU Città della Salute e della Scienza) from January 2019 to May 2022, we analyzed 53 pediatric cases (age 0-17 years), 20 males and 33 females. Albumin, IgG and FLC Kappa in serum and liquor were measured by turbidimetric method, while isoelectrofocusing was performed by oligoclonality test. RESULTS: in according to the clinical diagnosis we obtained four different groups: MS including 6 patients (12%), NID including 25 patients (50%), NNID with 13 subjects (26%) and 6 patients (12%) considered as negative controls (NC). First, ROC curve analysis carried out comparing the SM group to all the others (NID, NNID, NC) showed that a k-index cut-off of 6.2 yielded 83.3% sensitivity and 85.7% specificity, according to data from of the latest publications (Biomolecules 2022,12,677) and the cut-off used by our laboratory (6.15) (Journal of Neuroimmunology 339, 2020). The IgG index (result ³ 0.5, 50% of sensitivity and 45.5% of specificity) confirmed the poor analytical significance. Second, the comparison between NID and NNID group showed that k-index at a threshold of 3.3 was associated with a sensitivity of 80.6% and a specificity of 76.5, and seems an excellent cut-off for the discrimination of pediatric inflammatory diseases, in accordance with the latest publications (Biomolecules 2022,12,677). The IgG index poor quality value was similar to the first study. CONCLUSIONS: in our pediatric population we observed a low number of MS diagnosis. The k-index cut off (6.15) used by our laboratory for MS is confirmed also for pediatric cohort. Moreover, this study found that a lower cut-off of k-index, 3.3, may be applied for the differential diagnosis of inflammatory processes of the central nervous system.

SP01-CO02

URINARY MICROBIAL SIGNATURE BY 16S RRNA ANALYSIS IN BLADDER CANCER PATIENTS

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The role of microbiome in the development of cancer is still unclear. In particular, the presence of microbes in the genitourinary tract raises the question of how the urinary microbiome can influence the development and progression of bladder cancer or how the presence of the tumor can impact the microbial balance of the urinary tract. The study of genitourinary-associated bacteria by sequencing of the bacterial 16S rDNA hypervariable genes using Next Generation Sequencing (NGS) provides a valuable tool in screening, risk identification, and therapeutic possibilities in bladder cancer. The aim of our work was to study the bacterial populations present in the urine samples of patients undergoing transurethral resection of bladder tumor (TURBT), in order to characterize the urinary-associated bacterial profile of these patients. The microbiome was analysed in the samples collected from patients undergoing TURBT (n=34) at our hospital. Each subject provided two urine samples: a first-morning (TURBT-FM) and a catheterized specimen (TURBT-C). First-morning sample of volunteer subjects were used as controls (HC; n=11). Bacterial 16S rRNA hypervariable regions V3-V4-V5 were sequenced using NGS Illumina platform. The sequencing data were evaluated to identify the operational taxonomic units and statistical analyses were performed using dedicated pipelines. Firstly, no significant differences in microbial profiling between TURBT-FM and TURBT-C samples were observed, suggesting that these two samples are comparable. Therefore, we compared TURBT-FM vs HC samples, finding a statistically significant b-diversity (Bray-Curtis index, p-value < 0.011) and showing a notable differences in the bacterial community profile between patients and controls. Based on Univariate analysis data, Lactobacillaceae and Bifidobacteriaceae families resulted less abundant in TURBT-FM than controls. In addition, we found a reduced abundance of Moraxellaceae family, observed up to at genus (Enhydrobacter) and species (Moraxella osloensis) levels. Further insights are needed to explore the role of these taxa in bladder cancer; however, our results suggest the impact of microbiome in the pathogenesis of bladder cancer.

SP02 - La Medicina di Laboratorio nella vaccinazione contro COVID-19

SP02 - 01

COVID-19: RESEARCH IN PREVENTIVE MEDICINE

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At the end of 2019 a new human-transmitted pathogen was isolated in China, and it was named SARS-Cov-2. The World Health Organization (WHO) on 11 March 2020 declared COVID-19 (the illness caused by SARS-Cov-2) pandemic. In Italy, the first European country affected by Sars-Cov-2, the virus made its appearance at the end of February, overwhelming in particular the northern regions of Italy and causing heavy loss of life and public health crisis. To deal with the pandemic, the centralization of health resources has put the entire National Health System in difficulty, causing postponements and delays in specialist and diagnostic visits and also impacting primary prevention interventions.
Already in the first months of 2020, the WHO had stressed how important it was to keep vaccination services active and prevent further deterioration of vaccination coverage, already stalled in the pre-pandemic era, to prevent children and public health from being threatened by other diseases, risking to cause the transition from one health crisis to another. If this is fundamental in middle-high-income countries, when it comes to low-middle-income countries it becomes dramatic, suffice it to say that the global vaccination coverage data already reported a stalemate in the pre-pandemic era (85%) for DTP 3 and measles vaccines.
World Immunization Week and European Immunization Week are occasions to rekindle the spotlight on the importance of all vaccinations because, although today the focus is mainly on those against COVID-19, it is necessary to remember that vaccines are a preventive tool against numerous infectious diseases and every day allow millions of lives to be saved.
Now, two vaccines against SARS-CoV-2 that are available in USA and Europe, are based on mRNA encoding the SARS-CoV-2 spike protein, a key target of neutralizing antibodies. mRNA vaccine technology is not totally new as it has already been studied and developed before for flu, Zika, rabies, and cytomegalovirus. This new kind of vaccines are formulated in lipid nanoparticles allowing mRNA delivery into cells and they can induce the production of the viral spike protein. mRNA is just transitorily expressed and does not interact with the genome.
The European Medicines Agency (EMA) gave also its approval of two COVID-19 vaccines that consist of an adenoviral vector containing the SARS-CoV-2 spike protein gene and one vaccine that contains the spike protein produced by recombinant DNA technology, using a baculovirus expression system in an insect cell line.
Ema approved the sixth vaccine for protecting against COVID-19 on 24 June 2022; it is an inactivated, adjuvanted vaccine. The vaccine contains whole particles of the original strain of SARS-CoV-2 that has been inactivated and two adjuvants (aluminium and cytosine phospho-guanine).
Vaccination has been shown to contribute to reducing deaths and severe illness from COVID-19, and to reduce the transmission of COVID-19.

SP02 - 02

MONITORAGGIO DELLA RISPOSTA ANTICORPALE AL VACCINO

G. Lippi

Verona

The immune response versus infective agents, thus including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), comprised the so-called adaptive immunity, which encompasses the generation of antibodies by B cells and cytotoxic activity by T cells, compounded by the immune memory, which has the role to contrast recurrent infections by the same pathogen. Serological testing has been conventionally defined as a diagnostic procedure used for detecting an immune response against an infectious agent, which can develop following either natural or artificial immunization. The crucial question that has emerged since the beginning of the worldwide COVID-19 vaccination campaign is whether or not laboratory monitoring of COVID-19 vaccination may be clinically useful and economically sustainable. Nonetheless, there are several reasons that contribute to justify the utility of serological monitoring after vaccination, including the opportunity (i) to know in advance whether vaccine recipients have passed a recent SARS-CoV-2 infection, (ii) to monitor the individual humoral response after vaccination, (iii) to verify the humoral response in presumably “low-responder” populations, and, last but not least, (iv) for timely detecting a fast decay of humoral protection [1]. According to the current knowledge, a timeline for serological testing could be suggested, entailing pre-vaccine measurement to precisely identify whether or not the subjects has been recently infected by SARS-CoV-2, followed by at least 2 following tests between 1 and 6 months to detect faster decline of anti-SARS-CoV-2 antibodies. As concerns the technical aspects, it may be advisable to use immunoassays capable to recognize antibodies targeting the entire trimeric spike protein, its S1 subunit, or its receptor binding domain, along with techniques that generate accurate quantitative values (1). Due to low degree of harmonization so far, an identical assay shall be used for longitudinal monitoring of antibodies values, and we finally advise against performing these measurements outside of clinical laboratories. That said, a major concern is rising. This is specifically due to the fact that the antigen and epitopes of the prototype SARS-CoV-2 lineage used for coating some immunoassays could no longer mirror the sequence of the spike protein or the

RBD of some circulating variants, such as, for example, the highly mutated Omicron lineage. Contextually, the anti-SARS-CoV-2 antibodies elicited by these highly mutated SARS-CoV-2 lineages could be no longer reliably detected by some commercial immunoassays. Therefore, along with compulsory re-evaluation and revalidation of their methods against live virus neutralization assays, diagnostic companies must also embark in redesigning assays by replacing ancestral SARS-CoV-2 antigens with those of highly mutated SARS-CoV-2 variants (2).

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SP02-03

MONITORAGGIO DELLA RISPOSTA CELLULARE AL VACCINO

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La vaccinazione per COVID-19 e l'infezione da SARS-CoV-2 inducono sia una risposta umorale mediata dagli anticorpi prodotti dai linfociti B, di tipo neutralizzante (NAbs) e non-neutralizzante, sia immunità cellulare mediata da T linfociti e cellule B di memoria. Nello sviluppo e nella sorveglianza dell'immunità a seguito di vaccinazione, la ricerca si è inizialmente focalizzata sul ruolo degli anticorpi neutralizzanti con minor interesse per il ruolo delle cellule T e B di memoria, ed ancora con scarso interesse per il ruolo degli anticorpi non-neutralizzanti che, comunque, conferiscono protezione grazie a meccanismi di opsonizzazione e citotossicità cellulare anticorpo-dipendente. Vi è, però, crescente evidenza dell'importanza del contributo delle cellule T alla risposta immunitaria dell'ospite ed in particolare per la rapida e durevole protezione rispetto l'infezione o re-infezione da SARS-CoV-2, specialmente nei confronti delle nuove varianti di interesse (VOC). Vi è sempre maggior consapevolezza che la diminuzione della protezione verso malattia asintomatica o COVID-19 di lieve entità non sia accompagnata da un declino parallelo della protezione verso malattia severa, protezione che rimane elevata -attorno al 70%- anche a distanza di mesi dal completamento del ciclo primario di vaccinazione ed anche a fronte di una significativa diminuzione degli anticorpi circolanti anti-SARS-CoV-2. Una possibile spiegazione del fenomeno proviene da un numero sempre maggiore di studi che hanno valutato l'efficacia dell'immunità cellulare anche verso linee altamente mutate di SARS-CoV-2, in particolare Omicron, che concordano nel dimostrare che la vaccinazione determina una risposta immunitaria cellulare tale da cross-reagire con una moltitudine di varianti di SARS-CoV-2, inclusa Omicron. Dal punto di vista della medicina di laboratorio, vi sono molti aspetti che devono essere affrontati, in particolare le tematiche della riproducibilità e standardizzazione dei metodi di studio dell'immunità cellulare visto che molti studi finora sono stati eseguito con metodi sperimentali e su casistiche poco rappresentative. Tuttavia, alcuni di questi metodi sono oggi disponibili a livello commerciale e devono essere verificati e validati nei laboratori clinici.

SP02-CO03

EVALUATION OF QUANTIFERON SARS-COV-2 INTERFERONG (INF G) RELEASE ASSAY IN TWO COHORT OF BNT162B VACCINATED FRAGILE PATIENTS

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Background: Severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) vaccination is the standard of care for the prevention of COVID-19 disease, with a positive impact in countries in which vaccination has been promoted. Since the emergence of variants of concern (VOCs) European Medicines Agency (EMA) recommended an extra dose of the COVID-19 vaccines Comirnaty (BioNTech/Pfizer) and Spikevax (Moderna) for patients with severely weakened immune system and booster doses for subjects with normal immune system to ensure a lasting response. Although Vaccination triggers both humoral and cellular immune response, COVID-19 vaccination efficacy is evaluated by measuring antibodies only, whereas adaptative cellular immunity is unexplored. Our aim is to test this new kit QuantiFERON SARS-CoV-2 to evaluate the immune response after three doses of BNT162b vaccine in healthy donors compared to two cohort of fragile patients: Common

Variable Immunodeficiency (CVID) patients and Kidney Transplant Recipients (KTR) patients.
Methods: Blood samples were collected from eight health care workers in our department, fourteen CVID patients and eight KTR patients. All the individuals recruited were naïve to SARS-COV2 and immunized by three doses of BNT162b vaccine. We examined humoral responses to vaccinations using the LIAISON DIASORIN "SARS-COV-2 S1/S2 IgG". Next blood from all participants was subjected to the novel Interferon γ (INF-γ) Release Assay (IGRA) from Qiagen, measuring INF-γ release induced by two proprietary SARS-CoV-2 peptide pools (Ag1 and Ag2) encompassing the spike protein and designed to stimulate CD4+ and CD8+ T cells and induce the releases of INF-γ. Results:Using LIAISON "SARS-COV-2 S1/S2 IgG" assay from DIASORIN we confirm that in healthy subjects BNT162b third dose had successfully mounted humoral immune response with a S1/S2 IgG mean of 17100 BAU/ml. Conversely, the CVID group and KTR group shown a statistically significant reduction of IGg levels with a mean of 978 BAU/ml and 1029 respectively. Notably seven patients (five CVID and two KTR) presented IGg levels below the cut-off (33,8 BAU/ml). Next, we evaluated the INF-γ response to SARS-CoV-2 Ag1 and Ag2 founding seven non-reactive subjects (three CVID and four KTR). Surprisingly three of non-reactive patients shown a good humoral response, whereas three patients with a negative humoral immune response shown reactiveness to IGRA assay.
Conclusions: Assessing cellular immunity for SARS-COV-2 in addition to humoral immunity is important taking into account that cellular immunity plays a pivotal role against the virus and likely its variants. Some patients with weakened immune response have no correlation between humoral and cellular immunity, suggesting that the evaluation of T cell responses could be a more sensitive clinical marker of immunization. In this scenario the evaluation of cellular immunity might be informative for clinicians to identify patients more susceptible to a severe COVID-19 disease.

SP03 Telemedicina e Medicina di Laboratorio

SP0 3- 01

MACHINE LEARNING AS A TOOL FOR LABORATORY MEDICINE, TO REALIZE TRULY PERSONALIZED CARE.

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Machine learning (ML) promises to reduce errors and increase efficiency in cognitively complex tasks in every application domain where this general computational approach is proposed. Human beings stand to benefit from being able to delegate, partially or completely (with some risk or limitations), the execution of classification tasks to machines in situations where humans are either scarce or fallible resources, or both. As known, many medical specialties rely on the services of laboratory medicine (LM) to obtain responses and data that are characterized by unparalleled quality, reliability and stability (even considering imaging exams that are not dependent on operators), ranging from blood biomarkers and genetic profiles to bacterial cultures. The implementation of machine learning in this context may result in the enhancement of various classification tasks and have a variety of downstream effects on medical practice: once a patient has been defined in terms of relevant factors (such as gender, age, ethnicity, or metabolic characteristics and lifestyle), ML may assist practitioners determine whether the findings for that patient are abnormal and pathological, or normal; this can occur even based on earlier examinations of the same patient, therefore attaining the goal of personalized or precision medicine; in order to reach the ideal of the blood test as a "liquid biopsy," these methods may also assist in determining whether a particular patient is suffering from a certain disorder (whose "signature" was discovered in their tests); also, ML may assist in determining the validity of the equipment's data (1); and in determining if it is acceptable to prescribe further particular tests to the same patient from more common, faster, and less expensive procedures (such as complete blood count) (2). The potential effect of these applications is generating a lot of attention, as noted by literature reviews (3-4), which have lately attempted to account for the exponential growth in the number of articles detailing applications in the above-mentioned contexts (and others). However, the LM community must not repeat the mistakes of other communities that have been enticed by the potential of ML without assessing either the assumptions (asking whether these systems are truly accurate on cases other than those on which they were trained, i.e., whether they are robust and therefore trustworthy in the real world, in the wild); or the potential consequences (such as, for instance, the risk of increase in defensive medicine, of greater overuse and therefore overdiagnosis, as well as of the deskilling of diagnosticians and laboratory technicians (5)).The professional figure who is sometimes advocated to use these new machines and deliver the services that these will enable must therefore be able to evaluate the reliability of these systems, the appropriateness of the requests, and the plausibility and reliability of the results, for the greater patient safety and the long-term economic, social, and human sustainability of the health system into which they will be embedded.

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SP03-CO16

THE NEED OF INFORMATIC STANDARDIZATION IN THE MANAGEMENT OF LABORATORY TEST SEMANTICS

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A centralised public electronic health record system has been established in many advanced countries to ensure continuity of care. In order to enable interoperability between the different clinical data sources and to overcome their purely documentary level, the adoption of standard criteria is needed. Laboratory medicine can make a significant contribution to this goal through its mature and well-established digitisation.However, the effectiveness of IT support depends on the synergy achieved between both communities of health informaticians and laboratory staff.Since 2015, the Italian government, with DPCM 178/2015, has established the technical rules to ensure the semantic interoperability of the FSE (electronic health record), adopting the LOINC (Logical Observation Identifiers Names and Codes) coding system for laboratory examinations. The aim is to achieve unambiguous recognition of the type, method, and result description.To date, each Laboratory Information Systems (LIS) uses its own coding systems, thus making test results from different labs not comparable de facto. Translating laboratory codes into LOINC requires a deep knowledge of both coding system and local reality. Therefore, such translation can be obtained only by a multidisciplinary approach.We present two examples of this collaboration at the level of semantic interoperability, where it is most urgently needed.In early 2010s, we performed a pioneering study in Liguria Region on a subset of laboratory data obtained from HIV infected patients, to be used for regional observational studies. Starting from this limited experience we underwent a more structured project with the Veneto Region obtaining the translation of all laboratory analyses offered in that region. The lesson learned from both experiences was that a strong commitment from specialized personnel is needed and proper tools must be used to effectively monitor the evolving realities of both laboratory technology and of the code itself. Terminology management standards, as the Common Terminology Service (Version 2) and the FHIR Terminology Server, both supported by the international standards organization Health Level 7, are available and their use is strongly suggested to support this continuous updating effort.

SP03-CO17

TELE-CONSULTATION IN THE MANAGEMENT OF SYSTEMIC LIGHT CHAIN (AL) AMYLOIDOSIS: THE PAVIA AMYLOIDOSIS CENTRE EXPERIENCE.

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Light chain (AL) amyloidosis complexity requires specialized laboratories and use of highly sensitive methods to monitor the disease for hematologic (HR) and organ response (OR). After COVID-19 outbreak, access to our laboratory was limited and a tele-consultation (TM) program was built. During the first wave, all planned visits were converted to TM. When restrictions were eased, TM was proposed to patients that did at least one in- person visit. We asked to perform at a local lab: s. protein electrophoresis, s. and u. immunofixation and measurement of s. free light chains (FLC), creatinine, NT-proBNP (or BNP), troponin I, alkaline phosphatase and 24h proteinuria. Lab already connected with the Italian Society of Amyloidosis were suggested and we verified consistent use of the same FLC assay for HR. Biopsy samples can be shipped to Pavia for amyloid typing. In 2020, 369 TM were performed. In 102 (27%) this was the first evaluation. In 73 (71%) a final diagnosis was made: 18 systemic AL, 19 localized-AL, 33 wild-type transthyretin, 2 reactive AA and 1 LCDD. In 13 (13%) amyloidosis suspicion was not confirmed and 16 (15%) were already on treatment for AL and a second opinion was discussed. A TM was done in 276 (72%) patients previously seen. Of them, 214 (80%) had systemic AL with 99 (46%) on active chemotherapy [89 (41%) proceeded with therapy, 10 (4%) suspended due to sustained HR]. Seven (3%) started a new treatment due to loss of HR. In all other cases, HR was confirmed and treatment free interval was prolonged. In 2021, 109 patients performed TM: 54 (50%) systemic AL, 44 (38%) localized-AL, 5 (5%) AA, 6 (5%) monoclonal gammopathies of clinical significance. In 11 (19%) patients ongoing chemotherapy was proceeded and a new therapy was suggested due to increased FLC along with organ biomarkers in one case. In all other cases, follow-up was advised. TM allows for an integrated approach between lab and clinical management. We reached a final diagnosis in 71% of cases and we assessed HR and OR in local lab

making clinical decisions. Despite being a response to pandemic, this hub and spoke TM model proved effective in a complex disease. The use of standardized method in laboratory practice and in the clinic is crucial to the success of TM for patients' management.

SS01 - Il contributo del Laboratorio di Coagulazione nel monitoraggio delle terapie antitrombotiche: interazione Clinica-Laboratorio

SS01-01

LABORATORY ASSESSMENT OF THE ANTICOAGULANT ACTIVITY OF THE DIRECT ORAL ANTICOAGULANTS (DOACS)

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DOACs represent a class of direct-acting oral anticoagulants which, unlike vitamin K antagonists (AVK), act by inhibiting a single coagulation factor: thrombin (Dabigatran) or Factor Xa (Apixaban, Edoxaban and Rivaroxaban). These drugs have shown equal efficacy and safety compared to VKAs in the treatment and prevention of venous thromboembolism and systemic embolism in patients with non-valvular atrial fibrillation. DOACs are given at a fixed dose and therefore do not require dosage adjustments based on laboratory test results. The unnecessary dose adjustment has probably discouraged many laboratories from implementing specific tests for the assay of DOACs. In recent years, however, consensus has been increasing among experts and scientific societies on the usefulness of evaluating, by means of laboratory tests, the anticoagulant activity of DOACs in specific patient populations, usually in urgent/emergency situations. Indeed, the inter-individual variability of the levels is very high (around 70-80%) and both post-hoc analysis of the phase III clinical trials and some "real life" studies demonstrated the presence of a correlation between the DOAC level and the risk of bleeding and thrombotic complications.

The situations in which the measurement of DOAC is recommended (or at least suggested) are: 1) in presence of hemorrhagic or thrombotic complications; 2) in patients with ischemic stroke candidates for thrombolysis; 3) in case of surgical or invasive procedures; 4) if the use of antidotes is indicated; 5) in patients in whom an excess of anticoagulation is suspected; 6) in case of simultaneous use of interfering drugs; 7) in under- or overweight patients and in any case in frail/elderly subjects; 8) in case of worsening of renal/hepatic function.

Spectrometry/high-pressure liquid chromatography represent the reference tests to measure the concentrations of plasma DOAC levels, but these techniques are often not available in general laboratories and the turnaround time is not compatible with urgent/emergency situations in which the assay is recommended/suggested. In recent years, specific tests for the determination of DOAC activity have been developed, and are easily to be implemented in any laboratory, such as: diluted thrombin time, anti-FIIa activity and ecarin time (Dabigatran) and anti-FXa activity (Apixaban, Edoxaban and Rivaroxaban). These tests are simple, relatively fast, with acceptable inter-laboratory CV%, as demonstrated by the most recent EQA exercises. The results must be expressed as concentration in ng/mL. To date, the therapeutic ranges for these drugs are not known, so in the laboratory report may be indicated: 1) the expected levels at the steady state, at trough and at peak (i.e. 2 hours after the last intake), measured in patients enrolled in the phase III clinical trials or 2) a critical level, i.e. a value below which the bleeding risk can be considered negligible (<30 or < 50 ng/mL).

SS01-02

PLATELET FUNCTION TESTING CURRENT PRACTICE AMONG LABORATORIES: HOW, WHEN, WHY AND TO WHOM

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Platelets play a central role in physiological hemostasis and also in pathological thrombosis. Quantitative and/or qualitative platelet defects promote bleeding, whereas strong platelet reactivity may associate with thromboembolic complications.

The main use of platelet function tests has been traditionally to identify the potential causes of abnormal bleeding, to monitor pro-hemostatic therapy in patients with high risk of bleeding and ensure normal platelet function either prior to or during surgery. However, nowadays there is also much renewed interest in monitoring the efficacy of anti-platelet therapy and measuring platelet hyper-function or to predict thrombosis.

The current evaluation of a potential platelet defect usually involves platelet aggregation and/or measurement of granule content/release. These test are labor intensive, costly, time consuming and require a fair degree of expertise and experience to perform and interpret therefore are largely restricted to the specialized laboratory or center. Also additional expensive specialist tests are often required (flow cytometry, platelets nucleotides, Proteomics, Next Generation Sequencing).

A number of dedicated platelet function instruments that are much simpler to use and are now utilized as point-of-care (POCT) instruments have become available in last years. Some POCT instruments have been incorporated into routine clinical use and can be utilized not only as general screening tests of platelet function but as monitors of antiplatelet therapy and to potentially assess both risk of bleeding and/or thrombosis.

Given the advances in platelet function testing field new tests, mostly POCT, have been incorporated into the routine laboratory analysis. Because of the general consensus that in vivo bleeding time should be replaced, the development of reliable, sophisticated but simple to use whole blood tests that simulate in vivo hemostasis provides the ability to screen samples rapidly before

applying our existing test set and could be used as a reliable bleeding test substitute.
Many of the simpler platelet function tests could also be potentially utilized as POCT instruments to assess bleeding risk and to monitor antiaggregant therapy. Platelet function testing is therefore become increasingly utilized outside of the specialized laboratory or center.
Finally, important developments in the platelet genome and proteome are leading to advances which may have significant impact upon the diagnosis and management of patients with hemostatic and/or thrombotic defects.
In conclusion, many tests are available to assess platelet function and no one is perfect. New platelet function tests will continue to become available. Selecting the right test requires careful consideration: purpose, timeframe, available expertise and cost.

SS01-03

IL CLINICO E L'INTERPRETAZIONE DEI DATI DI LABORATORIO NEL MONITORAGGIO DELLE TERAPIE ANTITROMBOTICHE: QUALI PROBLEMATICHE, QUALI UTILIZZI?

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Not all treatments with anticoagulant drugs require monitoring. Heparin and low molecular weight heparins (LMWH) and Fondaparinux usually do not need any laboratory measurement when the drugs are administered for prevention of venous thromboembolism (VTE), or - generally in clinical practice - when administered at full doses in relation to the body weight. In contrast, vitamin K antagonists (VKAs) always require frequent blood test (INR) controls to reach and maintain over time the required anticoagulant effect. These drugs, however, are used for many decades and clinicians have learned how to monitor their anticoagulant effect. The direct-acting oral anticoagulants (DOACs), are currently the most frequently used anticoagulant drugs for the prevention and treatment of venous thromboembolism and systemic embolism in patients with non-valvular atrial fibrillation. Being given at fixed doses, these drugs do not require routine laboratory monitoring and dose adjustments. For this reason, most clinicians have so far overlooked the issue of DOACs laboratory measurement. However, in recent years consensus has been increasing among experts and scientific societies on the usefulness of laboratory tests, to assess the anticoagulant activity of DOACs in specific patient populations, especially in in urgent/emergency situations, such as in presence of hemorrhagic complications to decide on use of the now available antidotes, or to decide on use of thrombolysis in case of thrombotic complications, or when surgical or invasive procedures are needed in emergency. For the large and increasing use of DOACs in the general population, the frequency of patients in urgent/ emergency conditions or in other clinical states requiring an assessment of their anticoagulant activity will increase sharply. Subsequently, the number of clinicians who must cope with this issue will also increase. However, several obstacles are limiting their full participation and comprehension to this activity. The decision on which tests should be available is laboratory staff's concern; however, the turnaround time of tests should be compatible with the clinical needs. Though therapeutic intervals of DOACs are not available, intervals suggested as appropriate in relation to the different clinical condition should be clearly mentioned in laboratory reports, possibly as results of collaborative decisions. Some laboratory-specific aspects, that are important for the correct interpretation of laboratory results, should be clearly detailed in written and repeatedly circulated among clinicians and nurses (this is the case -among others - for the correct timing of blood sampling in relation to LMWH or DOAC administration, or for the interference of LMWH on activity results of anti-Xa DOACs).

SS01-CO04

EVALUATION OF HYPERCOAGULABILITY IN PATIENTS WITH MULTIPLE MYELOMA USING EXPERIMENTAL PARAMETERS OF ROTATIONAL THROMBOELASTOMETRY: MAXV, MAXVT, AUC

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INTRODUCTION: Haematological malignancies are, generally, associated with an increased thrombotic risk with a thrombosis rate 28 times higher than in healthy individuals. Multiple Myeloma (MM) is a haematological neoplastic pathology with strong thromboembolic repercussions. Thrombogenicity of MM is multifactorial and risk factors are traditionally divided into three groups: patient-related clinical risk, disease-related risk, and treatment-related risk. Venous thromboembolism in these patients occurs during the first months of treatment with immunomodulatory drugs (IMiDs) in combination with high-dose dexamethasone. Although therapy with IMiDs improves the clinical outcome, it is highlighted a concomitant increase of thromboembolic manifestations which make the anticoagulant therapy in MM patients difficult to manage. To date, the viscoelastic tests, assessing global haemostasis, allow to stratify the thrombotic risk in these patients. To evaluate the coagulation dynamics proprieties, we investigated experimental parameters [Maximum Velocity of Clot Formation (MAXV), Time to MaxV (MAXVt) and Area Under the Curve (AUC)], by using Rotational

Thromboelastometry (ROTEM).
METHODS AND RESULTS: The study included 35 MM patients (at diagnosis and follow-up) and 35 healthy subjects unrolled from Haematology Division of AOU Federico II of Naples. The EXTEM, INTEM and FIBTEM assays were overall investigated for both standard and experimental parameters. Particularly, among the experimental parameters, evaluating coagulation dynamics proprieties, we observed statistical significance differences between controls and patients for EXTEM (MAXV: p=0.003; MAXVt: p=0.01), for INTEM (MAXV: p=0.005; MAXVt: p=0.043) and for FIBTEM (MAXV: p=0.001; AUC: p=0.0005) tests.
CONCLUSION: To the best of our knowledge, this is an original study showing that these experimental parameters can give an additional contribution in the interpretation of the hypercoagulability state, both at diagnosis and at follow-up. This approach could be a valid tool for a prompt response of the coagulative status, compared to numerous and more investigating classic coagulation tests and allowing physicians to give a timely and adequate anticoagulant treatment.

SS01-CO05
FACTOR V DEFICIENCY REVEALED DURING DOAC THERAPY

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A 68-years-old-man, with a history of bleeding diathesis not investigated, after an adenoidectotmy and a post traumatic splenectomy, accessed to the first aid of the Pontedera Hospital for intense asthenia and discharge of black stools. Laboratory tests revealed severe anemia (hemoglobin 4 g/dL), Prothrombin Time (PT) 3,42, activated Partial Thromboplastin Time (aPTT) 3,8. In the previous months onset of exertional dyspnea, lower extremity edema and diagnosis of atrial fibrillation. It was administered to him low molecular weight heparin (LMWH) replaced later with dabigatran 150 mg BID to prevent stroke. Dabigatran was later suspended due to gastric intolerance and replaced with edoxaban
60 mg SID. The colleagues from the Pontedera Hospital suspended edoxaban and request second level coagulation tests, they sent us frozen citrated plasma samples. We first made sure there was no residual edoxaban in the circulation. Then we did the mixing tests for PT and aPTT, at room temperature and after incubation for two hours at 37°C. The mixing tests corrected coagulation times. Measures of coagulation factors revealed us severe deficiency of FV (1,1%). The other factors resulted: FVII 41%, FXII 51%, FIX 117%, FXI 107%, FVIII 171%, FX 65%, FII 62%. No antibodies against FV, FII and FVII were found. The patient was sent to Antithrombosis Centre in Florence to evaluate the most appropriate therapy to prevent stroke and the opportunity of the surgical closure of the left atrial appendage.Conclusions- When starting anticoagulant treatment, according to consolidated guidelines, knowledge baseline blood works (blood count, renal and liver function, full coagulation panel) is required. Our patient, which showed a likely congenital deficiency of FV, was treated with DOAC without having performed recommended laboratory test and his bleeding diathesis after surgery was probably not carefully evaluated. The collaboration between colleagues with different skills and experience is the most effective way to diagnose rare disease and to choice the most appropriate therapy in a comorbidity setting.

SS02 - Ruolo del monitoraggio terapeutico del farmaco nella moderna Medicina di precisione: considerazioni tra presente e futuro

SS02-01
IL MONITORAGGIO TERAPEUTICO DEL FARMACO (TDM): CRITICITÀ IN ETÀ PEDIATRICA

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A recent study published by Divya Hoon and colleagues (2019) has shown that in 44.5% of visits to office-based physicians who prescribed systemic drugs to children, these drugs were prescribed off-label (1). The term "off-label" use refers to use of a drug that is not included in the package insert (approved labeling) for that medication. Specifically, dugs are used off-label when administered for an unapproved indication or in an unapproved age group, dosage, or route of administration (2). Unfortunately, the majority of drugs administered to neonates and children are off-label due to the lack of clinical studies conducted on this special population. As consequence, dosing strategies adopted to treat children are often translated from adult studies by using allometric scales. However, pharmacological treatments applied to these patients should deserve more tailored dosing approaches. Therapeutic Drug Monitoring (TDM) is defined as assessing the adequacy of the drug plasma concentrations in relation to a target concentration or concentration window at a specific time in a dosing interval. Therefore, TDM represents an useful tool not only for monitoring plasma concentrations of drugs with a narrow therapeutic index but also to evaluate the PK behavior of different medications especially when administered to neonates

and children. Unfortunately, TDM-based studies are not always applicable to these patients due to ethical and physiological concerns that limit the number of samplings and the volume of blood withdrawable. In this brief communication, we will focus on the main criticisms in performing TDM in neonatal and pediatric patients. These will include:

- Age-related pharmacokinetic (PK) variability. In fact, developmental and physiological changes that characterize neonates and children can dramatically affect PK behavior of many drugs (3);
- Necessity of sensitive bioanalytical technologies. TDM should rely on analytical methods such as high-performance liquid chromatography coupled to UV (HPLC-UV) or to mass spectrometry (LC-MS/MS) characterized by fast detection, high accuracy and precision (4);
- Novel microsampling strategies that are not influenced by heamtocrit (Hct). Volumetric absorptive microsampling (VAMS) have been explored as alternative to dried blood spot (DBS) and allow to overcome the DBS-associated Hct effect (5);
- Need for therapeutic ranges tailored on neonates and children. Children are not small adults and evidence-based therapeutic ranges are arguably even more important in these subjects;
- The impact of excipients in pediatric formulations. Excipients such as propylene glycol should be closely monitored alongside to drugs' levels for their possible toxicity.
- The risk of drug-drug interactions during poly-therapy. TDM is particularly advisable when multiple drugs are administered in order to monitor PK interactions;
- The occurrence of metabolism-related adverse events. Some pharmacological treatments can interact with specific metabolic pathways leading to adverse reactions (i.e hyperammonemia).

Therefore, the aim of this presentation will be not only to describe these criticisms but also to provide possible suggestions to overcome them.

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SS02-02

PERSONALIZZAZIONE DELLA TERAPIA NEL PAZIENTE ADULTO E ANZIANO.

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Pharmacogenetics (PG) represents the "branch" of pharmacology that guides the personalization of pharmacological therapies. Alongside PG, Therapeutic Drug Monitoring (TDM) has been proposed and included in many different guidelines for numerous therapies in order to optimize the treatment regimen and personalize therapies in young, adult and elderly patients. The TDM of drugs is used daily in various clinical settings where it represents some help to reach a further possibility of clinical-therapeutic success, especially in particular clinical cases and setting, promptly personalizing the therapy. The elderly patient, specifically, has physiological characteristics (lower renal clearance, lower hepatic metabolism, etc.) capable of making the pharmacokinetics of the drugs substantially unpredictable. Moreover, in the elderly patient often could be present the more consistent problem of polytherapy due to the concomitant presence of various pathologies to be treated (as diabetes, hypertension, hypercholesterolemia, etc.). Other than the "small molecules", many of the new biological drugs, such as monoclonal antibodies, may also show different pharmacokinetic patterns depending on the patient's age. Therefore, both the methodological aspect of TDM, both the pharmacological profile obtained from it, and the rationale for its use, are extremely relevant for obtaining reliable results and, at the same time, helping the clinician in diagnostic and therapeutic decisions, with the final aim to personalize the therapies.

SS02-03

ANTIVIRAL DRUGS FOR THE TREATMENT OF COVID-19 PATIENTS: APPLICATIONS OF DRUG MONITORING

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In the last years, several studies on new and/or repurposing antiviral drugs were initiated to fight Coronavirus disease 2019 (COVID-19) pandemic. Three antivirals have so far been authorised in Italy for the treatment of COVID-19 in adults who do not need supplemental oxygen and

who are at high risk of progressing to severe COVID-19. Specifically, the drugs currently authorized are Remdesivir (intravenous route) and the orally administered Molnupiravir and Nirmatrelvir-Ritonavir (Paxlovid). Remdesivir is a prodrug of the nucleotide analogue (GS-441524) which inhibits the SARS-CoV-2 RNA-dependent RNA polymerase. Remdesivir has been shown to improve the COVID-19 outcome in different settings. Molnupiravir is a prodrug that after entering in the cells changes into an active form of triphosphate, ready to be incorporated into viral genome causing many errors in SARS-CoV-2 RNA. In a clinical trial, Molnupiravir compared to placebo showed a 30% reduction of COVID-19-related hospitalizations. Paxlovid is a combination of Nirmatrelvir and Ritonavir. Nirmatrelvir acts by inhibiting protease enzyme, essential step to transform some viral proteins into their final functional form. The relative risk reduction of hospitalization or all-cause death at day 28 for Paxlovid compared to placebo was 88%. To guarantee safe and effectiveness of the pharmacological therapies, the evaluation of patient's pharmacokinetics (PK) profile is mandatory. Therefore, the monitoring of drug concentration of antivirals against SARS-CoV2 could be pivotal to optimise drug regimens, increase efficacy and avoid drug-related toxicity and to evaluate intra-individual variability and drug-drug interactions. Actually, few data on antiviral drugs concentrations in COVID-19 subjects are published. Among them, the most interesting are the following: i) Remdesivir and its main metabolite showed high PK interpatient variability due to both age and renal function in COVID-19 inpatients. The PK variability may have a potential effect in determining the efficacy of Remdesivir administration in patients affected by COVID-19. ii) Molnupiravir metabolite penetration into upper airways and mucosal secretions was demostred. These data could support the use of molnupiravir in a prophylaxis for SARS-CoV-2 infection iii) Nirmatrelvir displays a short half-life, which could result in suboptimal drug exposure and difficulties in achieving efficacy. Therefore, there is the need to use ritonavir (CYP3A4 Inhibitor) to slow down the metabolism and to increase the plasma concentrations of Nirmetrelvir. Drug-drug interactions are expected when drugs metabolized by CYP3A4 are co-administered with Paxlovid. Further research on antiviral drugs concentrations in COVID-19 patients could help to define therapeutic strategies more efficient and appropriate to treat SARS-CoV-2 infection.

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SS02-04

THERAPEUTIC DRUG MONITORING IN ALTERNATIVE MATRICES: FROM LABORATORY TO CLINICAL PRACTICE

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Therapeutic Drug Monitoring (TDM) is a diagnostic tool used for years in daily clinical practice to optimize the exposure of patients to drugs, with the goal to maximize the therapeutic response while preventing at the same time the development of drug-induced toxicity.

TDM consists essentially in determining the concentrations of a drug in an easily accessible biological matrix, normally serum, plasma or whole blood obtained from venipuncture, on the primary assumption that systemic drug concentration is reflective of that attained in the target site. This information is then used to individualize dosage so that drug concentrations can be maintained within a therapeutic target range.

Since blood remains the gold standard for TDM, the majority of the therapeutic target ranges refer to this biological matrix. However, alternative matrices could provide complementary information and more often clinicians request drug determination in matrices other than plasma, as detailed below

The use of saliva as an alternative to conventional matrices has been a subject of interest for decades and has been proposed for several drugs. The main advantages of saliva are the ease of collection not requiring invasive procedures, the limited (if any) purification steps and the reduced costs. It has been proposed that salivary levels can represent a surrogate marker of the free drug concentration in the plasma (also referred as protein-unbound fraction), which is the active drug fraction responsible of the pharmacological effect. Actually, the correlation between saliva and plasma drug levels depends on many variables such as molecular weight and the negative log of the acid dissociation constant (pKa) of the compound, as well as its lipophilicity and the ionization status. In addition, the procedure of sample collection, the oral flow rate and the possible oral contamination, are key variables known to

impact on the variability of salivary drug concentration. All together, these factors have largely hampered the use of this matrix, limiting the application of saliva-based TDM to a limited number of analytes (mostly antiepileptic drugs) and not in clinics as a diagnostic tool.

Conversely interest has grown for the TDM using alternative biological matrices in special population, as in the case of breastfeeding women. Currently, nearly 90% of the pregnant women start breastfeeding after partum, and more than half of them take medications. This implies that a large number of breastfed infants are potentially exposed to medications in uterus and in human milk from breastfeeding women. While most of the drugs that women take during pregnancy or post-partum are not associated with known adverse outcomes of the infant, several case reports of serious infant toxicity have been published in literature reporting the importance of the determination of drug content in amniotic fluid and/or in breast milk. This atypical TDM can help physicians to evaluate the exposure of the fetus or infant to the drug, eventually establishing a causal effect with the development of the adverse event. In fact, during pregnancy, drug concentrations in fetal serum at steady state are in equilibrium with the maternal serum drug concentrations that, in turn, are governed by maternal dose per time and clearance. By contrast, infant drug exposures through breast milk are defined by infant dose via milk (per time) and infant drug clearance. For each mother–infant pair, it is, therefore, important to identify factors that can increase the excretion of the drug into breast milk (as decreased maternal clearance, maternal overdosing) or decreased the infant drug clearance (i.e. liver or kidney disease, prematurity). In fact, both these conditions may theoretically increase the infant serum drug concentration, and thus increase the risk of the adverse drug reaction(s).

In our center, the TDM requests for determination of breast milk drug concentrations mainly deal with drugs active on the central nervous system such as antiepileptic drugs, antidepressants and/or antipsychotics.

Another special population that may benefit from the TDM in alternative matrices is represented by critically ill patient treated with anti-infective drugs. Indeed, for these drugs, it is mandatory to quantify the amount of drug that reaches the site of infection and to correlate it with the microbiological data (i.e. the minimum inhibitory concentration, MIC). If the concentrations of the drug in the site of infection are higher than the MIC, there is a high probability of success of the antimicrobial therapy. For these drugs, the measurement of systemic concentrations is poorly predictive of antimicrobial response due to anticipated differences in distribution of antibiotics or antifungal to the tissue compartments.

Bronchoalveolar lavage (BAL) is a semi-invasive method used in both research and clinical practice as a way of quantifying drug concentrations from epithelial lining fluid (ELF) and/or alveolar cells (AC) in pulmonary infections. The drug concentration ratio between ELF or AC and plasma, is important to guarantee that sufficient drug concentrations reach the pulmonary tract.

Currently, there are a limited number of BAL studies measuring ELF concentrations in critically ill patients due to practical and ethical issues. However, in selected clinical situation, such as ventilator-associated bacterial pneumonia, where pathogens may have elevated MIC values, or in the case of patients with modified plasma protein content, the availability of BAL concentration can be important for optimizing dosage regimens of antibacterial agents.

In the last few years, the TDM of drug concentration in tissues has gained interest and popularity, because it can provide better correlation to therapeutic effect. This is the case also for immunosuppressive drugs. In fact, evidence suggests that intra-graft and intracellular concentrations may, more accurately, predict the outcomes of transplant recipients as it may provide a better understanding of drug distribution during graft rejection.

Unlike liquid matrices, bioanalysis in tissues offers unique challenges such as proper tissue sampling, appropriate tissue sample preparation, efficient extraction of the analytes from the tissue homogenates, and demonstration of stability and recovery of analytes in intact tissues. Therefore, apart some very restricted cases, the TDM in tissue is far from becoming a routine clinical practice.

A more easily available biological matrix is represented by the cells present in blood sample such as peripheral blood mononuclear cells (PBMC), lymphocytes and monocytes, isolated, counted and lysed for the determination of intracellular drug concentration

For antiretrovirals, immunosuppressants and antileukemic drugs, information about the intracellular concentrations and intracellular distribution has been proposed as innovative markers of therapeutic drug efficacy compared with the traditional blood-based TDM.

To date, all the intracellular assays described in literature do not discriminate between drug localized into cell membranes or into the cytoplasm, whether bound to intracellular proteins or unbound, which should be the effective drug moiety. Measurement of total cell concentrations is thus of limited value, but it is the only data available at this time. In addition, several technical challenge are still a problem for the spread in use of intracellular concentration as a diagnostic tool.

Remarkably, preliminary experiences have documented that, despite the above mentioned methodological limitations, this innovative TDM can predict the response of patients to pharmacological therapies.

LC-MS/MS is recognized as reference technique for determination of drug concentrations of low-molecular-weight molecules. The high degree of precision and accuracy makes this method suitable for measurements also in human alternative matrices even in case of very low concentrations. Recent instrumental developments of sample preparation techniques have enable faster and less complicated sample pre-treatment procedures. However, LC–MS/MS is not a gold standard procedure per se, independently of the efforts given during the development and validation steps.

Matrix effect represents a key factor in method development as ion suppression or enhancement negatively influence the accuracy and sensitivity of the method. The use of isotopic internal standards and

chromatographic separation of analytes from region of ion enhancement or suppression can take under control the effect of matrix effect.
In any case, international guidelines for bioanalytical method validation advise to validate the method with the same matrix of patient sample, in order to achieve reliable results that are necessary for proper decisions on drug dosing and patient safety.
Due to the relative difficulty in finding rare blank matrices, surrogate matrices may be acceptable for analytical method validation such as a suitable buffer medium, diluted plasma or water with addition of sample proteins. It should be kept in mind that if a modification in matrix specie of a fully validated method is introduced, a new validation of the method will be necessary. In addition, although every attempt is made to formulate standards and quality controls to be as similar as possible to the specimens to be analyzed, in vitro samples can significantly differ from patient samples and incurred sample reanalysis of patient samples is advocated.
The request from clinicians for the evaluation of drug concentration in "exotic" matrices is growing in particular settings, therefore the applicability of bioanalytical methods to different matrices from plasma and serum will become an important challenge in which the laboratory will have an important role.

SS02-CO06

THERAPEUTIC MONITORING AND PHARMACOKINETICS STUDY IN LC / MS-MS OF ELEXACAFTOR / TEZACAFTOR / IVACAFTOR, DURING THE PREGNANCY PERIOD

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Cystic Fibrosis (CF) is an autosomal recessive genetic disease due to mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene, which causes a deficiency in the protein channel responsible for chlorine transport, making secretions thicker causing severe damage to the lungs, digestive system, and other organs. Currently more than 2000 mutations have been identified of which the most frequently encountered in affected patients is the deletion of phenylalanine at position 508 (F508del). In 2020, a new drug called "Kaftrio" was approved by EMA for the treatment of CF patients from the age of 12 and carrying at least one F508del mutation, given by the combination of three molecules (Ivacaftor, Tezacaftor and Elexacaftor (ETI)) capable of correcting the malfunction of the mutated protein by partially restoring its activity. The improvement in clinical conditions and life expectancy, associated with an improvement in female fertility due to greater fluidity of the cervico-vaginal mucus, leads to an increasing number of women with CF to become pregnant. The Infectious and Tropical Diseases department and the Regional Reference Center for Perinatal Toxicology of the Careggi University Hospital of Florence requested the support of the General Laboratory to carry out the therapeutic monitoring and study of the pharmacokinetics of ETI in a 33-year-old patient suffering from CF during the pregnancy. This request derives from the lack of exhaustive guidelines and data in the literature that report the changes in the concentration of the active principles and its metabolites during the gestational period. In our laboratory, a quantitative method in LC / MS-MS was optimized and validated using 4000 QTrap mass spectrometer (ABSciex) for the therapeutic monitoring of the various analytes on plasma samples at 0h, 2h, 4h, 6h, 8h and 24h at each trimester of pregnancy. Thanks to the success of the results obtained, it was decided to extend the determination of these substances in a multicentre study involving women with cystic fibrosis during the gestational period to broaden the series and the assessment of drug concentrations, as well as in maternal blood, including in cord blood and breast milk.

SS03 - SIBioC Young Scientist Fight against COVID19

SS03-01

COVID-19: AN OPEN CHALLENGE BETWEEN RESEARCH AND INNOVATION

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Since the SARS-CoV-2 was isolated and identified as COVID-19 etiological agent, the traditional laboratory routine is dramatically disrupted. An enormous number of naso-pharyngeal swabs (NPS) has been tested every day for diagnosing COVID-19 and contact tracing. Although the NPS molecular testing immediately appeared to be the gold standard for diagnosing acute SARS-CoV-2 infections, it requires skilled personnel to collect NPS and performing tests, dedicated instrumentation and time to release results. In this scenario, laboratories and manufacturers worked a lot to search new and fast solutions to overcome these limitations. Saliva was proposed as a valid alternative to NPS, as its collection is easy, standardized if devices commercially available were used, independently obtained and well tolerated by subjects. Moreover, it was also demonstrated that sensitivity and specificity of both molecular and antigen testing on saliva was comparable to those obtained in NPS (1,2). These findings allow to save resources involved in sample collection and adopting saliva in active surveillance programs proposed as strategy to

limit virus spread (3). Process optimization was also object of research and innovation. New collection devices to catch SARS-CoV-2 from the subjects and environment were also developed to reduce at minimum samples handling before testing and risks for staff in the working area, especially in case of high drops dissemination (e.g. dentists). Different inactivation solutions (by liquid and lyophilized chemical solutions and heating) were also evaluated to limit contagion risk and ensure results accuracy and short turnaround time (TAT). Inactivation by heating seems to be the most effective strategy to reach the above cited aims (4). In addition to molecular testing platforms, a variety of antigen testing for SARS-CoV-2 detection (POCT and laboratory-based immunoassays) were also evaluated to identify the suitable, effective and affordable system. Lumipulse G-SARS-CoV-2 Ag CLEIA testing represents the best compromise between molecular testing and POCT, as it showed high sensitivity and specificity in NPS as well as in saliva samples, short TAT, did not require skilled personnel (1,5). These findings are very important in the current scenario. Vaccination has been demonstrated effective in reducing hospitalizations, but the number of daily infected subjects is still high due to the dissemination of highly mutated and more transmissible SARS-CoV-2 variants. Then, the continuous search for processes optimization and solutions to develop sustainable large scale screening programs remain a priority for economically activities opening and protection of fragile subjects. The prediction of host response to infection remains still difficult to understand, then the search of new biomarkers also is another interest and opened field. Proteomics in saliva appeared a promising tool for screening markers of disease occurrence and progression.

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SS03-S02

THE CLINICAL LABORATORY IN COVID-19 MANAGEMENT

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The outbreak of the COVID-19 pandemic declared worldwide1 due to the spread of SARS-CoV-2 has further highlighted the key role of Laboratory Medicine in the healthcare process. In this context, clinical laboratories needed to quickly reorganize their internal workflow to deal with the emergency and continue to ensure rapid and accurate responses. This was possible thanks to the contribution of all medical laboratory professionals, including administrative staff, nurses, clinical laboratory technician, pathologists and physicians who worked in synergy for an efficient and successful management of the COVID-19 pandemic in clinical laboratory setting. There are many activities in which clinical laboratories have been involved, not only concerning diagnostics.
In our Metropolitan Area of Bologna, the LUM (Laboratorio Unico Metropolitano) started to provide the diagnostic for SARS-CoV-2 detection since November 2020. Several diagnostic lines have been implemented, particularly those involving the detection of SARS-CoV-2 RNA and antigen by nasopharyngeal and salivary swabs. These methods have been made available in the context of urgency and routine for different types of users (inpatients and outpatients' diagnosis, healthcare workers and school screening, etc.). Results reporting also included extensive consultancy activities provided by laboratory professionals to internist, infectious disease specialists, public health departments staff. In addition to the activity of SARS-CoV-2 detection, our laboratory provided other emergency exams 24/7 from the beginning of pandemic, including the possibility to request specific inflammatory biomarkers such as serum IL-6. Moreover, through the outpatient service of our department the vaccination service has been implemented, especially for fragile and allergic subjects. In relation to this process, surveillance through dosing anti-SARS-CoV-2 antibodies was carried out; this activity allowed us to understand the dynamics of antibody response of a specific group of patients. At last, our laboratory participates in the regional and national surveillance program for detection of SARS-CoV-2 variants by sending positive samples to the regional reference laboratory. All these activities performed by clinical laboratories for the management of COVID-19 pandemic are constantly evolving and still requires effort, particularly for technological and organizational updating actions.

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SS03-03

IL RISCHIO DI REINFEZIONE DA SARS-COV-2 E LE IMPLICAZIONI PER LA DIAGNOSTICA

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One of the most interesting aspects of the COVID-19 pandemic is that a variable percentage of patients (from 2% to 69%) could have a repeated positivity following hospital discharge or even several weeks after clinical recovery (1). There are multiple reasons why a positive result to SARS-CoV-2, usually ascertained by RT-PCR, may be detected again, including reinfection, disease reactivation, prolonged viral shedding or false positive results (1-2). Since the beginning of the pandemic, several authors have reported the possibility of reinfection by SARS-CoV-2 or reactivation of a latent infection, calling for urgent attention from researchers, as well as public health policymakers. In our study we estimate incidence rate of 3.5% of reinfection in the Province of Modena in the first 6 months of 2021. Reinfection rates according to vaccinated or non-vaccinated subjects were 0.6% vs 1.1% ($p<0.0001$).

Multiple questions regarding reinfection associated with SARS-CoV-2 are still ongoing. What is the pathophysiological mechanism for reinfection? Who are the subjects with a higher risk of reinfection? What is the clinical burden for reinfected patients? Reinfection with the SARS-CoV-2 virus can be mainly attributed to two phenomena: decay of the immune response and viral mutations that favor the appearance of new variants (3-5). Currently, there are discordant rates of reinfection reported in SRs (ranging from 0-50%), which could partially be explained by the heterogeneous adopted definitions of reinfection. Today, there is still no universal agreement on the determination of the correct time period between positive results for SARS-CoV-2 for the definition of reinfection, although the definition provided by CDC is the most accredited (https://www.cdc.gov/coronavirus/2019-ncov/php/invest-criteria.html).

It has been pointed out that the severity of reinfection depends on the individual immune response, as well as both the viral load and the SARS-CoV-2 variants causing the reinfection. New virus variants could evade immune responses acquired in subjects with infections from previous variants or reduce the capacity for neutralization by polyclonal antibodies (4). This issue suggests the need to increase the current knowledge about the degree of protection provided against SARS-CoV-2, leading the development of vaccines and the creation and implementation of appropriate interventional strategies.

Because COVID-19 is a relatively new disease, several aspects of its progression and long-term health effects are unknown, one of the aspects that have become more relevant as time goes by is the impact that reinfections. There is a real, albeit rare risk of SARS-CoV-2 reinfection. Nevertheless, a standardized approach to identify and report reinfection cases should be developed.

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SS03-CO07

COMPARATIVE ASSESSMENT OF MAGLUMI SARS-COV-2AG TEST AND NUCLEIC ACID APMPLIFICATION TEST (NAAT): FIRST CONSIDERATIONS

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Introduction: NAAT is still the international reference assay for the diagnosis of Covid-19 due to high sensitivity and specificity; it is able to detect the pathogen even at low viral load, nevertheless a positive PCR result demonstrates the presence of nucleic acid in the sample, but not if it contains an infecting virus; the risk of unnecessarily isolate a person who is no longer infectious and a long TAT can preclude a screening utility. Conversely, immunoassays targeted to detect viral antigens have the potential to fit the requirements for a screening population test. In this study, we evaluated the consistency of results obtained by Maglumi SARS-CoV-2 Ag test in comparison with molecular test. Methods: 79 positive NAAT nasopharyngeal swabs (NPS) with Ct-threshold cycle (Ct) between 39 and 13 were selected and analysed with MAGLUMI CLIA assay targeted to nucleocapsid SARS-Cov-2 antigen (M) by Snibe Diagnostic. 37 NPS were also analysed with Liaijson XL (L), DiaSorin s.p.a., which is routinary used in our lab. 5 negative NPS were included in the experiment as negative controls. Results: all 5 negative NPS were confirmed negative with both CLIA assays. 33 of 79 (41,8%) positive NPS were confirmed positive on M, all these samples had a $Ct < 26$. The remaining 46 positive NPS, resulting negative on M, had a $Ct > 24$. Results for 37 NPS tested both with M and L agreed 100%. Conclusions: CLIA test demonstrated to be less sensitive to the viral presence

than NAAT, in particular M test seems to be able to detect a positivity to SARS-Cov-2 when the viral load is detected at a CT < 24. The grey area, where NAAT and CLIA were not in perfect agreement, seems to be Ct= 24 / 25. In this area M missclassified 67% of positive NPS, but further investigation with a larger number of samples is necessary to confirm this finding. While PCR positivity may persist for several weeks after the onset of the disease and the disappearance of symptoms, Ag tests reach satisfactory sensitivities when infected people are more likely to be contagious; together with the high throughput of the technology, makes them an extremely useful tool for screening population, especially during the pandemic.

SS03-CO08

QUALITATIVE AND QUANTITATIVE PERFORMANCE OF SARS- COV-2 NUCLEIC ACID DETECTION TESTS: RESULTS FROM THE 2021 EXTERNAL QUALITY ASSESSMENT IN LOMBARDY

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Introduction. Nowadays, the nucleic acid amplification tests, such as real time reverse transcription polymerase chain reaction (rRT-PCR) assays, are the gold-standard for detecting SARS-CoV-2. This study aimed to evaluate the qualitative test performance and inter-assay variations of SARS-CoV-2 nucleic acid detection tests by analysing the results of the External Quality Assessment (EQA) programme carried out in Lombardy in 2021.
Methods. 2021 EQA for SARS-CoV-2 molecular test consisted of 12 samples (cells culture supernatants): 5 SARS-CoV-2-negative and 7 SARS-CoV-2-positive (3 had viral load (VL)>5x103copies/ml, 4 VL=1-5x103copies/ ml). Each participating laboratory had to provide qualitative (i.e. positive/negative) and quantitative (i.e. Ct value) results obtained with each SARS-CoV-2 molecular test in use. Qualitative test performance was evaluated by positive/negative percent agreement (PPA/ NPA); inter- assay variation of quantitative results was evaluated by coefficient of variation (CV).
Results.79-90 laboratories participated to 2021 EQA for SARS-CoV-2 RNA detection by using 199-231 systems and returning a total of 2,623 results. PPA ranged between 99.5% (samples 1 and 7) and 100%, NPA between 69.9% (sample 4) and 100%. This latter range became 99.1-100% by ruling out the “invalid” results (n=89) due to low amount of human cells in two samples. Considering all systems, CV ranged between 10.8% and 13% for SARS-CoV-2-positive samples with VL=1-5x103copies/ml and between 11.9% and 14.2% for samples with high VL. We report here only the results of the two most used systems: system A (275 results) and B (245 results). For samples with VL=1-5x103copies/ml, CV ranges by viral target (vt) were: vt1) 6.4-9%; vt2) 6.6-7.8%; vt3) 2.5-4.3%; vt4) 2.4-3.8%. For samples with high VL, CV ranges were: vt1) 7.3-11.9%; vt2) 5.8-10.4%, vt3) 2.1-4.4%; vt4) 2.8-4.2%.
Discussion. Within 2021 EQA for SARS-CoV-2 molecular test, PPA and NPA were above 99.1%. However, the inter-assay variability among systems was notable and related both to VL and to viral target detected by the test. This variability - thought limited - needs to be carefully evaluated and continuously monitored within quality assurance programs.

SS04 - La stratificazione del rischio cardiovascolare: una sfida fondamentale per la Medicina di Laboratorio

SS04-01

CARDIOVASCULAR RISK EVALUATION IN THE GENERAL POPULATION: THEORETICAL CONSIDERATIONS, EXPERIMENTAL EVIDENCE AND CLINICAL RELEVANCE.

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Only during the last 10 years, the set-up of some immunoassay methods with high-sensitivity analytical performance (limit of detection between 1 and 3 ng/L) allowed a reliable determination of circulating levels of cardiac troponin I (cTnI) and T (cTnT) in the most part of healthy adult subjects, in accordance with quality specifications required by the most recent international guidelines. Several recent studies reported that circulating levels of hs-cTnI and hs-cTnT in healthy adult subjects show considerably lower intra-individual (from 4% to 12%) than inter-individual variations (about 50%) (1). Accordingly, the high-sensitivity methods for cTnI (hs-cTnI) and cTnT (hs-cTnT) should be considered an accurate estimate of the physiological myocardial renewal in healthy adult subjects (2). In particular, some experimental and clinical studies suggested that the 99th percentile value of biomarker distribution in the reference healthy population (i.e., 99th URL value) corresponds to the amount of hs-cTn contained in about 40 mg of myocardial tissue (2). Several experimental and clinical studies have recently demonstrated that cardio-specific biomarkers (such as cardiac natriuretic peptides and cardiac troponins) may help in the identification of apparently healthy subjects, who are at risk for accelerated progression towards symptomatic heart failure (3,4). Indeed, the cardiovascular risk progressively increases in the general population even for hs-cTnI and hs-cTnT values below the 99th percentile URL (i.e., the recommended cut-off for the detection of myocardial injury and diagnosis of myocardial infarction)

(3,4). In particular, the MORGAM/BiomarCaRe study investigated whether the change in 3 repeated measures of hs-cTnI collected 5 years apart improves 10-year prediction of cardiovascular risk in 3875 participants, aged 30–60 years at enrolment (51% female, disease free at baseline) (5). This study found that median hs-cTnI concentrations changed from 2.6 ng/L to 3.4 ng/L over 10 years. Furthermore, the change in hs-cTnI values throughout 10-year follow-up more accurately predicted the cardiovascular risk in the general population than the most recent measurement (5). Indeed, considering the results of the most recent clinical studies, it is conceivable that an increase in hs-cTnI concentrations, even of only 5-10 ng/L over some months in a patient with a suspect of cardiomyopathy, should suggest an initial myocardial remodelling, ultimately culminating in symptomatic heart failure (3,4). Of course, an early and effective treatment of individuals at higher cardiovascular risk may revert the initial myocardial remodeling and slow down heart failure progression (3, 4). n conclusions, the results of some recent clinical studies have demonstrated that hs-cTnI and hs-cTnT methods are able to identify individuals at highest risk to develop symptomatic heart failure (3,4). However, further studies are needed to specifically evaluate the cost-benefit of screening programs specifically designed with the aim to identify asymptomatic individuals of the general population at higher risk for progression toward heart failure.

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SS04–02

VALUTAZIONE DEL RISCHIO CARDIOVASCOLARE IN PAZIENTI IN TRATTAMENTO CON CHEMIOTERAPICI

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Pazienti trattati con chemioterapia per patologia oncologica sono a maggior rischio di sviluppo di patologie cardiovascolari. Tali patologie sono rappresentate da aritmie, disfunzioni ventricolari, scompenso cardiaco, trombosi arteriosa, ischemia miocardica. La valutazione cardiologica del paziente, prima della somministrazione della terapia anti-neoplastica, rappresenta un'opportunità unica per l'inquadramento del paziente, permettendo sia l'evidenziazione di patologie pre-esistenti, sia l'implementazione di strategie atte alla prevenzione delle complicazioni correlate alla chemioterapia, sia durante che dopo la somministrazione.

Nella valutazione basale del paziente la determinazione dei marcatori cardiaci, troponina e peptidi natriuretici, fornisce dati oggettivi per una stratificazione del rischio di sviluppo di complicanze cardiache durante e dopo la somministrazione, identificando pazienti che possono trarre beneficio da trattamenti preventivi anche durante il trattamento chemioterapico. Incrementi dei marcatori non vanno interpretati come fattori che inducano ad una sospensione/riduzione delle dosi di chemioterapico, ma devono indurre una rivalutazione congiunta tra oncologi e cardiologi del paziente per indirizzarlo ad approfondimenti strumentali o a terapie preventive. Esistono infatti oramai parecchie evidenze che la somministrazione di ACE inibitori o di beta-bloccanti determina una diminuzione dei valori dei marcatori e soprattutto una significativa riduzione del rischio di sviluppo di complicanze.

Un punto ancora oggetto di dibattito è rappresentato dal timing dei prelievi. Mentre la determinazione basale è da tutti accettata, il timing e la frequenza di valutazione durante la chemioterapia non è ancora stabilita per nessun marcatore. Una strategia spesso percorsa è quella di una determinazione in associazione alle altre valutazioni ematochimiche, in maniera da poter monitorare in maniera seriale eventuali variazioni di concentrazione.

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SS04-CO09

PREVENZIONE PRIMARIA DEGLI EVENTI CARDIACI NEI DONATORI DI SANGUE: RISULTATI DI UNA ESPERIENZA MONOCENTRICA

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Premessa: Le malattie cardiovascolari sono la prima causa di mortalità (10,4% di tutte le morti) e la prima causa di ricovero ospedaliero (14.5 % di tutti i ricoveri), con un trend destinato a crescere secondo le ultime stime OMS. Realizzare in modo concreto un'azione educativa e di prevenzione di queste patologie puo# essere fondamentale per prevenire eventi cardiovascolari, soprattutto in quei soggetti con rischio medio-alto. L'utilizzo di test di laboratorio ad alta sensibilita# e specificità d'organo, come lo sono i metodi di ultima generazione per la misura delle troponine cardiache, permettono un'accurata valutazione del rischio anche nei pazienti con impegno cardiaco asintomatico. La possibilità di poter rilevare anche piccole concentrazioni di troponin a I in una popolazione sana , ha consentito di creare delle tabelle per la stratificazione del rischio cardiovascolare, differenziate per genere, e con rispettive raccomandazioni per rischio basso, moderato ed elevato. Data l'importanza di considerare la stratificazione del rischio come un fondamentale improvement nella gestione della prevenzione cardiovascolare si è scelto di eseguire ai donatori che afferiscono alla ST AOOR Villa Sofia-Cervello di Palermo, il test della troponina I in aggiunta ai test di screening per la qualificazione biologica previsti per legge.
Metodi: sono stati testati 3450 Donatori (2429 uomini e 1021 donne). Il test è stato effettuato sulla stessa provetta di siero del campione prelevato per le indagini di qualificazione biologica. Il test della Troponina I ad alta sensibilità (hsTnI) è stato effettuato su piattaforma Alinity (Abbott) con metodica CMIA. Sono stati avviati a consulenza cardiologica tutti i donatoricon valore di Troponina medio-alto.
Risultati: N.89 donatori, di cui 79 uomini (età: 32-65 anni) e 10 donne (età: 55-60 anni), sono stati avviati a consulenza cardiologica. Il valore medio di Troponina riscontrato, era di 6,8 e 22,2 per gli uomini e di 6,6 e 23,8 per le donne, indicativirispettivamentedirischio moderatoedelevato.Tali donatori presentavano i seguenti fattori di rischio: obesità, familiarità per malattie cardiovascolari e diabete, ipertensione arteriosa. Tutti i soggetti avviati a consulenza cardiologica, sono stati sottoposti a visita specialistica ed elettrocardiogramma;
n. 34 soggetti, con valori di Troponina elevata e con più fattori di rischio cardiovascolare presenti, sono stati sottoposti a successive indagini strumentali, quali test ergometrico, ecodoppler cardiaco. N. 2 soggetti sono stati ulteriormente sottoposti a RM cuore. In tali soggetti sono state individuate lievi patologie a carico del sistema cardiovascolare, prima di allora sconosciute, e sono stati tenuti in follow up per 6 mesi. Per tutti gli altri donatori avviati a consulenza cardiologica, non sono stati riscontrati dati clinici rilevanti e si è provveduto a dare indicazioni su uno stile di vita sano
Conclusioni: l'introduzione del test hsTnI, ha permesso di identificare precocemente i soggetti a rischio cardiovascolare all'interno della nostra popolazione di donatori di sangue attivando così un percorso di prevenzione primaria degli eventi cardiaci

SS05 Medicina personalizzata in ematologia, nuove frontiere

SS05-S01

PERSONALIZED MEDICINE IN HEMATOLOGY

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Hematology has paved the way for therapeutic innovations in medicine. This is also happening now in the new frontier of personalized medicine.
The definition and scope of the term "personalized medicine" varies widely, ranging from the extremely broad to the very narrow. Here we report several different definitions.
"The use of new methods of molecular analysis to better manage a patient's disease or predisposition to disease" by the Personalized Medicine Coalition; "Providing the right treatment to the right patient, at the right dose at the right time" by the European Union; "The tailoring of medical treatment to the individual characteristics of each patient" by President's Council of Advisors on Science and Technology; "Health care that is informed by each person's unique clinical, genetic, and environmental information" by the American Medical Association; "A form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease" by the National Cancer Institute (NIH); "The use of genomic, epigenomic, exposure and other data to define individual patterns of disease, potentially leading to better individual treatment" by National Academy of Sciences (NAS).
Overall "precision medicine" is perhaps most synonymous to "personalized medicine".
But if we look closely at the evolution of hematology, the era of personalized medicine began several years ago.

- 1907: Reuben Ottenberg reports the first known blood compatibility test for transfusion using blood typing techniques and cross-matching between donors and patients to prevent hemolytic transfusion reactions.
- 1956: The genetic basis for the selective toxicity of fava beans ("favism") and the antimalarial drug primaquine is discovered to be a deficiency in the metabolic enzyme, glucose-6-phosphate dehydrogenase (G6PD).

- 1977: Cytochrome P450 2D6, a polymorphic metabolizing enzyme, is identified as the culprit for causing some patients to experience an “overdose” or exaggeration of the duration and intensity of the effects of debrisoquine, a drug used for treating hypertension.

Continuing through the years to the present day we can see many examples of personalized therapy that are current practice today: hemopoietic stem cell transplantation, infectious diseases prophylaxis and treatment, GvHD biomarkers, targeted drug levels, pre-emptive therapy on specific molecular targets, integration of targeted therapy inside transplantation program both as bridging therapy and early maintenance post-transplant particularly in acute myeloid leukemia because of the several mutation recognized determining clonal heterogeneity and drug resistance.

Real-world applications of immunotherapy are personalized medicine: allogeneic transplantation a platform for immunotherapy, checkpoint blockade treatments to pick-up anti-leukemia specific responses, CAR-T and TCR engineering, antibodies-redirected anti-leukemia specific responses, suicide-gene engineered T-cells and others.

Gene therapy, gene editing and CAR-T therapy are indubitably additional form of personalized therapy in which patients own cell, appropriately modified, are the therapeutic agent.

As final remark we must recognized limitations of personalized medicine. Several can be listed, in this abstract we wish to underline cost issue. Personalized medicine contributed to the recent rapid and impressive cost increment seriously impacting equity of resources distribution and therapeutic perspectives. As Physicians we should remember what may undoubtedly be the oldest but actual and most relevant definition of personalized medicine: it’s far more important to know what person the disease has than what disease the person has (Hippocrates).

SS05-CO10

SINGLE-MOLECULE REAL-TIME SEQUENCING OF THE M PROTEIN: TOWARD PERSONALIZED MEDICINE IN MONOCLONAL GAMMOPATHIES

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In patients affected by monoclonal gammopathies, tumoral B cells or plasma cells secrete a monoclonal antibody (M protein), which can be used to track the presence of the tumor itself. Moreover, the M protein can directly cause potentially life-threatening organ damage, which is dictated by the specific unique clonal light and/or heavy chain of each patient, as in patients affected by immunoglobulin light chain (AL) amyloidosis.

Patients’ specific M protein sequences remain mostly undefined and molecular mechanisms underlying M-protein related clinical manifestations are largely obscure. By combining the unbiased amplification of expressed immunoglobulin (Ig) genes through inverse PCR from cDNA with long-read DNA sequencing and bioinformatics analyses, we have established a method to unambiguously identify the full-length variable sequence of expressed Ig genes in patients with monoclonal gammopathies and to rank the obtained sequences based on their relative abundance, thus enabling the identification of the full-length variable sequence of M protein genes from a high number of patients analysed in parallel.

The assay, termed Single-Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq), has undergone an extensive technical validation, including comparison with gold-standard techniques of immunoglobulin gene sequencing, assessment of reproducibility and sensitivity, and validation through proteomics on amyloid deposits. Noteworthy, SMaRT M-Seq successfully identified the full-length variable sequence of M protein genes from a cohort of 86 AL patients, including cases with a small clonal burden and an undetectable M protein with conventional diagnostic assays, validating its throughput. Sequence information was then exploited to enable the sensitive detection of clonotypic sequences.

High-throughput sequencing disease-associated M proteins from large cohorts of patients has the potential for uncovering molecular mechanisms of M protein-related clinical manifestations which, so far, have remained largely unexplored, and could enable approaches of personalized medicine for early diagnosis using sequence-based predictive algorithms and for detection of minimal residual disease.

SS05-CO11

EVALUATION OF THE CBC-O TOOL: WHY DOES THE MCHC INCREASE?

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Introduction: the increase of MCHC (Mean Corpuscular Hemoglobin Concentration) in CBC (complete blood count) is a complex problem, which must take into account numerous factors. RBC impedance and HGB optical density (photometric) measurements can be influenced by various causes, which can impact on red blood cell indices and may depend on both biological variables, such as: cold agglutinins, lipemia, jaundice, abnormal proteins, hemolysis, red blood cell disease, dehydrated red blood cells; that from pre-analytical factors such as delayed sample processing, temperature variations during collection, transport and storage. This paper evaluates the CBC-O (optical) tool, which supports the clinical pathologists in identifying the cause and in the correct reporting of the CBC parameters, avoiding the default thermostating of the samples.
Materials and methods: for the evaluation of the CBC-O tool, the samples, collected with K2EDTA anticoagulant, of 121 patients for the execution of the CBC were used. The tool can be calculated after performing the RET (reticulocytes) reflex analysis of the XN hematology analyzer (Sysmex) when MCHC is> 365 g/L. The tool CBC-O uses the parameters: RBC-I, RBC-O, HGB, HGB-O to calculate delta RBC, delta HBG and the RBC score through which the tool suggests the cause of the MCHC increase by returning the blood cell indices corrected reds of the CBC.
Results: the samples that have been used can be divided into different groups according to the cause of the MCHC increase: patients with probable presence of cold agglutinins, whose RBC indices obtained after thermostating the sample and those calculated by the CBC-O tool are been compared with overlapping results; with hemolysis / lipemia and samples whose MCHC was increased due to a delay in sample processing and problems related to transport and storage. In the latter, the comparison between the results obtained from the thermostating of the sample and the tool are not comparable.
Conclusion: identifying the cause of the increased MCHC allows the clinical pathologist to make corrections or make appropriate decisions.

SS06 - Sostanze psicoattive: dall'uso clinico all'abuso

SS06 - 01

NUOVI TREND DI CONSUMO DI SOSTANZE D'ABUSO DURANTE LA PANDEMIA DA COVID 19

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Per contenere e contrastare il COVID-19 (COronaVIrus Disease 19), le persone sono state costrette a rispettare misure severe come il lockdown e il distanziamento sociale, modificando i propri stili di vita. Questo evento ha avuto conseguenze negative sul benessere fisico e mentale delle persone, provocando stati di ansia e di rabbia, disturbi del sonno, depressione e disturbi da stress post-traumatico. Ciò ha portato sempre più individui a ricorrere all'utilizzo di sostanze sia lecite che illecite, con un aumento del consumo di alcol e di sostanze psicotrope, aggravando le condizioni di chi, già, soffre di tossicodipendenza (1). Anche, il mercato delle sostanze stupefacenti si è dovuto adattare alle restrizioni connesse al COVID-19, specialmente dopo la chiusura delle frontiere e le restrizioni nei viaggi. L'utilizzo di corrieri umani è stato, pressoché, sostituito da contrabbando tramite container intermodali o tramite catene di approvvigionamento commerciali (2). Perfino le rotte commerciali hanno subito modifiche, abbandonando il transito via terra a favore del trasporto via mare, come nel caso del traffico della resina di Cannabis prodotta in Marocco e trasportata nell'UE, prima della pandemia, via terra attraverso la Spagna. Al contrario, dalla seconda metà del 2020, sono stati segnalati ingenti sequestri di resina di Cannabis in numerosi porti marittimi europei. La pandemia, ha inoltre, accelerato la digitalizzazione del mercato delle sostanze stupefacenti, sostituendo la vendita per strada con metodi alternativi che hanno portato i consumatori a rivolgersi al mercato illegale del dark web o all'utilizzo di servizi di messaggistica criptati o a piattaforme web come Telegram o infine a servizi di posta con consegna a domicilio. Anche il tipo di sostanze stupefacenti consumate si è modificato. Mentre, l'analisi delle acque reflue, condotta nel 2019 nelle principali città europee, aveva indicato un aumento complessivo di consumi di sostanze come cocaina, MDMA, amfetamina e metamfetamina, droghe solitamente associate a eventi sociali ricreativi, durante la pandemia, l'interesse del consumatore si è spostato su ansiolitici e narcotici da consumare in solitudine. Oltre ai tranquillanti, è stato, segnalato un crescente utilizzo di oppioidi e di nuovi oppioidi sintetici. In Canada e in Nord America, dall'inizio della pandemia, si è registrato un aumento netto dei decessi per overdose da oppioidi sintetici come il fentanil (3) (in Canada, tra aprile e giugno 2020 sono stati superiori del 58% rispetto allo stesso trimestre del 2019). In Europa, si sono registrati cambiamenti nei livelli di abuso dei farmaci per il trattamento sostitutivo degli

oppioidi dopo la prima chiusura (4) nonché un aumento dell'utilizzo di droghe psichedeliche e dissociative, come LSD, 1P-LSD, 2C-B, NBOMes, ketamina, DMT e GHB. L'aumento di utilizzo non terapeutico di benzodiazepine e farmaci Z ha, anche, sollevato particolari preoccupazioni, sia per il basso costo sia per l'elevata disponibilità nonché per l'aumento di utilizzo tra i consumatori di stupefacenti ad alto rischio non consapevoli del contenuto e della potenza delle compresse acquistate online.

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SS06-02

RECREATIONAL AND CLINICAL USE OF GHB

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γ-hydroxybutyrate (GHB) is an illicit recreational drug of abuse acting as a potent central nervous system depressant and is often encountered during forensic investigations both in living and post-mortem cases. The sodium salt of GHB is registered as a therapeutic agent (Xyrem®), approved in several countries for the treatment of narcolepsy-associated cataplexy and (Alcover®) is an adjuvant medication for detoxification and withdrawal in alcoholics. Small amounts of GHB are produced endogenously (0.5-1.0 mg/L) in various tissues, including the brain, where it functions as both a precursor and a metabolite of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA). Available information indicates that GHB serves as a neurotransmitter or neuromodulator in the GABAergic system, especially via binding to the GABA-B receptor subtype. Although GHB is listed as a controlled substance in many countries abuse still continues, owing to the availability of precursor drugs, γ-butyrolactone (GBL) and 1,4-butanediol (BD), which are often not under control. After ingestion both GBL and BD are rapidly converted into GHB (t½ ~1 min). The Cmax occurs after 20-40 min and GHB is then eliminated from plasma with a half-life of 30-50 min. Only about 1-5% of the dose of GHB is recoverable in urine and the window of detection is relatively short (3-10 h). This calls for expeditious sampling when evidence of drug use and/or abuse is required in forensic casework. The recreational dose of GHB is not easy to estimate and a concentration in plasma of ~100 mg/L produces euphoria and disinhibition, whereas 500 mg/L might cause death from cardiorespiratory depression. Effective antidotes to reverse the sedative and intoxicating effects of GHB do not exist. The poisoned patients require supportive care, vital signs should be monitored and the airways kept clear in case of emesis. After prolonged regular use of GHB tolerance and dependence develop and abrupt cessation of drug use leads to unpleasant withdrawal symptoms. There is no evidence-based protocol available to deal with GHB withdrawal, apart from administering benzodiazepines

SS06-03

NEW SYNTHETIC OPIOIDS

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In the last decades, several new psychotropic molecules mimicking the pharmacological effect of the classic drugs of abuse appeared on the illegal market, causing acute and fatal intoxications in more than 100 countries worldwide (1). These molecules are defined as new psychoactive substances (NPSs) by the United Nations Office of Drugs and Crime (UNODC) and their legal status is often controversial, although they pose an increasing public health threat (1) Recently, the subclass of new synthetic opioids (NSOs), in particular fentanyl and benzoimidazole analogues, stood out as an emerging class among NPSs and raised concerns due to the rapid increase of fatalities related to new analogues (1,2).
Similar to the classical opioids, namely morphine and heroin, NSOs selectively bind to the μ-, δ-, and κ-opioid receptors in the peripheral and central nervous system (CNS), thereby simulating the effects of endogenous opiates. These neurochemical interactions are mainly responsible of a classic opioid toxidrome characterized by miosis, sedation, coma, and even death, which can be reversed with the competitive antagonist naloxone (3)
In the early 2010s, USA were struck by the "opioid crisis" as NSOs flooded the illegal drug market and the number of opioid-related overdose deaths significantly increased in the USA, mainly involving fentanyl and analogues (3). In 2019, new NSOs subclasses (eg. benzimidazole opioids such as nitazene analogues), thiambutenes, and cinnamylpiperazines) emerged onto the illicit drug market, subsequent to the scheduling of fentanyl analogues in the USA and China (1).
In 2020, NSOs resulted the third largest group of NPSs identified by European Monitoring Centre for Drugs and Drug Addiction after stimulants and cannabinoid receptor agonists (1)

Similar situation was reported in Italy by the National Early warning system, in charge of
early detecingt emerging NPS threats in Italy, acquiring information from police forces and collaborative centrist raise an international alert (4).
From 2016 to 2019, 4 fentanyl analogs and 1 synthetic opioid (U47-700) were identified for the first time in Italy, following fatal intoxications. Among the identified fentanyl analogs, it is worth mentioning 4-furanyl fentanyl, identified for the first time not only in Italy but also in Europe.
The expansion of these NSOs constitutes an important challenge in clinical and forensic toxicology. First of all, most of these substances are not detected in laboratory routine screening tests and confirmation methods. Also, due to the low doses employed of these highly potent drugs, the concentrations expected in the biological samples are in the concentration ranges of low ng to pg/mL or ng to pg/g range, requiring extremely sensitive methods of detection (5).
The rapid identification of NPS is instead essential to stop the health and social problems related to the spread of these new dangerous and highly addictive substances in the general population and in conscious and unconscious consumers.

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SS06-CO12

THERAPEUTIC DRUG MONITORING OF MEDICAL CANNABIS IN PEDIATRICS: THE EXPERIENCE OF GIANNINA GASLINI INSTITUTE.

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The growing interest in medical cannabis-based therapies has increased in recent years and treatment of drug-resistant epilepsy is one of the most relevant applications. Medical cannabis is composed by the dried female flowering tops containing among others at least 90 cannabinoids. D9-tetrahydrocannabinol (THC) is the only psychotropic compound while cannabidiol (CBD) is the main non-psychotropic constituent. In recent years, a plant-derived pharmaceutical formulation of purified CBD oral solution (Epidiolex®) was approved as adjunctive therapy in conjunction with clobazam for drug resistant seizures associated with Dravet or Lennox-Gastaut Syndrome. The PK of CBD shows extensive variability posing the bases for treatment optimization TDM . Starting from 2007, we administered different available CBD preparations (eg. Italian FM2, Epidiolex®) and started performing TDM on dispensed products. We have developed several analytical methods for the measurement of THC and CBD and their main metabolites: 11-hydroxy-D9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-D9-tetrahydrocannabinol (THC-COOH) for THC and 7-hydroxy-cannabidiol (7-OH-CBD), cannabidiol-7-oic acid (7-COOH-CBD), 6-alpha-hydroxy-cannabidiol (6-a-OH-CBD), and 6-beta- hydroxycannabidiol (6-b-OH-CBD),for CBD. , in plasma as well in microsampling volumetric devices (VAMS) using ultra performance liquid chromatography coupled
to tandem mass spectrometry (UHPLC-MS/MS) and validated them following international guidelines. Several clinical samples derived from pediatric patients under treatment with both medical cannabis and CBD-based pharmaceuticals were analyzed and differences in blood levels of THC and CBD and their respective metabolites as well as correlation with administered dosages were studied. VAMS have been also validated in capillary blood posing the bases for further developing the possibility of home sampling and minimizing the discomfort caused

by venipuncture. Results put in evidence the high inter-individual variability and large differences in bioavailability between purified and galenic preparations underlying the need to implement TDM for personalization of medical cannabis treatments.

SS06-CO13

HS-SPME-GC-MS ASSESSMENT OF THE EXPOSITION TO THC DURING SMOKING BY A PORTABLE VAPORIZER FOR CANNABIS

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The emerging hemp industry is interested by the new tabletop or portable vaporizers as they appear less harmful of the mainstream cigarettes. They work heating dried materials at low temperature in order to achieve an aerosol full in active compounds like phytocannabinoids, flavonoids, and terpenoids without combustion by-products. Investigate the performance of a new pocket pen vaporizer was the aim of this study. In order to evaluate the potential exposure of vapers, a HS-SPME- GC-MS method was developed for the characterization of vapor phase produced by cannavaping. Vaping was realized under standard conditions and vapor phase was collected in quick and direct manner to avoid any degradation and contamination. Analytes sampling were carried out by a DVB/CAR/PDMS fiber, and their identification was performed through gas chromatography coupled to mass spectrometry. This approach allowed to monitor active ingredients and to verify absence of harmful compounds which characterize the mainstream cigarette smoke. In addition, the quantification of the main cannabinoids, THC and CBD, in the vapor phase was performed in order to evaluate their potential inhalation by vapers. Results confirm the ability of this new portable vaporizer to release cannabinoids and terpenes in a vapor that is free from the by-products of combustion making it a valuable tool for cannabinoids administration in medicine.

SS07 I POCT: uno degli strumenti "per una nuova normalità"

SS07-01

ESPERIENZA DI IMPLEMENTAZIONE E GESTIONE DEI POCT IN UNA ASL ABRUZZESE CON 5 PRESIDI

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Scopo del lavoro è stato quello di creare una rete territoriale di POCT nei presidi di pronto soccorso degli Ospedali periferici dislocati nel territorio interno abruzzese. Come preparazione del lavoro è stata completata una ricognizione su tutti i presidi e successivamente sono stati installate 6 strumentazioni di POCT nelle sedi di Atessa, Casoli, Guardiagrele, Gissi ed Ortona.Su ciascun presidio si è provveduto ad installare uno strumento per l 'esame emocromocitometrico, uno per gli esami di base di chimica-clinica, uno per l' esame delle urine, uno per i test di base della coagulazione, uno per la troponina ed un emogasanalizzatore.Si sono poi definite le procedure per l 'esecuzione dei controlli sulle varie strumentazioni. Inoltre è stata definita una procedura aziendale per la Gestione dei POCT.Tutto il progetto, per il suo avvio, ha richiesto 6 mesi da settembre 2021 a marzo 2022. La rete territoriale installata sui vari presidi viene gestita dall'ospedale di riferimento,ossia quello dell 'Ospedale Clinicizzato SS.Annunziata -Chieti dove personale dedicato si occupa della verifica di tutte le strumentazioni.

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SS07 - 02

SOLUTIONS AND PATHWAYS IN A LEVEL III PEDIATRIC HOSPITAL

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In compliance with ISO standards 15189 and 22870, the governance of decentralised diagnostic (POCT) is one of the new challenges for many laboratories in Europe. The path requires organisation and specific skills, and the aim is to ensure analytical systems that combine three fundamental aspects: ease of use, analytical quality, and process traceability. POCTs are needed in intensive care, including neonatal and pediatric emergency/urgency wards, ensuring immediate clinical picture framing and rapid assessment and prognosis, essential for patient outcome. The appointment of a POCT Manager for

A.O.U. Ospedali Riuniti Ancona allowed a reorganisation process to be undertaken, starting from the Pediatric Hospital G. Salesi. A step-by-step process was implemented that provided the establishment of a POCT group, training in the use of the system to support and assist the departments in managing the critical testing phase. Crucial elements of the project were: the careful selection of instruments, which tend towards an analytical "operator-independent" solution; the connection to the middleware, guaranteeing the management of the Acute Care systems present; the compulsory acceptance of tests on the order entry system, with bar-code printing for the sample/patient's identification; the preparation of an ad hoc report for the tests. For performance monitoring, indicators relating to percentage of accepted and correctly identified samples; adverse events due to pre-analytical errors; effectiveness of training of department operators, through specific Key Performance Indicator (KPIs) were identified. The intention of the new organizational model is to move from a vertical logic of care, that is summative of individual performances and services, to a horizontal patient-oriented, socio-assistance logic, using the most suitable technological and IT solutions. Diagnostic and IT technological supervision, by the Analysis Laboratory, allow complete control of the decentralised location, with guaranteed analytical quality and traceability of the services provided. The new trends taking place in the health context, demographic change, increased user expectations of good health, development of new health technologies, produce a greater demand for health services with an increase in treatment and care costs. This diagnostic uncertainty, if not well addressed, could lead to inappropriate prescriptions, unnecessary additional tests, and to an increase in potentially avoidable hospital admissions. POCTs can improve the diagnostic process and medical prescriptions, increase the quality of care and, indirectly, relieve pressure on healthcare systems, by shifting the axis of care from the hospital to the territory ('comfort zone'). At the same time, for chronic pathological conditions (frail patients), these tests provide an immediate result with early adaptation of therapy. For children, clinical decision-making process and its complexity are different from those for adults. The impact of POCTs on patient outcomes and healthcare processes in pediatric care will therefore need further investigated.

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AACC Guidance Document on Management of POCT JALM July 2020

SS07-03

POINT OF CARE TESTING, CONNECTIVITY AND REGULATIONS: ALL TO BE IMPLEMENTED AT THE SAME TIME AS A "KEY SOLUTION"

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In a changing world Health reform (especially primary care reform) is now a focal point of attention in many countries, for a number of reasons. Access to care is frequently limited due to disability, distance, service is fragmented and disconnected, error rates are unacceptable, evidence and adherence to guidelines are poor and patient experience is irrelevant. Managers and policymakers should identify inefficiency and ineffectiveness in primary care, services should be redesigned through the use of a care pathway-based approach, physicians and other caregivers should be in position to make decisions and take action at the first point of contact with the patient at local clinics, nursing homes and primary care facilities. POCT can play an important role in "problem solving" policy, because they offer numerous advantages. However, using those devices in settings outside of the hospital pose critical and important clinical risks. The most important aspect for POCT is how to use them, to address many of the problems that arise from disjointed services and delays in delivering vital information, such as medical test results, everyplace those results are needed. Delocalization does not imply absence of control, which is always mandatory, but without connectivity it is impossible to obtain an effective management. The integration of POCT data into the patient's electronic medical record is a critical component of management. Connectivity of POC devices supports documentation of results, continuity of care, and enables data review for quality assurance compliance. The Clinical and Laboratory Standards Institute (CLSI) produced guidelines on the requirements that should be met when manufacturers are developing the connectivity features of the device. Incongruously, even the ISO 15189 and 22870 do not recognize connectivity as a mandatory requirement for accreditation, despite the strong commitment to traceability and quality control. In Italy a national rule is lacking and local regional rules are rather divergent: some of them contain only essential indications while others are too detailed and difficult to apply, moreover most of them are timeworn. Only recently (2021) the Regione Campania emanated a regional decree. The lack of harmonization and regulation among Italian regions makes it necessary to issue of a national rule aimed at minimizing the problems associated with POCT management. Moreover, if not taking part in development and management of POCT Systems, a big opportunity is being lost for laboratorians to increase their professional role, experience, knowledge and scientific contribution to the POCT domain.(CLSI POCT01-A2 Point-of-Care Connectivity, 2006; CLSI AUTO09-A: Remote Access to Clinical Laboratory Diagnostic

Devices via the Internet; Approved Standard, 2006 CLSI AUTO11-A2: Information Technology Security of In Vitro Diagnostic Instruments and Software Systems; Approved Standard – Second Edition, 2014).

SS07-CO14

IL POCT NELLO SVILUPPO DI UN NETWORK CLINICO ASSISTENZIALE NELLA MEDICINA DI PROSSIMITÀ

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Point of care (POC) are tests conducted near the site of patient care, outside of laboratory, usually performed by patients or healthcare personnel not trained in laboratory medicine. POC testing (POCT) require small sample volumes, minimize pre-analytical errors, and reduce alterations of labile analytes. Furthermore, when used appropriately, could improve the patient's outcomes providing faster results and earlier therapeutic strategies. Instead, its over or incorrect use could lead to a patient risk and potential increase of healthcare costs.In the Province of Modena, a decentralized and multi- professional POCT diagnostic system was developed to integrate the clinical and diagnostic pathways supporting fast clinical decisions, to improve outcomes and to optimize the clinical patient pathways. We installed 129 POCT (77 emogasanalyzers, 9 coagulometers, 9 instruments for haematological tests, 9 instruments for chemical analyses, 10 counters, 6 for immunoassay, 3 cube-PCR and 6 for rapid diagnosis of COVID-19). These instruments were installed not only in emergency rooms of three Hospitals but also in specific setting as the OSCO-Community hospital, oncological and emergency setting and Neonatal Intensive Care. Furthermore, 264 glucometers were located in various departments of three hospitals. This system provides multiple advantages for: (i) patients reducing waiting time and long-distance travelling from home, (ii) healthcare professionals improving skills, autonomy and responsibility, reducing workload and promoting collaboration, and (iii) the healthcare system giving continuity in the therapeutic and assistance pathways ensuring a high analytical quality and costs reduction. To implement a successful POCT network is vital to develop a multidisciplinary team composed by physicians, nurses and laboratory technicians working together. Furthermore, the continuous training and education of operators guarantees the efficiency and productivity of the system.In conclusion, the POCT network represent an opportunity for the health care system to consolidate the patients-based laboratory medicine, reducing for diagnostic response, clinical decision and the hospitalization.

SS07-CO15

ANALYTICAL PERFORMANCE EVALUATION OF A POINT OF CARE TESTING SYSTEM FOR THE COMPLETE BLOOD COUNT

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Introduction. Point of care testing (POCT) is an alternative approach to laboratory testing, that should be preferred only when the test TAT must be so short that samples management in laboratory is not suitable. However, POCT should generate results comparable to those obtained in laboratory. Purpose of the study. In our study we estimated the concordance of the blood cell count carried out on POCT analyzer Norma Icon-5 with that performed in laboratory on automated blood cell count Sysmex XN1000, to evaluate the POCT implementation in Emergency Department (ED). Methods. Venous blood samples were collected in tubes containing K3EDTA anticoagulant to 40 patients admitted to the ED. All samples were assayed on Norma Icon-5 and, within 10 minutes, on Sysmex XN1000. The degree of concordance between POCT results of red blood cells (RBC), white blood cells (WBC), and platelets (PLT) count and haemoglobin (Hb) concentration was evaluated. Data were compared with Passing-Bablok regression and Bland-Altman plots. Results. The PoCT vs. XN1000 bias was -2,44% (95% IC: -3,45÷-1,43), 0,16% (95% IC:-0,70÷-1,92), 1,22% (95% IC: 0,612÷1,83) and -10,45 (95% CI -12,33÷8,67)% for RBC, WBC, Hb and PLT respectively. PoCT vs XN1000 absolute bias was -0,11 (95% IC -0,15÷-0,06), 0,07 (95% IC -0,01÷0,16), 1,65 (95% IC 0,80 ÷ 2,45) e -21,25 (95% IC -24,40÷-18,11) for RBC, WBC, Hb and PLT respectively. Passing- Bablok regression equation was y= -0,015 (-0,309÷0,240) + 0,976x (0,915÷1,0435), y= -0,186 (-0,404÷0,012) + 1,038x (1,008÷1,068), y = -1,889 (-7,224÷1,500) + 1,030x (1,000÷1,069) e y=-12,098 (-21,500÷-3,824) + 0,964x (0,924÷1,000) for RBC, WBC, Hb and PLT respectively. Conclusion. The POCT blood cell counter results were statistically concordant with those of the laboratory system only for WBC e RBC count, but showed a clinically acceptable bias also for Hb concentration values. However, PLT showed an underestimation of PLT statistically significant and also exceeding the minimum clinical allowable bias. Therefore, the POCT blood cell counter can be used for clinical purpose only for RBC, WBC and Hb determination.

SS08 - Diagnostica delle crioglobuline

SS08-01

CRYOPROTEINEMIA: THE NEW FRONTIER FOR THE LABORATORY

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Cryoglobulinemia is a rare pathologic condition that can be difficult to diagnose both clinically and, in the laboratory, which is why close collaboration between the clinic and laboratory is essential. The laboratory needs the skills and experience to interpret the laboratory tests and the clinician should not hesitate to contact the laboratory when the result is not supported by the clinical signs. To strengthen this collaboration, the Protein Study Group of the Italian Society of Clinical Biochemistry (SIBioC) in collaboration with the Italian Association for the Fight against Cryglobulinemia (ALCRI) have established a fruitful partnership.
The laboratory plays a central role in the cryoproteinemia diagnosis, but the laboratory criticalities are well known. In the pre-analytical phase, maintenance of the heat chain is a critical issue; from the withdrawal until the arrival in the laboratory the sample has to stay at a temperature of 37°C. In the analytical phase, the sample must be kept at 4°C to allow the cryoproteins to precipitate, then, the cryoprecipitate must be washed, dissolved and immunotyped. The post-analytical step is cryoprotein type identification which may be subject to the operator's interpretation.
Cryoprotein testing remains totally manual and operator-dependent so it was important to identify areas where best practice guidance or even harmonisation of the laboratory investigation would be beneficial.
Patients with untreated cryoproteins can suffer significant tissue damage leading to limb amputation or even death, so the importance of cryoprotein analysis cannot be underestimated. Cryoproteinemia is in fact a pathological condition whose diagnosis cannot be made without laboratory findings. However, it is undeniable that cryoprotein research suffers from numerous pitfalls involving all stages of the analytic process. These criticalities are exacerbated by the strong inter-laboratory heterogeneity often conditioned by local technical and organisational resources. It is, therefore, crucial to urge laboratories to participate in External Quality Assessment (EQA) programmes for cryoproteins, bearing in mind that the only one currently available is the one proposed by UK-NEQAS.
Still uncommon is the cryofibrinogen analysis, the determination of which would be complementary to that of cryoglobulins, not least because of the overlap in symptoms between cryoglobulinemia and cryofibrinogenemia. This test, however, which is not included in the Essential Levels of Assistance of Italian healthcare, is slightly or not at all requested by clinicians, and consequently, laboratories rarely equip themselves for this determination, thus deterring demand.
In summary, there is a need to start a process of harmonising analytical procedures for cryoproteins testing by a continuous dialogue between clinics and laboratories with the frontline commitment of scientific societies. In particular, the path taken by SIBioC and ALCRI is precisely that of collaboration in order to draw up recommendations useful to the laboratory technician, the clinician and above all the patient.

SS08-02

CRYOGLOBULINEMIA AND THERAPY

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Abstract: Treatment of patients with cryoglobulinemic vasculitis (CV) should be tailored to the single patient, considering the severity of the disease. Expertise, knowledge of biology, and multidisciplinary cooperation are needed (1). Furthermore, the approach is substantially different in HCV-related or -unrelated CV, as well in the course of CV with type II or III versus type I cryoglobulinemia (2). Comorbidities and the previous therapies for CV, in particular corticosteroids and cyclophosphamide, and for HCV infection must be considered. Moreover, the irreversible organ damage, ie., in the case of peripheral neuropathy or nephritis, caused by parenchymal fibrosis after vasculitis requires supportive therapy (1).
In life-threatening manifestations, early diagnosis and prompt treatment are mandatory (3). High-dose corticosteroids and plasmapheresis represent the most rapid approaches currently available (3). Rituximab and cyclophosphamide remain further options to spare corticosteroids. In very severe, HCV-related CV, antiviral therapy is not the first priority. The risk of infections during treatment is high. Prophylaxis and early recognition and treatment of infectious complications are mandatory. Motor neuropathy and rapidly progressive glomerulonephritis also deserve an aggressive approach. In severe manifestations of CV and in patients recovering from a life-threatening CV, rituximab represents the best immunosuppressive treatment. Mycophenolate mofetil may be an alternative in some patients with milder renal disease. The efficacy and safety of rituximab in CV are supported by controlled, randomized trials (1) and worldwide 10-year real-life experience. The concomitant use of corticosteroids can be substantially reduced (1). Rituximab does not worsen HCV infection in the long term. By contrast, rituximab may induce severe reactivation of HBV infection (4). In about half of patients, the duration of the response to a single cycle of rituximab may be quite long (4). Regular maintenance schedules appear advisable, however, when a relapse would be hazardous. Cryoglobulins and rheumatoid factor (RF) decrease but usually do not disappear after rituximab treatment, however, controlling the burden of the B-cell

lymphoproliferation is usually sufficient (2). Antiviral therapy with Direct Antiviral Agents (DAAs) has the strongest biologic rationale in HCV-related CV. They may be added even ab initio to rituximab. Such a combination might show synergistic effects. In HCV-related CV, DAAs should be given whenever possible. Complete clinical response requires a sustained virologic response. Cryoglobulins and RF usually decrease over time and may even disappear, although CV may also persist, consistent with a true autoimmune disease (2).
Many patients with CV present non-severe manifestations, consisting of purpura, arthralgias, and weakness. Because the clinical picture may be highly variable based on disease chronicity, the frequency of relapses, the age of the patient, the disease activity, the concomitant liver disease, and comorbidities, the treatment must be individualized (5).

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SS09 - CASI CLINICI selezionati da Abstract

CC01

UNA COMPLICATA DEFINIZIONE DEL RISCHIO NEOPLASTICO LEGATO A BRCA

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Low-frequency constitutional gene variants represent alterations that inform inherited cancer risk definition and precision medicine strategies in cancer. Patients diagnosed with ovarian cancer should be first tested for mutations in BRCA1/BRCA2 genes on the neoplastic tissue to predict the response to platinum-based agents and PARP inhibitors.
In case of a positive result, the pathogenic mutation is searched for in the matched DNA isolated from peripheral blood. The constitutional origin and the zygosity of the mutation have to be ascertained to define hereditary cancer risk, implement surveillance and preventive strategies and assess cancer risk for family members. If a pathogenic genomic variant is present but with a low frequency in the DNA from peripheral blood, this may be ascribed to an event of clonal hematopoiesis of indeterminate potential or to a mosaicism. Analyses of secondary normal tissues are therefore necessary to exclude sequencing artifacts and confirm the presence of the low-frequency pathogenic variant in blood or even in other non-transformed tissues. The clinical case of an ovarian cancer patient with a triple negative breast cancer history was analyzed along with her family members.
The DNA from the patient's ovarian carcinoma was isolated and NGS-sequenced for BRCA1/BRCA2 genes. A BRCA1 nonsense pathogenic mutation was identified and its presence confirmed in the DNA from peripheral blood, though with lower-than-expected heterozygous frequency. Further NGS-based analyses in secondary normal tissues revealed the patient as a constitutional mosaic for this variant. In addition, both her breast and ovarian neoplastic tissues harbored this variant with high frequency, demonstrating that the mosaic variant contributes to tumor etiology. A screening of family members revealed that both parents were negative for the pathogenic mutation, confirming therefore the mosaic as a post-zygotic event. One of the daughters, finally, resulted as a previously undiagnosed heterozygous carrier for the pathogenic variant, indicating that the germline tissue of the mosaic patient was involved and the low-frequency BRCA1 variant was heritable.
Constitutional mosaicism is a renowned mechanism for multiple hereditary cancer-associated genes and enables access to personalized therapies and preventive cancer strategies. Therefore, to improve the current standard of constitutional analysis, we propose a new algorithm for the BRCA diagnostic routine in order to increase the sensitivity of germinal assessment and decrease the number of false negatives when pathogenic or likely pathogenic variants occur at low-frequencies.

CC02

UN CASO COMPLESSO DI ATTRIBUZIONE DI PATERNITÀ

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The analysis of STR loci is one of the most powerful methods to estimate relatedness between individuals. In the current case, we describe a complex paternity case arrived in Forensic Genetics Laboratory of Ceinge. A woman requested a paternity testing to know whether a man was the father of her child. Buccal swabs were used to obtain DNA specimens. Autosomal profiles of all subjects were obtained with GlobalFiler™ Amplification kit. Our results showed that child's autosomal profile presented a mixed profile with a maximum of three alleles per locus. Excluding possible sources of contamination, this scenario was compatible with a mother/son mixture. In fact, the child, because affected by leukaemia, previously had undergone to an allogeneic hematopoietic stem cell transplantation with his mother as donor. The PowerQuant® System used to quantify DNA samples, highlighted a concentration autosomal/Y ratio of almost 2. This finding was also underlined with the GlobalFiler™ system, which showed, in Amelogenin locus, a peak height ratio XX/XY of 1.8. We did not have the recipient's autosomal profile, prior the transplant, therefore, we compared locus by locus, the known maternal profile and the mixed child profile. We identified the pairs of mother-son alleles for each locus applying the deconvolution rules of two contributors in the mixture, where one of them is known. Hence, we chose the most probable pair, considering the profile mixture ratio woman/man of 1.8. According to International Society for Forensic Genetics guidelines (ISFG), the biostatistical interpretation was based on a likelihood ratio (LR) approach. Furthermore, LRmix software was used to interpret the mother/son mixture and Familias 3 to comprehend the kinship on the autosomes analysis. In the end, the father/son relationship was confirmed by Y-STRs analysis. Our case highlights that, although buccal swabs represent the most used tool to collect DNA in forensic investigations, we should bear in mind the possible occurrence of chimerism in reference material derived from these samples, if a subject had an allogeneic hematopoietic stem cell transplantation. Therefore, in similar case, other specimens, like hair roots, could be used if they are available.

CC03

IL RUOLO DEL LABORATORIO NELLA VALUTAZIONE DI UNA INASPETTATA ALTERAZIONE DEL APTT

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Acquired haemophilia A (EAA) is a rare hemorrhagic syndrome with autoimmune pathogenesis, due to the development of autoantibodies directed to various epitopes of the Factor VIII molecule. Early recognition of the pathology could help in the adeguate management of clinically suspected EAA, thus a specific laboratory diagnostic algorithm is needed in case of prolonged APTT.Clinical case: on February 2020, patient S.L., 80 years old female patient, accesses to emergency department due to lipothymia, and diffuse hematomas on legs and shoulder since about a week. She underwent standard hematologic tests which show Hb dosage of 7.1 g/dl and normal coagulation parameters except APTT ratio =1.6 . She was not under anticoagulant therapy. APTT ratio was ridetermined the following day, confirming a value 1.4. EAA is suspected thus mixing test and Factor VIII dosage were requested. The mixing test was performed after an incubation time of two hours at 37 ° of a 1:1 mixture of patient and normal plasma confirming a prolonged an APTT ratio of 1.9, thus without correction, and Factor VIII dosage of 5%. Obizur was administrated with excellent clinical follow up. However, the observed delay in performing an optimal diagnostic test could have compromised patient's prognosis. To overcome this output, a laboratory diagnostic algorithm has been proposed as a kind of reflex testing that can be automatically performed by the laboratory staff in case of extended APTT, when a suspicion is referred by clinicians based on clinical and anamnestic (i.e. no anticoagulant therapy) information. The use of APTT/ mixture test could represent a firstlevel approach that should be performed, even in an emergency mode. Failed or incomplete correction of the APTT after mixture test performance suggests the presence of an interfering antibody and orient the choice of appropriate therapy.Acquired haemophilia A is a rare autoimmune disease (1.5 cases / 106 inhabitants / year) secondary to the production of autoantibodies inhibiting factor VIII and is burdened by a mortality of up to 30%. Diagnosis must be timely. We propose a scheme of reflex testing immediately performed by the lab when the clinical and anamnestic information collected by the doctor in charge of the patient rise the suspect a coagulation problem of unjustified or not known cause.

CC04

UN POSSIBILE EFFETTO AVVERSO DELLA VACCINAZIONE ANTI SARS-COV-2

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Background: SARS CoV-2 vaccines, which demonstrated a high efficacy and a beneficial safety profile, could also represent a trigger factor for immune-mediated disease. We report a case of severe anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis following the mRNA vaccine for COVID-19, diagnosed de novo in February 2022 in a Nephrology Unit of Southern Italy.
Case Report: A 21-year-old man of Moroccan origin, with a history of bronchial asthma, entered the hospital for respiratory distress and low-grade fever, occurred few days after the first administration of the BNT162b2 vaccine. He was discharged with oral steroid and antibiotic therapy. One day after the second dose, he entered the hospital again due to worsening of respiratory symptoms, fever, edema and papulo-erythematous/purpuric lesions on limbs and trunk. He underwent routine blood tests with these findings: hypereosinophilia (6.63x10³cell/µL), increased creatinine (2.1 mg/dl), proteinuria (6 g/24h) and microhematuria (>1.0 mg/dL). Further laboratory tests showed increased levels of total IgE (6474 IU/mL, normal value <100 IU/mL) and Eosinophilic Cationic Protein (109 µg/L, normal value <13 µg/L). Autoantibodies anti Myeloperoxidase (Anti-MPO) were positive with high levels (740 CU/mL, cut-off value <20 CU/mL). Additional instrumental examinations and renal biopsy confirmed the diagnosis of ANCA-associated vasculitis. The patient was treated with metilprednisolone (3 boluses of 1g/day), then prednisone (1mg/kg/day) and 2 boluses of Rituximab (375mg/m²). After two months, the follow-up exams revealed normal serum creatinine level (0.8 mg/dl), reduction of proteinuria (3 g/24h), negative anti-MPO and complete remission of the respiratory status.
Conclusions: Among the complications of anti COVID-19 vaccines, several cases of de novo or relapsed ANCA-associated vasculitis were recently reported. We described a new case, onset in a younger manthan previous studies. Even though the relationship between COVID-19 vaccination and development of autoimmune disease has only been suggested, an appropriate laboratory approach, in support of clinical surveillance for immunological complications, should be helpful to diagnosis, monitoring and clarification of possible physiopathologic connection.

CC05

GAMMAPATIA MONOCLONALE E INFEZIONE DA SARS-COV-2

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We report the clinical case of a 90-years-old woman with a history of arterial hypertension, hypothyroidism, obesity, and chronic obstructive pulmonary disease (COPD), who referred to our center (Pederzoli hospital, Peschiera del Garda, Verona, Italy) for dyspnea in January 2022 after tested positive for SARS-CoV-2 infection. The patient received primary vaccination with mRNA vaccine in October 2021. At the time of hospital admission, chest high resolution computed tomography (HRCT) revealed bilateral ground-glass opacities compatible with interstitial pneumonia. Blood tests revealed lymphopenia (0,7 10^3/ uL), iron (33 ug/dL) C-reactive protein (CRP) (28.1 mg/dL) and erythrocyte sedimentation rate (ESR) (79 mm). Treatment with oxygen therapy, steroids, and immunosuppressants (Baricitinib) was started. Two weeks after hospital discharge, during a routine examination, a totally unexpected alteration of the electrophoretic pattern was found on serum protein electrophoresis (SPEP) performed with Capillarys 2 (Sebia, Lisses, France). This alteration is visible as apeak in the g region and it was characterized as IgG- kappa by immunofixation performed with Hydrasys 2 (Sebia, Lisses, France). In this case, the unforeseen occurrence of such alteration in SPEP caught our attention because the patient had no previous evidence of monoclonal gammopathy, and her SPEP was normal in January 2022.A few studies describe the occurrence of a monoclonal spike in association with acute inflammatory illnesses, especially in viral infections. Considering that during severe COVID-19 there is a release of IL-6, which plays an important role in B-cells differentiation into plasma cells, a correlation could be hypothesized between the COVID 19 disease and the alteration in SPEP, without excluding the possible interference given by pharmacological therapy.In conclusion, our findings underline the need of further studies in order to evaluate the degree of immune hyperactivation in patients with severe COVID-19 and the prognostic role to improve the management of patients in this clinical setting.

CC06

UN LIQUIDO SINOVIALE DA INDAGARE ATTENTAMENTE

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Paziente di 82 anni con gonalgia ingravescente, deambulazione difficoltosa e dolore alla flessione al ginocchio destro portatore di protesi totale. Il ginocchio è instabile agli stress in varo-valgo e alla radiografiasi presenta mobilizzazione franca di entrambe le componenti tibiali e femorali. Si pone indicazione alla revisione dell'impianto. Ves elevata, scollamento di entrambe le componenti e storia clinica di dolore rendono necessario escludere un'infezione periprotesica. Si esegue aspirato di liquido sinoviale per conta cellulareed esame colturale. Il liquido, di aspetto torbido e colore paglierino, è raccolto in provette K3EDTA trattato con ialuronidasi e analizzato con contaglobuli XN 2000 Dasit secondo le raccomandazioni CLSI e ICSH. La conta automatizzata mostra un numero elevato di cellule 85000 uL di cui 83% mononucleate e 17% polimorfonucleate senza allarmi morfologici o strumentali. I dati suggeriscono una infezione periprotesica. Lo scattergram strumentale è di colore grigio e forma allungata e mostra una popolazione unica di elementi cellulari la cui peculiarità allerta gli operatori di laboratorio. Si allestisce quindi camera di Burker e cytospin. Sia la conta in camera che il vetrino mostrano assenza totale di elementi figurati e presenza in grande quantità di materiale amorfo, di colore biancastro, somigliante a materiale plastico. La conta al contaglobuli sebbene validata e affidabile soffre di alcune interferenze. In questo caso frammenti plastici amorfi generano segnali captati dal contaglobuli come cellule fornendo un dato numerico errato. Questo evento va considerato se il tipo di impianto è soggetto a usura precoce o se instabilità e mobilizzazione generano alterata cinematica articolare con formazione di particolato anomalo. Basandosi solo sulla conta automatizzata il paziente sarebbe stato classificato come infetto esponendo il clinico ad una diagnosi errata ed il paziente ad una procedura chirurgica aggiuntiva non necessaria. L'approccio ragionato al campione è fondamentale per gestire il rischio di diagnosi errate. In questo caso l'approfondimento al microscopio ottico ha permesso di evidenziare la presenza dell'interferenza ed evitare un danno al paziente.

CC07

I REPERTI INSOLITI NECESSITANO DI INDAGINI APPROFONDITE

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Factitious disorder imposed on another (FDIA), also known as Munchausen syndrome by proxy, is a relatively rare, underreported, but serious form of child abuse. Deception is the core of this behavioral pattern. It is characterized by the falsification of signs/symptoms or induction of injury or disease by a caregiver (generally the mother) without external reward (1).Here, we report a case of FDIA in which laboratory findings were crucial for a timely diagnosis. A 1.5-year-old male child was hospitalized multiple times, mainly because of "intermittent gross hematuria". The urine samples, collected at home and brought in by the mother, appeared red, but, in some occasions, also pink, gray and violet. In two cases the mother reported the presence of blood in the stools and fever. Urinary tract echography, laboratory tests for renal function and metabolic diseases were unrevealing. When once again accessing the emergency room, the mother provided two urine samples with a highly concentrated sediment of red color and a diaper with fine sand-like 'brick dust'. After centrifugation the supernatant was clear yellow and free hemoglobin and myoglobin were absent. Microscopic sediment examination showed viscous large red spots suggesting the presence of an exogenous contaminant. Urine infrared spectroscopy (IRS) revealed traces of seed, crumb and bread crust, and chromatographic analysis showed high levels of lycopene, a major component of tomato sauce and tomato-based food products. A diagnosis of FDIA was done and actions to protect the child's health were taken. Despite the presence of highly suggestive warning signs or red flags in both the caregiver and the victim, FDIA diagnosis is generally difficult and often complicated by a real underlying disease, and can take several years of observation. The reported mortality rate related to FDIA ranges between 6 and 9%. Therefore, early diagnosis is of great importance in order to limit morbidity and mortality. Major efforts to identify exogenous contaminants of biological fluids, if suspected, should be undertaken.

1) Bass C, Glaser D. Lancet. 2014 Apr19;383(9926):1412-21.

CC08

LA SPETTROSCOPIA PROTONICA DI RISONANZA MAGNETICA NUCLEARE COME BIOPSIA BIOCHIMICA VIRTUALE

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Introduction

Myotonic dystrophy type 1 (DM1) is a multisystem disease caused by a (CTG)n expansion in the gene coding for DM protein kinase (DMPK) on chromosome 19q13.3 [1]. Diffuse morphological and functional brain white matter (WM) changes [2], mainly in frontal, temporal and parietal lobes, are reported in neuroimaging and pathological studies [3].
In vivo proton MR spectroscopy (1H-MRS) is a quantitative technique that allows to assess non- invasively the metabolism of targeted brain volumes of interest [4] (VOIs).
Our case pointed to the diagnostic role of 1H-MRS as "virtual biochemical biopsy" for the characterization of WM changes in a DM1 patient.
Methods: The patient (M/36 years) presented a juvenile form of DM1 (genetic class E2) with a disease duration of 24 years. As part of disease monitoring that included clinical and neuropsychological evaluations, he performed a brain morphological MRI that showed a suspected low grade glioma in the right parieto-occipital WM.
Using a standardized protocol on a high field 3T scanner, 1H-MRS was localized in four VOIs [5] (8ml, each scan duration ~3min): 1. suspected glioma, 2. likely DM1 related occipital WM alteration, 3. and 4. contralateral to 1. and 2. morphological healthy tissue. Metabolites content was quantified with the automatic software LCModel 6.3. A sample of 10 matched healthy controls (HC) were selected from our Laboratory database.
Results: VOI 1. and 2. showed a decrease of N-acetyl-aspartate (NAA), neuronal-axonal marker, and an increase of mio-Inositol (mI), glial marker (NAA/mI, 1.10 and 0.69) compared to HC (2.86±0.61). No significant differences were found for VOI 3. and 4. compared to HC. Interestingly the patient had deficits in visuo- spatial and -constructional abilities related to the WM changes location. At one-year follow-up the DM1 disease showed a relative stability.
Conclusions: The absence in the spectrum of an increase in choline, marker of cell turnover and density, and the absence of lactate and mobile lipids allowed to exclude a brain tumor [4].
This case report highlights that 1H-MRS is a reproducible and sensitive technique for in vivo metabolism evaluation that should be included in the diagnostic work-up of brain focal lesion and in selected cases it may replace the need of biopsy procedure.

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Genova, 5-7 ottobre 2022

Riassunti Poster

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• **EP011, EP013, EP070**	Diagnostica cardiovascolare
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Codice E-Poster	Argomento
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• **EP005, EP015, EP020, EP027, EP029, EP030, EP032, EP069, EP089, EP098, EP112, EP129, EP137, EP139, EP156, EP179, EP182, EP193, EP200, EP205, EP218, EP223,EP234, EP238, EP244, EP257, EP269**	Varie

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

EP001

EMOGLOBINOPATIE ALLA LUCE DEI NUOVI FLUSSI MIGRATORI: RUOLO DEL LABORATORIO TRA REALTA' E PROSPETTIVE FUTURE

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PREMESSA: Con l'introduzione dello screening delle emoglobine patologiche nel primo trimestre di gestazione, prestazione introdotta nel libretto di gravidanza secondo il DPCM del 12/01/2017, è stato osservato un incremento dell'incidenza di emoglobinopatie. Negli ultimi decenni si è registrato un aumento dei flussi migratori di soggetti provenienti in gran parte da Paesi extra Unione Europea, in particolare dal Nord e Centro Africa, dall'Est Europa, dall'Asia e dal Sudamerica, zone geografiche in cui l'incidenza dei difetti emoglobinici quali l'HbS, l'HbC, HbD Punjab e le diverse mutazioni beta talassemiche, sono elevate. Lo scopo di questo lavoro è quello di valutare la percentuale di donne in gravidanza, residenti nella provincia di Lucca, portatrici di mutazioni per varianti emoglobiniche in funzione del paese di provenienza, al fine di verificare la correlazione con la distribuzione geografica ed etnica delle forme più comuni di emoglobinopatie. METODI: Lo studio è stato realizzato valutando i risultati delle richieste per diagnosi di emoglobine patologiche dal 2016 fino a dicembre 2021. I campioni di sangue intero sono stati processati con G7 (TOSOH) e Sistema Premier Resolution (Menarini), per la quantificazione e l'identificazione delle diverse frazioni emoglobiniche.RISULTATI: La presenza di numerosi gruppi etnici ha determinato, nella zona di Lucca, un incremento dell'identificazione di varianti emoglobiniche e mutazioni beta talassemiche a partire da febbraio 2019. Confrontando i risultati ottenuti in termini di frequenza e tipologia di variante, è stato osservato che esiste una collerazione con la distribuzione geografica delle più comuni tipologie di emoglobinopatie. Le talassemie e le sindromi falcemiche costituiscono le forme più frequenti a livello globale. Un aumento dei difetti HbS e HbC è frequente nelle donne di origine africana, mentre la β-talassemia è endemica nell'area del Mediterraneo, nel Medio Oriente, in India e nel Sud-Est Asiatico.CONCLUSIONI: L'introduzione dello screening delle emoglobinopatie evidenzia come i flussi migratori rappresentano una sfida di adeguamento per il Sistema Sanitario, che è chiamato a prendere atto delle diverse peculiarità biologiche che possono contraddistinguere le varie etnie, presenti sul territorio.

EP002

Urinary 2,8-dihydroxyadenine (DHA) crystals: a case report

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We describe here the case of a 73-years-old female with suspected 2,8-dihydroxyadenine (DHA) crystals during morphological urinalysis. Urinalysis was performed with Menarini Sedimax Contrust Pro (A. Menarini Diagnostics, Florence, Italy) and bright field microscopy (Nikon Corporation, Tokyo, Japan) (400x magnification). The presence of crystals of spherical shape and brownish color was observed. The patient had a recent history of sclero-skin jaundice. With blood testing, hyperbilirubinemia and impaired liver function was detected. Subsequent abdomen computed tomography (CT) exam with intravenous contrast highlighted a 3 cm lesion in the hepatic isthmus and the presence of multiple hepatic cysts. Biopsy examination characterized the lesion as a pancreatic ductal adenocarcinoma.
Adenine phosphoribosyl transferase deficiency (APRT) is a rare autosomal recessive genetic disorder caused by generation and hyperexcretion of 2,8-DHA in urine that causes urolithiasis and crystalline nephropathy. APRT deficiency is caused by multiple mutations in the APRT gene (16q24) encoding the APRT enzyme catalyzing AMP synthesis from adenine and 5'-phosphoryibosyl-1-pyrophosphate. The disease is generally independent of gender or age, but has an over double burden in Caucasians compared to Asians. Symptoms are those typical of urolithiasis, with precipitation of stones along the urinary tract. The stones are typically radiolucent, and the onset is estimated between infancy and the 4th decade of life, but sometimes even later. Some patients could remain asymptomatic, though precipitation of 2,8-DHA can lead to acute renal failure or an end-stage renal disease requiring dialysis and transplantation in the worst scenario. Diagnosis is primarily based on identifying 2,8-DHA crystals or stones by morphologic examination combined with infrared spectrometry and/or X-ray crystallography.

EP003

Improved UPLC-ESI-MS/MS method for analysis of plasma amino acids by precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (ACQ)

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Analysis of free amino acid (AA) profiles in human plasma is crucial for the diagnosis and monitoring of patients with inherited metabolic disorders. Heterocyclic aromatic carbamate compounds are new generation precolumn derivatization reagents, able to obtain stable adducts of AAs with characteristics suitable for LC-MS analysis. The most widely reagent used is 6-aminoquinoline-N-hydroxysuccinimide carbamate (AQC). The AQC reacts directly with the primary and secondary AAs, the reaction occurs in a matter of seconds and leads to a single product for each AA, without interference of by-products (1). However, the application of AQC amino acid (AQC-AA) derivatives in LC/MS is not fully standardized and the derivatization reaction still needs complete optimization. The aim of this study was to improve, optimize and validate an ultrahigh-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) method for the quantification of clinical relevance AQC-AA derivatives in human plasma.The complete derivatization of all AQC-AA derivatives was performed in a 21 mM AQC solution, at 55°C and an optimal pH value of 8.8. The same daughter ion (m/z=171) corresponding to the cleavage of ureide bonds of the AQC adduct in each AA derivative was produced in a reaction time of 10 min. Optimization of derivatization matrix effect (dME% less than 20%) allows excellent reversed phase chromatographic separation and ESI-MS/MS spectrometric detection of 28 AQC-AA derivatives in 9 min, including isomers, such as leucine and isoleucine, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). A small sample amount (10-50 µl) and the inclusion of 20 isotopes marked as internal standards allows high linearity (up to 4000 µM) and sensitivity. The limits of quantitation is of 2.53 - 5.52 µM, the intra- and interday precision is less than 15%, and the accuracy is 83-118%, at three quality control levels. This robust analytical method makes it suitable for high-throughput targeted UPLC-ESI-MS/MS metabolomic analysis in clinical and epidemiological environments.

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EP004

ESPERIENZA DI APPLICAZIONE DI UN PROTOCOLLO PER COMMISSIONI MEDICHE LOCALI PER IDONEITA' ALLA GUIDA NEI CONDUCENTI SANZIONATI PER l'art.186 C.d.s.

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Scopi ed obiettivi:Lo stato derivante dalla dipendenza da alcool compromette i requisiti fisici e psichici richiesti nella valutazione d'idoneità alla guida."La Commissione Medica Locale deve accertare l'idoneità alla guida e a tal fine: può avvalersi di singoli consulenti oppure di istituti medici specialistici appartenenti a strutture pubbliche, con onere a carico del soggetto esaminato (D.P.R. 495/92, art. 330, c.6)".Per tali accertamenti è stato predisposto in Regione Liguria un Protocollo (Delibera 321 del 19.12.2018) che vede oltre alla tradizionale quantificazione della CDT su siero, quella dell'Etil-glucuronide (ETG) su matrice cheratinica, con l'esclusione di tutti i marcatori indiretti tradizionali (transaminasi, MCV ecc.). Tramite uno studio retrospettivo andremo a dimostrare come l'utilizzo del nuovo Protocollo, sia uno strumento efficace nel monitoraggio per l'idoneità alla guida dei soggetti sanzionati per l'art.186 del C.d.s. e come questo ci abbia permesso di semplificare la procedura rendendola al contempo più efficace. Materiali e metodo: Lo studio retrospettivo è stato condotto su 1276 utenti sanzionati per art.186 CDS esaminati dalle Commissioni Mediche Locali della Liguria (ASL 5-Spezzino; Asl 4-Chiavarese; Asl 2–Savonese) nel 2021 attraverso un Protocollo che includeva la quantificazione di ETG (Etil-glucuronide) su matrice cheratinica e CDT (Carboidrati-Transferrina carente) sul siero.Le analisi sono state eseguite presso il Laboratorio Tossicologico di Riferimento Regionale del Levante Ligure, sito a Sarzana presso l'Ospedale San Bartolomeo.La quantificazione di ETG su matrice cheratinica è stata eseguita utilizzando un cromatografo liquido (Agilent infinity 1260) accoppiato ad uno spettrometro di massa tandem (Agilent QQQ 6470) mentre il CDT è stato valutato in cromatografia liquida ad alta pressione (Thermofisher Ultimate 3000) con detector UV-VIS.Il campionamento della matrice cheratinica è stato eseguito su un segmento di capelli di 3-6 cm prelevato dalla zona nucale, in alternativa sono stati campionati i peli del torace o degli arti (SOHT Consensus 2019).Per la refertazione abbiamo considerato i cut-off suggeriti dalle Linee Guida dell'ISS, dei tossicologi forensi e del SOHT.

Risultati :Dall'osservazione dei nostri dati relativi alle Commissioni Medico Locali della Liguria, emerge che su 1276 utenti monitorati nel 2021 per guida in stato di ebrezza a cui è stato applicato il nostro Protocollo, circa il 17.16% ha presentato valori di ETG (Etil-glucuronide) superiori a 30 pg/mg, soglia che indica un consumo elevato di alcool.Confrontando la positività al marker ematico tradizionale di abuso alcolico CDT (1.02%) con quella dell'ETG (17.16%) , abbiamo riscontrato un aumento di casi di inidoneità alla guida del 16.14 % Possiamo concludere che l'introduzione del nostro Protocollo, esteso a tutta la Regione Liguria,

ha rappresentato un sicuro ausilio per le Commissioni Medico Locali, fornendo nuovi e più efficaci elementi valutativi per l'idoneità alla guida.

EP005

Valutazione delle performance analitiche di test immunoturbidimetrici per il dosaggio dell'elastasi pancreatica

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Introduzione: La valutazione dell'elastasi pancreatica fecale è importante per la determinazione della funzionalità pancreatica. Si considera fisiologica una concentrazione >200μg/g, si evidenzia la presenza di insufficienza pancreatica di grado lieve 100-200μg/g, di grado severo <100μg/g. In commercio sono disponibili diversi test. In questo studio abbiamo valutato le performance analitiche di tre metodi in turbidimetria per la determinazione dell'elastasi pancreatica su campioni di feci.

Metodi: Tra ottobre 2021 e febbraio 2022, presso il laboratorio analisi dell'Ospedale di Alessandria, sono stati raccolti i campioni di feci di pazienti con sospetto clinico di insufficienza pancreatica e analizzati con i kit Buhlmann fPELA® turbo Kit (BLM), Pancreatic Elastase Turbilatex CerTest (CT) ed Elaprest turbo Eurospital (EUR) su analizzatore Optilite (The Binding Site). I campioni sono stati sottoposti alla procedura di estrazione lo stesso giorno della seduta analitica.

Risultati: Sono stati valutati 40 pazienti con un'età mediana di 69 anni. La concentrazione media dei tre metodi è risultata di 259,06 + 166,67 per BLM, 489,64 + 250,22 per EUR e 364,20 + 218,06 per CT. È stata riscontrata una buona correlazione analitica tra BLM e EUR (coefficiente di spearman rs=0,955), con una sovrastima di EUR rispetto a BLM e la presenza di un bias significativo (+230,58). La concordanza clinica è stata del 53%, con 16 campioni discordanti. La correlazione tra BLM e CT è risultata buona (rs=0,924), con una sovrastima di CT e la presenza di un errore sistematico di 105,15. La concordanza clinica è del 78%, con 9 campioni discordanti. È stata riscontrata una buona correlazione analitica tra CT ed EUR (rs= 0,950), con una sottostima di CT (-128,59) ed una concordanza clinica dell'83%.

Conclusione: è stata riscontrata una buona correlazione analitica tra i tre saggi turbidimetrici. Sebbene le diagnosi dei pazienti non siano disponibili, sono emersi dei risultati discordanti rispetto al cut-off decisionale, che potrebbero essere dovuti dalla variabilità della fase preanalitica o dalla mancanza di uno standard di riferimento internazionale. È necessario approfondire questo studio valutando la metrologia del calibratore, l'accuratezza e il grado di imprecisione dei singoli metodi.

EP006

SF3B1, NOTCH1 AND ASXL1 VARIANTS BY NGS ANALYSIS ON UNMUTMED IGHV GROUP OF SARDINIAN PATIENTS CLL # SUBSET 1 IN CHRONIC LYMPHATIC LEUKEMIA.

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Introduction. Chronic Lymphocytic Leukemia(CLL) is a chronic B-cell malignancy. Recent studies have revelated recurrent mutations for NOTCH1 and others genes specially in patients with poor prognosis.In western countries.Recent data suggest that SF3B1 has been reported as one of the prognostic markers in CLL.The tumor suppressor gene ASXL1, which is located in 20q and is commonly mutated in malignant myeloid diseases and occasionally in CLL , it is a member of Asxl family involved in recruitment of epigenetic and transcriptional regulators genomic locus. ASXL1 may activate oncogenes.CLL patients associated at subset CLL#1 unmutaded IGHV and 1/5/7family expressing stereotyped share, poor prognosis and and biological features . Aim of study: Study by NGS on unmutated IGHV families of CLL, with subset# 1, in Sardinian people group, with 32 genes panel. Materials and methods:Were been studied 166 subsets of IGHV patients at starting of CLL, in Cancer Hospital of Cagliari, Ematology Department; all DNA samples were been evaluated from 2016 to 2022. Polymerase chain reactions amplifications for NOTCH1 and PCR and Sanger sequencing analysis were been performed for to evaluate IGHV somatic mutational status. Analysis, by NGS methodology, was performed on panels with 32 customized genes in use in our laboratory Results: In 166 patients studied, the 10,24% have showed the subset CLL# expression in IGHV unmuted. In summary, the frequency of type of subset# show: the 52,9% were CLL#1 unmuted, 11,76% CLL#6 unmutated, 11,76% CLL#4 mutated, 5,8% CLL#7 unmutated, 5,8% CLL#64b unmutated and 5,8% CLL#8 unmutated. The frequency of all CLL subset was more too in relations at normal frequency by Arrest tools. The analysis of mutations of NOTCH1 were found in 7 patients with subsets expresssion. Ngs variant analysis show 62,5% of NOTCH1 variant, SF3B1 and ASXL1 variant present in 50% of patients, CHD2 variants in 62,5 % ; IKZF3,MGA,BRAF presents in 25% of samples and TP53 with XPO1 in 12,5%. All variants were pathogenetics Tier3 and Tier 2C , and allelic fractions between 2 and 36%. In conclusion pathogenetics Tier3 and Tier 2C were presents for CHD2 variants, SF3B1 and ASXL1 variants and NOTCH1 variant in more 50% of sardinian patients.

EP007

LOW JAK2 V617F ALLELE BURDEN, POSITIVE : DIAGNOSTIC STUDIES AND INDICATIONS

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The myeloproliferative neoplasms (MPNs) are neoplastic pathologies that affect hematopoietic stem cells and are characterized by uncontrolled proliferation of mature myeloid cells. Approximately 70% of patients with myeloproliferative neoplasms carry a point mutation in the JAK2 gene. The JAK2 V617F mutation was discovered in 2005 and it consists in a point mutation in exon 14of JAK2, which involves the replacement of the amino acid valine with the amino acid phenylalanine at the level of codon 617 of the pseudo-kinase domain (JH2). The result of this mutation is the interruption of the autoinhibitory activity of the pseudokinase domain and the consequent constitutive activation of the kinase domain (JH1). The purpose of this studies the quantification of the JAK2 V617F mutation in patients with suspected MPN from 2017 to 2019 in the S.C Hematology and CTMO of the Businco Hospital in Cagliari. The analyzes were carried out on 593 peripheral blood samples and, after being analyzed, they were divided into 4 groups based on the percentage of mutated JAK2 V617F alleles: negative, gray zone, positive with low JAK2 V617F allele burden, positive. Particular attention was paid to the group of patients whose JAK2 V617F allelic quantification value fell in the gray area (2% of patients). Further molecular investigation performed on these patients showed the presence of the W515L mutation in the MPLgene in 12,5% of the samples and an insertion in the CALR gene in 14,2% of the patients.

EP008

Optimization of the workflow in the emergency sector of the clinical chemical analysis laboratory of Ss. Trinità Hospital of the Local Health Authority of Novara (ASL NO)

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Introduction: the clinical chemical analysis laboratory of the ASL NO is organized into sectors of activity, among these there is the emergency sector, characterized by a workflow distinct from the other sectors of the laboratory, called routine sectors. This laboratory has recently undergone a technological renewal, this has brought tangible improvements in all laboratory sectors but the workflow has never been revised.
Aim: to propose a revision of the emergency sector workflow within this laboratory, in order to enhance and make more agile the work of the human resources working within the laboratory, even in the wake of the spread of the operations management function that in recent years is affecting all the production areas of healthcare companies, in order to maximize the efficiency and effectiveness of the activities carried out within them.
Materials and methods: research in the literature of organizational models of the analysis laboratories, documentary analysis concerning the regulations for 24-hour workers, national and regional regulatory guidelines for clinical laboratories, company directives for the laboratory and laboratory documentation, direct observation of the workflow, statistical processing of the tests processed by the laboratory and interviews with some internal stakeholders.
Results and discussion: a new organizational model was developed through information obtained from these surveys; this new model is characterized by a partial modification to the current workflow and by a new rotational shiftwork. The changes to the current workflow include the handling of a portion of the emergency samples from the urgent sector to the routine sectors; these allow the emergency sector operators to have shorter work shifts and to work for a period in the routine sectors. The routine laboratory technicians will not be overloaded with work because they are supported by the new technology park that has speeded up the procedures.
Conclusions: the new organizational model aims at collaboration and integration between emergency sector and routine sectors through the changes to the workflow and the new rotation; the latter enhances the emergency sector lab technicians towards the sectoral specialization through the period of work in the routine sectors.

EP009

Abnormal levels of serum/urine serotonin and metabolites of catecholamines by monoamine oxidase deficiency A (MAO A)

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Defects in the biosynthesis, metabolism, and homeostasis of serotonin (5-HT) dopamine, norepinephrine, and epinephrine can cause a heterogeneous group of rare inherited neurometabolic syndromes usually manifesting in childhood, with a broad spectrum of neurological and psychiatric symptoms (1). The typical behavioural dysfunction associated with these disorders might be misdiagnosed as with a hyperactivity attention deficit syndrome, the most common disorder of the developmental age (childhood and adolescence). Consequently, these genetic disorders are often under-recognized.We here describe a 4-year-old male patient with a diagnosis of autism spectrum disorder and mild dysmorphic features, such as macrocephaly and bilateral epicanthus palpebralis. The whole exome sequencing analysis of the monoamine oxidase A (MAOA) structural gene, led to the identification in the 4 exon a point mutation homozygous c.410 A>G (p.E137G), which modifies a glutamine to a glycine. Brunner syndrome, a recessive X-linked disorder, associated with a complete and selective deficiency of enzymatic activity of MAOA was suspected (OMIM 300615). Sanger sequencing confirmed that his unaffected mother carried the same mutation in the heterozygous state, while his father did not carry the same mutation. A reduction of MAOA activity was assessed by measuring of serum and urinary monoamine catabolites. In the proband and his mother, the levels of serum 5-HT and of urinary normetanephrine (NMN), two specific MAOA substrates, were higher than normal, whereas the products 5-hydroxyindoleacetic acid (5-HIAA) and vanillylmandelic acid (VMA) was below the limit of normality. The identification of this new point mutation confirms the monogenic implication of the MAOA gene in behavioural dysfunctions The identification of an altered monoamine metabolism should be taken into account when prescribing psychoactive drugs in such patients. Brunner syndrome should be considered as a cause of abnormal behavioural symptoms. Early clinical suspicion and appropriate investigations, including serum/urine 5-HT and catecholamines metabolites measurement, are essential for accurate clinical diagnosis.

1) Cheung N W et al Intern. Med. 161: 2503-2504, 2001

EP010

Monitoring sputum properties in cystic fibrosis patients by means of Low-Field NMR before after kaftrio treatment

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Background. Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), responsible for chloride and sodium ion exchange across epithelial membranes. Dysfunctional CFTR induces the production of thick/viscous mucoid secretions in multiple organs, in particular the airways, where an augmented mucus viscosity is determined by the pathological increase in proteins, mucin and biological polymers. This process impairs muco-ciliary clearance, promoting chronic inflammation and bacterial infection leading to airway remodeling; this, in turn, can progress to respiratory failure, the most common cause of death for CF patients. The alteration of mucus composition can be easily assessed by evaluating the fraction that patients can expectorate (sputum). As sputum composition depends on lung condition, the determination of its properties represents a relevant parameter to indirectly monitoring lung disease.We previously (Abrami et al Magn Res Med, p.427, 2020; Abrami et al Magn Res Med, p. 2323, 2018;) employed Low Field Nuclear Magnetic Resonance (LF-NMR) to measure the spin-spin relaxation time (T2m) of the water hydrogens present in the CF sputum. These data showed that T2m measured in CF sputum indirectly correlated with circulating/local inflammation markers and directly correlated with FEV1 (forced expiratory volume in the first second, i.e. the amount of air that is exhaled in the first second purposefully trying to breath out as much air as possible). Thus, the assessment of sputum by T2m, provides a useful tool for the indirect monitoring of lung disease in CF patients. Here, we explore the possibility of T2m to detect lung function improvements following treatment by the novel drug Kaftrio. This is a triple modulator (Tezacaftor/Ivacaftor/Elaxacaftor) able to improve CFTR protein function. Approved by E.M.A in 2020, Kaftrio has been shown to have beneficial effects not only in CF patients with mild-to-moderate disease but also in those with more severe pulmonary status.

Methods. Sputum samples from 17 CF subjects, provided by Burlo Garofolo Hospital -Centro Regionale Fibrosi Cistic-, following a procedure approved by the Ethics Committee (prot n. 0005431/2020, CEUR-2019-Em-408), were collected (by voluntary expectoration) before and after Kaftrio administration (total samples 34). Sputum samples were studied by LF-NMR to determine T2m. The data obtained were related to lung function evaluated by FEV1.

Results. In 58% of cases, Kaftrio administration improved both FEV1 and T2m, thus indicating a positive effect of the drug on lung function. In 25% of cases Kaftrio determined a reduction of both FEV1 and T2m, indicating no significant benefic effect on lung function. Finally, in only 17% of cases, Kaftrio treatment resulted in discordant variation of FEV1 and T2m, i.e. FEV1 increase and T2m decrease. This odd observation may be attributed to the fact that Kaftrio caused the dehydration of the mucus (sputum) reducing its quantity (FEV1 increase) but worsening its nano-structure thus resulting in T2m decrease.

Conclusions. Here we demonstrate that T2m can be effectively used to evaluate CF lung function following Kaftrio administration, in addition to be able to monitor lung disease exacerbation (our previous results). Considering that T2m determination is very inexpensive does not require specialized personnel and it is not of any discomfort for the patient, it can become a valuable monitoring tool of lung function in CF diseases.

EP011

PREDICTORS OF Acute kidney injury (AKI) in ST-elevation myocardial infarction patients: extracellular matrix biomarkers

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BACKGROUND: Acute kidney injury (AKI) is a frequent complication in patients with ST-elevation myocardial infarction (STEMI), associated with increased morbidity and mortality.Structural and functional changes in kidneys leading to AKI may be due to different pathological mechanism, including renal hypoperfusion, ischemia and nephrotoxicity.Collagen turnover and extracellular remodeling are regulated by matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Another interesting group of candidate biomarkers for extracellular matrix remodeling are procollagen peptides, which represent molecules that are cleaved from collagen precursors. In this study we focused on the relationship of blood concentrations of TIMP-1 and protocollagen type III aminoterminal peptide (PIIINP) with the occurrence of AKI in STEMI patients.MATERIAL and METHODS. 117 consecutive STEMI patients admitted to Centro Cardiologico Monzino (65% males, mean age 63+/-13years) were enrolled in the study. The routine laboratory tests, particularly creatinine, eGFR, TIMP-1 and PIINT were performed in all population at admission on Atellica Solution, (Siemens Healthineers Diagnostic, NY, USA). Twenty healthy subjects served as controls.STATISTICS Continuous data will be expressed as mean +/- standard deviation (SD) and compared by unpaired t-test. Pearson's correlation coefficient will be calculated to assess the correlation between the variables of interest. A p-value <0.05 will be considered significant. RESULTS We observed no differences between TIMP-1 and PIINT by sex, while both correlated with age (p<0.01). Timp-1 and PIIINT were significantly higher in STEMI patient compared with controls. Timp-1 and PIIINT significantly correlate both with creatinine and eGFR (P<0.001). Timp-1 was also significantly higher in patients with AKI (increase in creatinine >0.5 mg/dl between hospital admission and the following 72 hours) CONCLUSIONS We report a consistent positive correlation of both Timp-1 and PIIINT with STEMI.

Development of AKI, one of the most important STEMI complications, seems to be associated with matrix remodelling biomarkers.

EP012

Combination of protein induced by vitamin K absence or antagonist-II and aspartate aminotransferase-lymphocyte ratio index for the prediction of hepatocellular carcinoma development in patients with hepatitis C virus-related cirrhosis treated with di

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Background and aims: Patients with hepatitis C virus (HCV)-related cirrhosis treated with direct-acting antivirals (DAA) are still at risk of developing hepatocellular carcinoma (HCC); the identification of biomarkers able to improve the surveillance of patients at risk is an unmet need. Here, we investigated the performance of protein induced by vitamin K absence or antagonist-II (PIVKA-II) compared or combined to different systemic inflammatory indices (SIIs) for the prediction of HCC development in patients treated with DAA on long-term follow-up (FU).
Patients and methods: We analysed data from 629 consecutive patients (median age 65, 57–76 years; 364 males/265 females) with cirrhosis and no history of HCC who achieved a sustained virologic response to DAA therapy. PIVKA-II and SIIs were measured at 3 months after the end of DAA therapy. PIVKA-II was measured in serum by fully automated chemiluminescence immunoassay Lumipulse G 600 II system (Fujirebio, Tokyo, Japan). The SIIs evaluated were neutrophil to lymphocyte ratio (NLR), lymphocytes to monocytes ratio (LMR), platelet to lymphocyte ratio (PLR), and aspartate aminotransferase-lymphocyte ratio index (ALRI).
Results: During a median FU of 36.8 (34.3–39.0) months, 62 (9.9%) patients developed de novo HCC. PIVKA-II (HR = 2.58, p < 0.001), LMR (HR = 0.77, p = 0.007), PLR (HR = 0.99, p = 0.020), ALRI (HR = 1.01, p < 0.001) but not NLR (HR = 0.98, p = 0.847) resulted significantly associated to HCC occurrence. The higher overall performance for HCC prediction was observed for the combination of PIVKA-II + ALRI (C-index: 0.72); the corresponding diagnostic accuracies at 1-, 2-, and 3-years of FU were AUC = 0.89, 0.79, and 0.74, respectively. Furthermore, PIVKA-II + ALRI allowed the stratification of patients into 3 risk categories (low-, medium-, and high-risk) with significantly different incidence rates of HCC (4.2%, 6.2%, and 19.1%; p < 0.001); notably, none of the patients of the low- and medium-risk groups developed HCC during the first year of FU.
Conclusion: PIVKA-II + ALRI may represent a valuable and inexpensive tool for risk stratification and personalization of HCC surveillance strategy for patients with HCV-related cirrhosis successfully treated with DAA.

EP013
Rischio aterosclerotico residuo oltre l'LDL

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Background: L'aterosclerosi è una malattia vascolare cronica progressiva nell'età adulta e responsabile di mortalità cardiovascolare nella maggioranza dei paesi del mondo. L'approccio diagnostico indicato per la stratificazione del rischio di tali pazienti, include i dosaggi del colesterolo sd-LDL e quello della Lipoproteina associata alla Fosfolipasi A2. Obiettivo dello studio: Una delle attuali frontiere della Diagnostica di Laboratorio è rappresentata dall'individuazione di specifici markers circolanti per inquadrare i parametri infiammatori e marcatori specifici di rischio cardiovascolare. Materiali e Metodi: Lo studio monocentrico ha arruolato 40 pazienti presso l'ambulatorio di Cardiologia dell'AORN "Monaldi" di Napoli, tra Ottobre 2021 e Maggio 2022. I criteri di inclusione erano: presenza di malattia aterosclerotica cronica (CAD e/o PAD) con storia di aterosclerosi in almeno 2 distretti vascolari, o in presenza di fattori di rischio cardiovascolare. I pazienti sono stati sottoposti ad un prelievo ematico al T0 e dopo 6 mesi di terapia con statine ad elevata intensità (torvastatina e rosuvastatina) sono stati dosati i livelli ematici di alcuni markers di laboratorio: Adrenomedullina (ADM), Copeptina (COOP), Proteina C Reattiva (PCR), Interleuchina 6 (IL-6), Lipoproteine a bassa densità (sd-LDL), Lipoproteina associata alla Fosfolipasi A2 (Lp-PLA2). Risultati: Abbiamo analizzato i dati relativi a 40 pazienti, di cui 27 affetti da CAD e 26 da PAD. I parametri infiammatori hanno evidenziato un significativo decremento dall'inizio della terapia, come i valori di colesterolo e trigliceridi. Conclusioni: Dall'analisi dei dati, i valori di ADM, COOP e PCR non hanno riscontrato drastiche variazioni per l'assenza di uno stato settico. La significativa riduzione dell'IL-6, potrebbe indicare un miglioramento dello stato infiammatorio sistemico. Il decremento dei valori di colesterolo e la riduzione di sd-LDL e della Lp-PLA2 a 6 mesi dall'inizio della terapia, rappresentano un importante risultato che evidenzia come i livelli di Lp-PLA2 siano validi nella stratificazione del rischio cardiovascolare tra i pazienti che sono a rischio intermedio o alto. Bibliografia: Ai M. et al. Small Dense LDL Cholesterol and Coronary Heart Disease; 2010-PubMed

EP014
Screening rapporti Proteine/Creatinina ed Albumina/ Creatinina urinari: quanto possiamo fidarci del dipstick?

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Introduzione
I rapporti proteina/creatinina (PCR) e albumina/creatinina (ACR) su urine estemporanee sono importanti per la valutazione quantitativa della proteinuria nella diagnostica e nel monitoraggio delle malattie renali ed in altri stati patologici. La chimica secca su dipstick è il metodo più usato per la determinazione di PCR e ACR, tuttavia, in considerazione dei limiti di questa metodica oltre che del fatto di fornire dei risultati semi-quantitativi è da considerarsi un metodo di screening che necessita di essere confermato con la determinazione mediante l'utilizzo di metodiche in chimica liquida.
Metodi
Sono stati estratti dal LIS, valutando un periodo di 5 mesi, tutti i risultati relativi ad ACR e PCR dell'esame chimico-fisico delle urine eseguito su strumentazione Sysmex UC-3500 (Sysmex Corp., Kobe, Giappone) in chimica secca/dipstick (semi-quantitativo) per i quali erano disponibili anche ACR e PCR eseguiti in chimica liquida (quantitativo, metodo di riferimento) su strumentazione Roche Cobas c702 (Roche Diag. AG, Rotkreuz, Svizzera). Sono state quindi valutate sensibilità e specificità del test dipstick rispetto al test in chimica liquida ai valori di cut-off indicati dalle linee guida KDIGO 2012.
Risultati
L'analisi è stata condotta su 172 valori di PCR e 3353 di ACR. Al cut-off di 30 mg/g, ACR eseguito su dipstick ha mostrato una sensibilità del 43,4% ed una specificità del 91,5%; al cut-off di 300 mg/g la sensibilità è risultata del 53,8% con una specificità del 95,9%.Al cut-off di 150 mg/g, PCR eseguito su dipstick ha mostrato una sensibilità del 48,5% ed una specificità del 91,8%; al cut-off di 500 mg/g la sensibilità è risultata dell'85,0% con una specificità del 96,1%.
Conclusioni
Sebbene le performance di specificità siano buone, non si può dire altrettanto a riguardo della sensibilità, anche considerando che il test dovrebbe avere finalità di screening. Si sottolinea quindi l'importanza dell'esecuzione del test in chimica liquida nei casi patologici o in caso di sospetto clinico di albuminuria o proteinuria.

EP015

IMPROVED DIAGNOSIS OF ADENOSINE DEAMINASE-2 USING A FUNCTIONAL ENZIMATIC TEST BY LC-MS/MS FROM DRIED PLASMA SPOT: A SINGLE CENTER EXPERIENCE

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Adenosine Deaminase 2 Deficiency (DADA2) (OMIM: 607575) is a monogenic autoinflammatory disease caused by biallelic loss of function mutations in ADA2 gene (previously CECR1, Cat Eye Syndrome Chromosome Region 1). A timely diagnosis is crucial to start anti-TNF therapies that are effective in preventing vasculitic complications. The confirmation of DADA2 is based on DNA sequencing and enzymatic assay. It is thus very important to have robust and reliable assays that can be rapidly utilized in specialized laboratories that can centralize samples from other centers. ADA2 activity was determined in dried plasma spot (DPS) by a LC-MS/MS method that allows the accurate determination of the ADA2 enzyme activity starting from very small amounts of plasma. Since May 2021 at Giannina Gaslini Institute ADA2 enzymatic activity has been tested for 94 patients whose 40 healthy subjects, 30 DADA2 patients and 24 carriers. 26 samples were sent from other centers. ADA2 activity, expressed as mean ± SD, was 1.46 ± 1.35 mU/mL in healthy controls, 0.02 ± 0.03 mU/mL in DADA2 patients and 0.21 ± 0.10 mU/mL in carriers. The Mann–Whitney test showed statistically significant differences ($p < 0.0001$) between the three groups. The method allows to significantly distinguish healthy controls from affected patients and carriers. It is of great help in implementing the diagnostic workflow of DADA2. The availability of a fast and reliable functional assay is particularly important in order to offer a rapid alert to clinicians even before the results of the genetic analysis and to promptly treat patients. Moreover, the use of DPS and the possibility to store and deliver samples at room temperature offers the advantage of inter-site shipments at room temperature allowing a rapid diagnosis of suspected subjects in specialized centers and timely treatment start.

EP016

Therapeutic drug monitoring of cystic fibrosis modulators Ivacaftor, Tezacaftor and Elexacaftor: development and validation of a new LC-MS/MS method and its application to CF patients treated with Kaftrio

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INTRODUCTION: Ivacaftor (I), tezacaftor (T) and elexacaftor (E) is a novel drug combination for cystic fibrosis (CF) that directly modulates the transmembrane conductance regulatory protein (CFTR). Unprecedented positive outcomes have been demonstrated on patients bearing at least one copy of the F508del allele. Interpatient variability has been observed in terms of response to treatment. More data on dose-concentration-response relationship would be therefore useful to better understand the mechanisms of response.

METHODS: We have developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously quantify I, T and E in plasma using a rapid organic extraction and separation on a reversed-phase C-18 HPLC column after addition of deuterated internal standards. Accurate analytes quantification using SRM detection was obtained. The method was validated following EMA guidelines for bioanalytical method validation and was applied on 29 samples obtained at Cmin from 27 patients treated with T, E and I at Giannina Gaslini Institute. Response assessment was established measuring FEV1(%) before and after treatment. Patients were classified in non-responders ($\Delta FEV1 \leq 6\%$), poor responders ($6\% < \Delta FEV1 < 14\%$) and high responders ($\Delta FEV1 \geq 14\%$).

RESULTS: The LC-MS/MS assay is linear over wide concentration ranges (0.12 -12 mg/L) in plasma accurate and reproducible in the absence of matrix effects. We have obtained a very specific, sensitive, and rapid quantification of the three CFTR modulators starting from very low volumes (50 µL) of plasma samples. The stability of analytes in plasma for at least 30 days allowed for a cost effective shipment and storage at room temperature. Plasma levels obtained (mean ± SD) were 3.56 ± 1.43 µg/mL for T, 4.82 ± 2.13 µg/mL for E and 0.79 ± 0.13 µg/mL for I. They were found to be not significantly different in

the 3 groups of patients (P>0.05) and no correlation could be found between Cmin and drug response.
CONCLUSIONS: Our new LC-MS/MS method is helpful in support to clinical studies on dose- exposure-response that are needed to further optimize treatments with Kaftrio in CF patients.

EP017
Neonate capillary blood gases, looking for reference values.

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Background
We report the experience of the last year with neonate capillary blood gas analysis performed by a POCT blood gas analyzer (Siemens RAPIDPoint 500®) in neonatology ward.
Methods
We collected blood gas analysis performed during 2021 selecting 42 healthy term (39,6 ± 1,3 weeks of gestation) neonates aged 0-5 days.
We have considered the following analytes: pH, pO2 (mm Hg), pCO2 (mm Hg), capillary Oxygen Saturation % (ScO2%), Sodium (mmol/L), Potassium (mmol/L), Hemoglobin (g/dL), Glucose (mg/dL), Chlorine (mmol/L), Lactate (mmol/L), Ionized Calcium (mg/dL). They were simultaneously measured with selective electrodes on blood gas analyzer.
Results
pH: Mean: 7,366; 1 SD: 0,038; 2,5 %ile: 7,290; 97,5 %ile: 7,442.
pO2 (mm Hg): Mean: 47,6; 1 SD: 8,1; 2,5 %ile: 31,3; 97,5 %ile: 63,9.
pCO2 (mm Hg): Mean: 39,6; 1 SD: 5,1; 2,5 %ile: 29,4; 97,5 %ile: 49,9.
ScO2%: Mean: 89,3; 1 SD: 5,8; 2,5 %ile: 77,6; 97,5 %ile: 100.
Sodium (mmol/L): Mean: 136,8; 1 SD: 3,6; 2,5 %ile: 129,5; 97,5 %ile: 144,2.
Potassium: Mean (mmol/L): 5,4; 1 SD: 0,5; 2,5 %ile: 4,4; 97,5 %ile: 6,4.
Chlorine (mmol/L): Mean: 103; 1 SD: 2,8 3 %ile: 97 %ile: 109.
Hemoglobin (g/dL): Mean: 19,8; 1 SD: 1,9; 2,5 %ile: 15,9; 97,5 %ile: 23,8.
Glucose (mg/dL): Mean: 62; 1 SD: 10; 2,5 %ile: 42; 97,5 %ile: 62.
Lactate (mmol/L): Mean: 2,8; 1 SD: 0,6; 2,5 %ile: 1,5; 97,5 %ile: 4,2.
Ionized Calcium (mg/dL): Mean: 4,9; 1 SD: 0,3; 2,5 %ile: 4,2; 97,5 %ile: 5,6.
Conclusions
The collected data reflects partially just some of the most recent studies available in literature.
The lack of recent extensive studies about neonate capillary blood gas analysis requires the collection of further data. These studies should include the analysis of pre-analytical (e.g. capillary hemolysis overestimating Potassium, presence of air microbubbles overestimating pO2/ScO2), analytical and post-analytical variables.
In conclusion, pointing out that even in common reference texts there are reference values often elaborated with outdated technologies, further studies and meta-analyses are necessary for the elaboration of more reliable reference values.

EP018

Identification of appropriate cut-off value for the use of protein induced by vitamin K absence or antagonist-II for surveillance of patients with cirrhosis at risk of hepatocellular carcinoma development

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Background and aims: Patients with liver cirrhosis should undergo semestral surveillance by ultrasound (US) due to the risk of hepatocellular carcinoma (HCC) development. Although debated, the use of tumor biomarkers is common in clinical practice. We aimed to identify the appropriate cut-off value for the use of protein induced by vitamin K absence or antagonist-II (PIVKA-II) for surveillance of patients with liver cirrhosis at risk of HCC. Patients and methods: A cohort of 1187 patients with liver cirrhosis (median age 64, 57–74 years; 720 males/467 females) were enrolled in the study; 205 out of 1187 (17.3%) had a diagnosis of HCC. Patients without HCC at baseline (n = 982; 82.7%) had a median follow-up (FU) of 34.6 (11.4–43.7) months; during the FU, 118 (12.0%) developed HCC. Baseline serum PIVKA-II was measured by fully automated chemiluminescence immunoassay Lumipulse G 600 II system (Fujirebio, Tokyo, Japan). The area under the curve (AUC), sensitivity (Se), specificity (Sp), were assessed by receiver operating characteristic curve analysis. Hazard ratios (HRs) were calculated by Cox regression and survival curves analysed by Kaplan-Meier method.Results: At baseline, median serum PIVKA-II values were significantly different between patients with and without HCC (128, 58–435 mAU/mL vs. 45, 33–59 mAU/mL respectively; $p < 0.001$); the performance for the detection of HCC was 0.802 (AUC). The most appropriate cut-off value maximizing Se without penalizing Sp was 50 mAU/mL (Se = 80%, Sp = 64%). Among cirrhotic patients without HCC at baseline, PIVKA-II > 50 mAU/mL resulted significantly associated with the development HCC during the FU (HR = 1.73; $p = 0.003$); consistently, 52 out of 607 (8.6%) with baseline PIVKA-II $\leq$ 50 mAU/mL developed HCC in comparison to 66 out of 375 (17.6%) of patients with baseline PIVKA-II > 50 mAU/mL ($p = 0.003$).Conclusion: The measurement of serum PIVKA-II showed a good accuracy for the detection of HCC; a cut-off value > 50 mAU/mL had appropriate performance for the purpose of surveillance of patients with liver cirrhosis at risk of HCC development.

EP019

Urinary free light chains: assessment of the upper reference limit in healthy subjects

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Background: The International Myeloma Working Group includes the quantification of Bence Jones protein (BJP) among the parameters for monitoring disease and therapy in monoclonal gammopathy (MG). The quantitative assay is performed by densitometric technique on 24-hour urine, subject to several biases. Even 24-hour urine can be inaccurate due to difficulties with collection in fragile patients and temperature maintenance. We propose to measure urinary free light chains (uFLC) by immunochemical method on the first-morning urine to urinary creatinine (uCr) ratio, instead of BJP by densitometry on the 24h urine. Since the amount of uFLC in a healthy population is very low or absent, we aim to establish the reference upper limit of uFLC concentration.

Methods: Paired samples of serum and urine of 126 healthy subjects were collected among laboratory workers and their acquaintances. The eligibility criteria for the study were: serum FLC ratio = 0.26-1.65 negative urinary immunofixation; serum creatinine: Female < 1.2 - Male < 1.4 mg/dl; eGFR > 60 ml/min/1.73 m2; urinary total proteinuria < 150 mg/g-uCr. The 97.5th percentile reference upper limits of uFLCs concentration were calculated according to CLSI EP28-A3c. uFLCs were measured with immunoturbidimetry (Binding Site Optilte).

Results: Of 126 subjects investigated, 48 were males (age 18-69) and 78 were females (age 22-64). We found a statistically significant difference between the concentration of uFLCs in males and females, while no correlation was found with age. The results, in mg/uCr, are as follows for males and females respectively: uFLC-κ, 34.35 (27.48-39.99 CI 90%) and 23.18 (17.92-27.63 CI 90%); uFLC-λ, 3.59 (2.51-4.48 CI 90%) and 1.96 (1.60-2.33 CI 90%).

Discussion: Current technologies allow for automated and more reliable results than densitometry, thus enabling more accurate follow-up of the patient. Detection of uFLC can be performed only after confirmation of monoclonal free light chains by immunofixation and if the patient's renal function is preserved. Results from this study, although preliminary, encourage us to carry out a broader investigation by enlarging the sample size in terms of gender and age, validating the results obtained with a prospective, multicentre study.

EP020

SARS-CoV-2 and blood groups at ARNAS (Highly Specialized National Relief Hospital) - Palermo

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The disease caused by the SARS-CoV-2 virus has spread in China, in the city of Wuhan (Wuhan Jinyintan Hospital), since the last quarter of 2019. From China it spread to Italy in early 2020 until to assume the character of a pandemic.The biological factors influencing this remarkable diversity in the course of the disease and, therefore, in the severity of the prognosis are largely unknown but a work by Jiao Zhao et al. suggests that blood type AB0 can influence Covid-19 infection and also disease severity. In the study by Zhao et al., conducted on 2173 patients, it was observed that individuals of group A have an increased risk of approximately 20% compared to individuals of other blood groups.These data do not yet have a real scientific value but only statistical evidence, other studies will be needed to confirm these data. However, in a study conducted on patients admitted to the Covid departments of the ARNAS of Palermo, we saw data that were comparable with the Chinese results, in fact, 69% of serious patients, who needed transfusions of concentrated red blood cells are group A, while the other blood groups are less represented up to no case of patients with group AB.Furthermore, the percentage of blood group distribution in serious Covid positive patients is significantly different ($p < 0.01$) when compared with the blood group distribution in donors in Palermo and in the normal population in Palermo. We have seen that individuals of group A have an increased risk of approximately 27% compared to individuals of other blood groups. Further studies will be needed to confirm the relationship described and in any case the results will not change clinical management. To date we do not have solid scientific evidence to demonstrate that the blood group has a direct relationship with the Covid-19 infection, however, partial confirmation comes from a recent study published by the GWAS group, conducted on 1980 patients of 7 Italian and Spanish hospitals, which confirmed this relationship. In fact, the susceptibility of the genetic cluster 3p21.31 was observed with severe pulmonary forms of Covid-19 infection, thus confirming a potential involvement of the AB0 blood group system.

EP021

Blood cytometry parameters in Sars Cov-2 patients: potential markers of disease characterization

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IntroductionThe usefulness of leukocyte cell population data (CPD) derived from optical signals of new hematology analyzers is currenctly being investigated. In Covid-19 pandemic several reports showed the clinical importance of functional and quantitative blood parameters. Our study aimed to assess CPDs in positive Sars Cov-2 patients as potential disease markers.MethodsFrom February to April 2020 (1st wave), 540 patients (490 negative and 50 SARS CoV-2 positive) were enrolled in this retrospective study, as well as 2821 patients from September to December 2020 (2nd wave) (2762 negative and 59 SARS CoV-2 positive). SARS CoV-2 infection diagnosis was carried out by Multiplex rRT-PCR from nasopharyngeal swabs and clinical information collected in cardiology emergency department (ED). CPDs were detected by XN 2000 hematology analyzer (Sysmex Corporation) considering a single determination on whole blood. Comparisons between disease waves and SARS CoV-2 negative and positive patients were performed. Additionally, C-reactive protein (CRP) and lactate dehydrogenase (LDH) were assayed. Statistical analysis using the univariate and multivariate general linear regressions were made.ResultsLeukocyte CPDs were classified into: cell complextity (NE, LY, MO X-axis), DNA/RNA content (NE, LY, MO Y-axis) and abnormal sized cells (NE, LY, MO Z-axis). We detected cytometric parameters increased from the reference population for all cell types for both 1st and 2nd wave ($p \leq 0.03$). However, smaller quantitative alterations were found in the 2nd vs 1st wave: 5 CPDs vs 9 CPDs. In addition we found higher CPD values of the 1st compared to 2nd wave: (NE-SFL) (p=00004), (LY-Y) ($p \leq 0.0001$), (LY-Z) ($p \leq 0.0001$), (MO-X) ($p \leq 0.0001$), (MO-Y) ($p \leq 0.0001$). These findings were confirmed by the higher concentrations of CRP and LDH in the 1st vs 2nd wave: 17.3mgdL (8.5-59.3) vs 6.3 mg/dL (2.3-17.6) (p=0.0003) and 241.5 U/L (201-345) vs 195 U/L (174-228) (p=0.0005) (median, interquartile range) respectively.Conclusions Leukocyte CPDs showed increased cell activation in patients of 1st wave confirmed by biochemical data, correlated with worse clinical conditions of hospitalized patients. Our results highlighted the CPDs as disease characterization markers or useful for a predictive risk model.

EP022

DIAGNOSI DI CELIACHIA: APPROPRIATEZZA DELLA RICHIESTA ALLA LUCE DELLE NUOVE LINEE GUIDA DELL' ESPGHEAN (The European Society for Paediatric Gastroenterology Hepatology and Nutrition)

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PREMESSA: In Italia i celiaci diagnosticati sono circa 225.000, di cui circa 52 mila nella fascia di età 6 mesi- 17 anni; tuttavia si stima che in realtà siano il triplo, perché molti pazienti non sono diagnosticati. In presenza di sospetto diagnostico per malattia celiaca, i test di screening appropriati da eseguire, in base alle nuove linee guida (ESPGHEAN 2020), sono il dosaggio della concentrazione di IgA diretti contro l'enzima transglutaminasi 2 (TGA-IgA) e della concentrazione di anticorpi IgA totali. Solo per concentrazioni di TGA-IgA superiori alla norma al primo riscontro, si procede alla conferma mediante ricerca degli anticorpi antiendomisio (EMA). Nei bambini sintomatici e asintomatici con valori di TGA-IgA maggiore o uguale a 10 volte il limite superiore della norma, confermati da EMA, la diagnosi di celiachia può essere posta senza necessità di biopsia intestinale, esame più impegnativo in età pediatrica.
Lo scopo di questo lavoro è quello di valutare i soggetti adulti e bambini, residenti nella provincia di Lucca, a cui sono stati richiesti gli esami di screening per sospetta celiachia, valutando l'appropriatezza della richiesta e confrontando il numero e la tipologia di richieste dal 2017 fino al 2021, in particolare durante la pandemia da SARS-CoV-2.METODI: Lo studio è stato realizzato analizzando campioni di siero dei pazienti, mediante il dosaggio di TGA-IgA e IgG con strumentazione Phadia 250 Thermo Scientific, metodica FEIA (Fluorescence Enzyme ImmunoAssay) e allestimento di vetrini IFA (fegato scimmia IgA) con sistema IF Sprinter Euroimmun.RISULTATI: I dati studiati dimostrano che le richieste per la diagnosi di celiachia risultano inappropriate rispetto alle più recenti linee guida. Nel periodo analizzato infatti, si osserva come richiesta prevalente (circa 90% dei casi) l'associazione dei dosaggi di TGA-IgA, IgA totali e EMA. Inoltre, dall'analisi dei risultati si evidenzia che sono state prescritte numerose richieste inappropriate di TGA-IgG nei bambini, senza preliminare riscontro del deficit di IgA totali, soprattutto nel 2021 rispetto agli anni precedenti. Nel 2020, in piena pandemia da Sars-CoV-2, il numero delle richieste totali per diagnosi di celiachia si è ridotto, pur mantenendo l'inappropriatezza del pannello dei test di screening.CONCLUSIONI: La Celiachia è una malattia autoimmune di complessa classificazione e diagnosi, che coinvolge molteplici specialisti, in quanto necessita di un approccio multidisciplinare. La complessità della patologia può portare alla ridondanza dei test richiesti, determinando l'esecuzione di esami inappropriati. Nella diagnosi iniziale è importante confermare il test di screening (TGA-IgA/G) mediante EMA, mentre nel follow-up dei pazienti è sufficiente il dato quantitativo. Risulta, quindi, fondamentale la sinergia tra clinici e laboratoristi al fine di ottimizzare il percorso dei pazienti celiaci nell'ottica dell'appropriatezza ed efficacia diagnostica.

EP023
RICERCA DELLA MACROPROLATTINA: VALUTAZIONE DI ALCUNI PARAMETRI OPERATIVI

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Introduzione
La prolattina (PRL) umana è classificabile in tre frazioni con diverse masse molecolari: una di 23 kDa, proteina monometrica, libera o solo lievemente legata, un'altra di 40-60 kDa, in genere dimeri, detta grande PRL (big PRL) e una terza superiore a 100 kDa detta macroprolattina (macroPRL). La distribuzione di PRL nel siero di pazienti normali consiste nel 60-90% monomera, 15-30% dimera e 0-10% macro.
La macroPRL predomina in alcuni soggetti con elevati livelli sierici, ma assenza di sintomi. Si tratterebbe della combinazione di PRL monomerica con autoanticorpi circolanti anti PRL e tale forma non sarebbe biologicamente attiva.
Gli IVD sono stati ottimizzati per minimizzare la reattività con la macroprolattina, ma non esistono diagnostici che non reagiscano con qualsiasi forma di macroPRL.
Un metodo a disposizione del laboratorio per discriminare la presenza di macroPRL è la precipitazione con PEG.

Materiali e metodi
Sono stati selezionati 60 campioni con PRL superiore ai valori di riferimento.
Le aliquote di ogni campione sono state trattate in parallelo mediante precipitazione con una soluzione di PEG 6000 in tampone fosfato e di PEG in acqua.

Risultati
Confrontando i risultati ottenuti sui campioni trattati con PEG in H2O e PEG in PBS è stata ottenuta un'ottima correlazione (R2 = 0,9895), intercetta 0,20. Il coefficiente angolare (0,8797) è indicativo di valori superiori nei campioni trattati con PEG in acqua. Per questi campioni si è avuto un alto numero di recuperi superiori al 100%.
Sono state confrontate le percentuali di recupero ottenute su campioni trattati con PEG-H2O e PEG-PBS rispetto ai valori di PRL sul campione originale e sul campione diluito con stesso rapporto e solvente usato per il PEG. I rapporti ottenuti dai campioni diluiti con lo stesso solvente hanno mostrato una migliore concordanza diagnostica. Con la diluizione con PBS si ottiene un'ottima correlazione con i dati in letteratura del metodo di riferimento.

Conclusioni
I risultati permettono di concludere che per ottenere risultati clinicamente affidabili, la procedura di dosaggio della macro PRL (analisi e uso dei dati per il calcolo) deve essere attentamente definita attraverso l'uso di tampone PBS e dei dati ottenuti in uguali condizioni di matrice e diluizione.

EP024
EFFETTO DELLA PANDEMIA COVID SULL'APPROPRIATEZZA DEL MONITORAGGIO DEL PAZIENTE DIABETICO MEDIANTE L'EMOGLOBINA GLICATA

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Introduzione
Per il monitoraggio del paziente diabetico esistono Linee Guida ufficiali quali la American Diabetes Association (ADA) e la Società Italiana di Diabetologia (SID).
Tutte le linee guida sottolineano l'importanza di una cadenza corretta nelle visite periodiche di controllo e nella determinazione dell'emoglobina glicata rispetto ai limiti decisionali di 53 mmol/mol (pessimo controllo) e di 42 mmol/mol (adeguato controllo).

Materiali e metodi
Sono stati estratti i dati delle emoglobine glicate dal 2017 al 2021 eseguite dal nostro laboratorio e riguardanti i pazienti ambulatoriali diabetici o con emoglobina glicata patologica e che avevano eseguito almeno due determinazioni.
Ognuno degli 88860 risultati è stato elaborato in modo anonimo classificandolo secondo il grado di coerenza alle linee guida e ottenendo una suddivisione in richiesta corretta o anticipata (sovra-richiesta) o in ritardo (sotto-richiesta) rispetto ai limiti decisionali previsti.

Risultati
Dall'elaborato si è ricavato un andamento annuale dal 2018 al 2021 di 18810, 19548, 16440, 18672 determinazioni.Il numero di soggetti seguiti nel corso degli anni è stato di 10442, 12325, 12970, 11595, 12023.
Nel 2020 vi è una riduzione del 15,4 % rispetto al 2019 e i giorni di apertura dei servizi prelievo si sono ridotti a causa del lockdown di circa 40 giorni, pari al 16%.La corretta applicazione delle scadenze dei controlli è stata mantenuta rispettivamente per il 30,0%, 25,2%, 21,7%, 22,4%. Le determinazioni anticipate sono state: 8,2%, 7,3%, 7,6%, 8,4%. I ritardi: 61,8%, 67,4%, 70,7%, 69,2%.I primi contatti sono stati 4800, 3121, 2418, 1217.

Conclusioni
Dai risultati delle determinazioni delle glicate rispetto ai giorni di apertura degli ambulatori si rileva che il numero delle determinazioni ha risentito della pandemia COVID verosimilmente solo a causa delle chiusure totali dei servizi durante il lockdown.
Inoltre, nonostante la pandemia, i tempi delle scadenze dei follow up hanno mantenuto un buon livello.La Struttura Sanitaria ha quindi attuato una politica di gestione dei Servizi che ha affrontato in modo efficace la Pandemia e nonostante la pandemia è stata garantita la presa in carico dei nuovi pazienti.
Per l'elevato numero di controlli in ritardo (circa 67%) permane un ampio margine di miglioramento nella gestione dei pazienti, in particolare per garantire un'efficace valutazione del controllo della patologia.
Significativa la progressiva riduzione dei primi contatti, verosimilmente dovuta al consolidamento continuo dell'acquisizione dei nuovi pazienti dopo l'ampliamento

del bacino d'utenza introdotto dalla Riforma Sanitaria Regionale.

EP025

Kappa Index: A Possible New Marker For Multiple Sclerosis

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INTRODUCTION

The detection of oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) has been the gold standard in the diagnosis of multiple sclerosis (MS) for many years. However, OCBs are a relatively complex laboratory assay with a great intra- and inter-operator variability in analysis; therefore, recent studies focused on the search of new biomarkers to improve the diagnosis of MS. In particular, the detection of kappa free light chains (kFLC) in the CSF (Kappa Index) is an indicator of intrathecal immune activation. In this study, we evaluated the performance of Kappa Index in the diagnosis of MS.

PATIENTS AND METHODS

The CSF and serum kappa FLCs and albumin were analyzed on the Optilite turbidimetric analyser (Freelite MX Kappa Free and Low Level Albumin assays, The Binding Site Group, Birmingham, UK) in 45 patients with suspected MS referred to the University Hospital of Udine between January and May 2022. The CSF and serum samples were also evaluated for OCBs. The Kappa Index was then calculated as follows: [(CSF kFLC/serum kFLC)/ (CSF albumin/serum albumin)].

RESULTS

MS diagnosis was confirmed in 14/45 patients, whereas 31/45 patients were diagnosed with other neurological conditions. Both OCBs and Kappa Index tested positive in 13/14 MS patients. Among non-MS patients, 24/31 were negative for OCBs and 26/31 were negative for Kappa Index. No difference was found in the sensitivity between the two tests (0.9285), while Kappa Index showed a higher specifity compared to OCBs (0.8387 vs. 0.7742). The concordance between the two tests was 95%.

CONCLUSIONS

Similarly to OCBs, the Kappa Index was found to be highly sensitive for MS diagnosis, while showing a slightly higher specificity. However, compared to OBCs, the kFLC assay has two advantages: simplicity of analytical methods and objective read-out by instrumental concentration measurement. In conclusion, considering the complexity of the OCB assay, Kappa Index could be a valid marker in support of MS diagnosis, especially in cases where OCBs interpretation is difficult.

EP026

L'ESPERIENZA DEGLI INDICATORI IFCC PER L'IDENTIFICAZIONE DELLE AREE DI MIGLIORAMENTO

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Introduzione

L'applicazione delle norme ISO 9001 e 15189 prevede il miglioramento continuo come obiettivo permanente.
Gli obiettivi qualità devono essere raggiunti e mantenuti e monitorati con indicatori di qualità costruiti usando dati che ne assicurino l'efficienza e l'efficacia.
E' ancora frequente che le Organizzazioni utilizzino i loro dati storici per costruire i limiti, avendo difficoltà ad eseguire l'analisi comparativa (benchmarking) con entità esterne.Un progetto autorevole sugli indicatori è promosso dal Working Group Laboratory Errors and Patient Safety dell'IFCC e prevede la raccolta sistematica di risultati dei partecipanti e la loro elaborazione.

Materiali e metodi

Nel 2021 un piano di miglioramento ha portato all'iscrizione del nostro SMeL al programma IFCC e standardizzato la gestione delle non conformità (NC).
E' stata rivista la registrazione con uso del LIS; è stato approntato un sistema esterno home-made d'interrogazione del LIS per estrarre e categorizzare le NC; sono stati emessi schemi per elaborare e presentare i risultati, consultabili dal personale.

Risultati

La prima fase del piano ha portato alla gestione di 24 indicatori (tra revisionati e nuovi) di cui 18 con analogo in quelli dell'IFCC.Essi riguardano il monitoraggio dei processi di lavoro (accettazione richieste, produzione referti, CQI, VEQ), di elementi di supporto (funzionamento LIS, eventi avversi del personale), di NC specifiche (NC riguardanti i campioni), di outcome (pazienti coinvolti in NC).
La prima elaborazione delle NC ha riguardato i pazienti ricoverati, i loro campioni e richieste; per gli ambulatoriali l'elaborazione è stata effettuata nella seconda metà del 2021 per coordinarla con le procedure del Servizio del Centro Prelievi.
La prima elaborazione dei pazienti ambulatoriali ha generato 9 indicatori specifici.
I dati evidenziano risultati ottimi in 10 indicatori, buoni in 3 e possibili aree di miglioramento in 5, ad esempio il TAT di alcuni esami urgenti.

Conclusioni

Il programma IFCC ha dato robustezza ai nostri indicatori in quanto non autovalutati, ma confrontati verso entità esterne.
L'inserimento in IFCC dei dati 2021 dei campioni ha riguardato solo i ricoverati, il contesto più a rischio di NC; per gli ambulatoriali si è proceduto ad un'elaborazione interna.
Alcuni indicatori IFCC (CQI, LIS) hanno un basso numero di partecipanti: l'auspicio è che altri laboratori capiscano l'utilità del programma e superando le difficoltà, come ad esempio le carenze di alcuni LIS e l'impegno richiesto, incrementando i dati e migliorando la robustezza statistica.

EP027

Transcriptional level evaluation of Osteopontin/ miRNA-181a axis in hepatocellular carcinoma cell line-secreted extracellular vesicles

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Recent evidence suggested the role of secreted extracellular vesicles (EVs) in the intracellular signalling within the liver becoming a promising candidate as biomarker in hepatocellular carcinoma (HCC). Osteopontin (OPN) seems to play a relevant role both for early diagnosis of HCC than on the mechanisms that drive oncogenesis but, to date, information on the expression levels of OPN in EVs secreted by HCC tumor cell line are missing. The study aimed to verify, by transcriptional and proteomic study, the presence of OPN in EVs secreted by tumorigenic (HepG2) and non-tumorigenic hepatocyte cell line (WRL68), and to analyse the expression variations of OPN, its isoforms and miRNA-181a in both these EVs. “In silico analysis” was also performed via the Gene expression Profiling Interactive analysis (GEPIA) and Hepatocellular Carcinoma Database (HCCDB). An up-regulation of OPN in EVs secreted by HepG2 with respect to WRL68 was found in line with the results obtained by the “in silico analysis”. The study demonstrates, for the first time, the OPN isoforms and its modulator miRNA-181a expression in EVs secreted by both cell lines, highlighting high levels of OPN isoforms in EVs secreted by HepG2 and identifying OPN as a promising biomarker for HCC diagnosis.

EP028

DETERMINAZIONE DI CISTINURIA IN HPLC E DEFINIZIONE DEGLI INTERVALLI DI RIFERIMENTO

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Introduzione

La cistinuria è una malattia ereditaria che si trasmette con modalità autosomica recessiva dovuta ad un trasporto difettoso degli amminoacidi dibasici cistina, ornitina, lisina e arginina attraverso la membrana delle cellule tubulari renali. Il mancato riassorbimento della cistina ne determina la sua precipitazione nelle urine a valori fisiologici di acidità sotto forma di cristalli e calcoli causando l’ostruzione delle vie urinarie, infezioni e malattie renali croniche.

La diagnosi di cistinuria viene posta in seguito al riscontro di frequenti coliche renali a cui fanno seguito indagini chimico cliniche e accertamenti strumentali. La valutazione microscopica al sedimento urinario può evidenziare la presenza di cristalli esagonali di cistina mentre l’analisi del calcolo con tecnica spettroscopica agli infrarossi ne identifica la natura.

Per la misura quantitativa di cistinuria nel nostro laboratorio è stato messo a punto un metodo in HPLC utilizzando un test commerciale per la determinazione plasmatica di omocisteina (Eureka Lab Division srl). Lo scopo di questo studio è valutare la capacità del sistema in HPLC di poter eseguire uno screening e monitorare la terapia in pazienti cistinurici.

Materiali e metodi

Per l’identificazione dell’esatto picco di cistina sono stati utilizzati i campioni di urina di alcuni pazienti già diagnosticati cistinurici; inoltre il picco è stato confermato usando una soluzione di cistina pura preparata ad una concentrazione analoga a quella fisiologica urinaria.

Sullo strumento HPLC LC1260 Agilent Technologies sono stati processati campioni urinari random di pazienti non cistinurici e cistinurici.

La concentrazione di cistina è espressa come rapporto cistina/creatinina in µmol/mmol; il valore di creatinina è stato misurato su ADVIA 1800 (Siemens). Con programma Excel è stata eseguita una prima stima degli intervalli di riferimento.

Risultati

L’analisi dei grafici evidenzia una coincidenza del picco della cistina con quanto già presentato in letteratura.

I dati hanno evidenziato per i pazienti non cistinurici un valore medio del rapporto cistina/creatinina µmol/mmol di 19,25+/-10,69 ds (n 25), mentre i pazienti cistinurici presentano valori superiori a 147 µmol/mmol (dato di letteratura).

Conclusioni

Il metodo in HPLC messo a punto, risulta essere specifico, economico e semplice; se associato ad altre indagini di laboratorio (spettroscopia agli infrarossi e valutazione microscopica del sedimento) può essere utile per la diagnosi. La corrispondenza rispetto alla letteratura degli intervalli di riferimento ne conferma l’utilità e la possibilità dell’uso per la valutazione della terapia in atto.

EP029

Expression profile of Adrenomedullin and its specific receptors in liver tissues from patients with hepatocellular carcinoma and in tumorigenic cell line-secreted extracellular vesicles.

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Background and aims: the transcriptional profile of adrenomedullin (AM), a new metastasis-related factor involved in hepatocellular carcinoma (HCC), and its specific receptors (CLR, RAMP1, RAMP3) were evaluated in liver tissues of HCV-positive HCC subjects undergoing liver transplantation (LR) and in donors (LD). Materials and methods: AM and its specific receptor expression were also assessed in extracellular vesicles (EVs) secreted by tumorigenic (HepG2) and non-tumorigenic (WRL68) cells by Real-Time PCR. Results: AM expression resulted significantly elevated in LR concerning LD (p=0.0038) and, for the first time, significantly higher levels in HCC patients as a function of clinical severity (MELD score), were observed. RAMP3 and CLR expression increased in LR as a function of clinical severity while RAMP1 decreased. Positive correlations were found among AM, its receptors, and apoptotic markers. No AM mRNA expression difference was observed between HepG2 and WRL68 EVs. RAMP1 and RAMP3 resulted lower in HepG2 concerning WRL68 while significantly higher levels were observed for CLR. Conclusion: while results at tissue level characterize AM as a regulator of carcinogenesis-tumor progression, those obtained in EVs do not indicate AM as a target candidate, neither as a pathological biomarker nor as a marker involved in cancer therapy.

EP030

Tossicità da eccesso di Vitamina D: problema clinico raro ma importante

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Confusione, apatia, vomito, addominalgia, poliuria, polidipsia, disidratazione sono i sintomi osservati in condizioni di tossicità da Vitamina D (Vitamin D Toxicity-VDT), associati a livelli di 25(OH)D >150 ng/mL (>375 nmol/L), grave ipercalcemia/ipercalciuria e ridotta attività del Paratormone. Possibili cause possono essere: a) eccessiva, protratta assunzione di vitamina D (VitD) per somministrazione involontaria/impropria di dosi elevate; b) alterazioni vie metaboliche della VitD; c) presenza di patologie ipercalcemiche per eccesso di produzione locale di 1,25(OH)2D (disturbi granulomatosi, linfomi, ipercalcemia infantile idiopatica). Pur rara, gli effetti della VDT possono essere gravi se non prontamente identificati. Gli elevati livelli di prescrizione o assunzione di VitD per supplementazione o terapia (ad alto dosaggio) aumentano il rischio di VDT, soprattutto in assenza di corretto monitoraggio clinico-laboratoristico. In uno studio dello “status” di VitD, nel periodo Maggio–Dicembre 2021 abbiamo misurato con metodo LC-MS/MS ad alta automazione (Thermo Scientific™ Cascadion™SM Clinical Analyzer) la 25(OH)D Totale (25(OH)D2 + 25(OH)D3) in 56.044 pz. ambulatoriali afferenti alle strutture del CDI-Milano (F=43.019; M=13.025; rapporto F/M=3,3; età (media+DS)=59+18 anni; intervallo età=0-104), classificati in severa insufficienza (25(OH)D<10 ng/mL; 2.237 pz.=3,99%), insufficienza (25(OH)D 10-19 ng/mL; 7.877 pz.=14,06%), sufficienza-livello non ottimale (25(OH)D 20-30 ng/mL; 19.897 pz.=35,5%), sufficienza–livello ottimale (25(OH)D 31-100 ng/mL; 25.910 pz.=46,23%); potenziale eccesso (25(OH)D 101-150 ng/mL; 101 pz.=0,18%), tossicità (25(OH)D>150 ng/mL; 22 pz.=0,04%). I pz. (100 F=0.23%; 23 M=0.18%) con livelli indicativi di eccesso/ tossicità si caratterizzano per età (media+DS:65+16 anni; intervallo età:23-90), rapporto F/M (4,34) superiori rispetto ai pz. totali, con patologie multiple (oncologiche, neurologiche, endocrine, ossee) associate. I livelli massimi (>320 ng/mL) sono presenti in pz. con Sclerosi Multipla in terapia (non evidence-based) ad altissime dosi di VitD. Le nostre osservazioni confermano che la VDT, rara ma ad elevata severità, deve essere ricercata, in particolare in pz. in età avanzata, in trattamento protratto con alte dosi di VitD.

EP031

Maintainance of diagnostic Laboratory during pandemic emergency (SARS-CoV-2) in a Small Hospital.

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The SARS-CoV-2 disease has then inundated the hospital system with unpredictable impact on healthcare organization. In this unprecedented moment of crisis, laboratory services, as an integral part of multi-specialty hospitals, had to face the outbreak, in particular, guaranteeing the diagnostic activity despite the fact that the laboratory is equipped to urgently provide the Covid19 tests. Therefore, the Covid19 pandemic has forced the Laboratory Medicine Services to address sudden organizational changes, putting them at risk maintaining adequate analytical quality. We describe a real-life experience at the our laboratory, which underwent a progressive adaptation in response to the rapidly evolving Covid19 emergency in the Emilia-Romagna Region.
This experience started on March, when our regional government authorized laboratories to perform PCR assays and Rapid antigen tests to detect SARS-CoV2 RNA in nasopharyngeal swab samples. In 10 days in our laboratory a room was set up for the execution of COVID tests. On 1th June, the laboratory examined approximately 42.122 samples using Real-time PCR, (4.42% were positive for the presence of viral RNA, of these 56.2% were male) and 35.348 Rapid antigen-tests (1.93% were positive for the presence of viral RNA, of these 51% were male) and 50.206 IgG/IgM assays for qualitative and quatitative assessment of SARS-CoV2 antibody. Under the strict and multi-domain protective measures implemented at our Laboratory very early, not one staff member has been diagnosed with COVID19 infection. Our results have been obtained with a proactive approach to the presenting difficulties, by sharing all decisions with the staff and by adopting transformation measures. Detailed knowledge of the workflow allowed rapid changes and resilience perspective allowed the re-addressing of our organization focused on staff's need of safety and tranquility. We have successfully developed internal guidelines and preventive strategies, adapting the existing SOPs and transforming the existing facilities as quickly as possible, to face the new situation, even before institutions reacted. In conclusion, our findings support the concept that the our laboratory can be extraordinarily responsive to emergencies like the one we are experiencing. As Prof. Plebani said "do not miss the opportunity, thanks to the visibility gained from the pandemic, to provide further evidence of the central role played by laboratory medicine in modern, personalized medicine".

EP032

Heat Shock Proteins 90 evaluation in hepatocellular carcinoma cell line-secreted extracellular vesicles.

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Background: Primary hepatocellular carcinoma (HCC) does not usually show any symptoms at the early stage and the use of biomarkers is necessary to aid in diagnosis. Recently extracellular vesicles (EVs), submicron membrane-bound structures secreted from different cell types containing a wide variety of bioactive molecules, have increased the attention in many cancers, including HCC, becoming an auspicious candidate as biomarkers and therapy in the scenario of limited diagnostic and treatment option. Many indications have shown that heat shock proteins (Hsps) are important modulators in treatment resistance and invasion of HCC becoming attractive therapeutic targets. In particular, Hsp90α/β isoforms have been found to play critical roles in regulating the proliferation, apoptosis, and metastasis of tumor cells, suggesting for these proteins a role as targets for modern anticancer therapies. Methods. The aim of the study was to verify the presence of Hsp90α/β in EVs secreted by an HCC tumor cell line (HepG2) and by a non-tumorigenic hepatocyte cell line (WRL68), both at protein and mRNA levels, and to analyze their expression variations. Results. The result showed that Hsp90s are transported by the EVs as protein but not at the mRNA level. Conclusions. To build new therapeutic targets using EVs or other organelles as performed on exosomes in recent studies, it is essential to evaluate the action at the pre or post-transcriptional level given their different behavior in transporting proteins or mRNA.

EP033
Case report: a sospected hypervitaminosis A

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Vitamin A toxicity is very uncommon, but when it occurs, it can be serious and even fatal. Manifestations can be seen on the skin, in the gastrointestinal tract, the liver, the skeleton, and central nervous system. Often when present with acute toxicity, it is not readily recognized and can be confused with viral hepatitis or other intoxications. There is no specific treatment for vitamin A intoxication, but prompt cessation of the drug often results in complete resolution. Here, we report a case vitamine A intoxication with high levels in liver tests, thrombocytopenia and apperance virosis. The patient showed several clinical signs abdominal pain, including mild anemia and thrombocytopenia. The patient is a 32-year-old female with no significant past medical history presents to the emergency department twice with abdominal pain. In January 2022, the patient has performed diagnostic tests were carried out and the lab values were normal. The first time, in the emergency department, the patient's initial vital signs were a temperature of 36.8°C, heart rate of 131 beats per minute (BPM), blood pressure of 114/75 mmHg, respiratory rate of 37 breaths per minute, an oxygen saturation of 99% on room air, and negative COVID-19 test.
The complete blood count revealed the following values: hemoglobin concentration, 10.8g/dl; hematocrit reading, 35.6 percent; leukocyte count, 7.33x10^3/ul; and platelet count, 85x10^3/ul . Laboratory tests showed normal serum bilirubin, AST 25U/L, ALT 15U/L and alkaline phosphatase of 69U/L. The patient was discharged with the recommendation that if the pain persists, return to the emergency room. After 2 days the patient returned to the emergency room and the laboratory tests were: hemoglobin concentration, 10.5 g/dl; hematocrit reading, 35.1 percent; leukocyte count, 13,08x10^3/ul with lymphocyte 68 percent and platelet count, 77 x10^3/ ul. Laboratory tests showed high levels in liver tests: bilirubin 0,24mg/dL, AST 10 U/L, ALT 82U/L, GGT 107U/L and alkaline phosphatase of 272U/L. She was admitted to surgery for further investigation and following a thorough medical history, vitamin A toxicity was diagnosed. After four days of the suspension of vitamin A, the laboratory tests were resulted normal. The interruption of vitamin A treatment was immediately followed by clinical and biochemical, therefore thrombocytopenia and elevated transaminases are thought to be due to hypervitaminosis A, in agreement with colleagues Arzu Ataseven et all.

EP034
Farmacia point-of-care: l'approccio del laboratorio ospedaliero nella validazione dei pannelli diagnostici

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Complice la pandemia degli ultimi anni, la Medicina di Laboratorio si sta avvicinando sempre di più al paziente ed ai suoi bisogni, uscendo dall'ambiente ospedaliero per diventare innovazione sul territorio.I sistemi Point-Of-Care Testing (POCT) permettono di eseguire analisi in prossimita' del paziente fornendo risultati in tempi brevi senza ricorrere al laboratorio ospedaliero. L'obiettivo di questo studio è stato quello di sviluppare un protocollo di validazione POCT per l'utilizzo di nuovi test di prossimita' nelle farmacie del territorio. Il protocollo e' stato applicato allo strumento POCT "Allegro" (NOVA Biomedical, Waltham, USA) ed ha coinvolto un confronto tra con gli strumenti in uso presso il laboratorio di Biochimica Clinica del Policlinico Agostino Gemelli (Roma). Sono stati confrontati 100 campioni per il dosaggio di Glucosio, Proteina C reattiva, profilo lipidico (Colesterolo, trigliceridi e HDL) ed Emoglobina glicata.I confronti dei risultati ottenuti sono stati valutati mediante analisi della regressione di Passing e Bablok e mediante diagrammi di Bland e Altman. I risultati di questo studio hanno mostrato una buona correlazione per tutti i parametri esaminati, che permettono la delocalizzazione intraospedaliera in aggiunta al territorio. Anche i reparti ospedalieri possono infatti beneficiare delle tecnologie POCT come Allegro nei casi in cui divengaimportante ottenere dei risultati in tempi brevi per consentire una dagnosi e una decisione medica veloce.A livello territoriale il progetto prevede l'utilizzo dello strumento nelle farmacie permettendo uno screening di base e un monitoraggio di pazienti cronici o a rischio dove l'accesso alla struttura ospedaliera sia limitato o problematico.Gli strumenti POCT decentrati nelle realtà intra ed extraospedaliere possono essere sotto diretto controllo del laboratorio centrale attraverso il collegamento al sistema informativo ospedaliero per la validazione e la firma digitale.Il sistema Allegro fornisce una soluzione per la gestione e i controlli dei test di laboratorio decentrati e una base solida per la Medicina di prossimità che , attraverso la creazione di soluzioni POCT volte ad aiutare le categorie più fragili.
Bibliografia: M. Zaninotto, G. Miolo, A. Guiotto et al. Quality performance of laboratory testing in pharmacies: a collaborative evaluation.
Clin Chem Lab Med 2016

EP035

A fully automated ELISA monotest system for the detection of drug and antidrug antibodies in patients with rheumatic diseases: a comparison with ELISA test routinely used

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Background Infliximab (IFX) and adalimumab (ADL) are biological drugs widely used in rheumatic diseases. Some patients generate anti-drug antibodies associated with reduced drug levels resulting in loss of efficacy, clinical failure and increased risk of adverse effects. We evaluated an automated quantitative method applied to the DIESSE CHORUS TRIO instrument for determination of IFX and ADL drugs and anti-drug antibody levels comparing it with enzyme-linked immunosorbent assay (ELISA) Lisa-Tracker Duo Adalimumab and Lisa-Tracker Duo Infliximab, routinely used in our laboratory.
Methods The study was performed on 83 patients with rheumatic diseases who did not respond to biological therapy, attending the Rheumatology Unit, San Giovanni di Dio Hospital, Florence. All sera were analyzed using the fully automated assays CHORUS Promonitor IFX, ADL, anti-IFX and anti-ADL (DIESSE Diagnostica Senese) and manual Lisa-Tracker Duo Adalimumab and Infliximab (Theradiag). Spearman correlation coefficient was performed to compare the two methods for drugs determination, while Cohen kappa was used to compare the positive / negative anti-drug antibody results.
Results The two ELISA methods showed an excellent agreement with Spearman coefficient for the dosage of IFX and ADL of 0,91 and 0,88 respectively. Agreement between the methods for the detection of anti-drug antibody levels showed Cohen kappa close to 1.0 for anti-ADL and 0,7 for anti-IFX antibodies. The two discordant samples out of 58 for ADL and three out of 25 for IFX showed borderline results. The analysis of anti-ADL revealed one discordant result, positive for Theradiag and negative for DIESSE; the sample is drug positive for both systems. The analysis of anti-IFX antibodies revealed two discordant results, one positive for Theradiag and negative for DIESSE and one positive for DIESSE and negative for Theradiag; samples are drug positive for both systems. Clinical data will be evaluated for the correct interpretation of laboratory results.
Conclusion The CHORUS Promonitor IFX and ADL and anti-IFX and anti-ADL assays showed similar performance to the Theradiag ELISA assays and they are a suitable tool in therapeutic drug monitoring thanks to the fully automated monotest system.

EP036

Nicotinamide N-methyltransferase, a promising marker for human osteosarcoma

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Osteosarcoma (OS) represents a primary bone neoplasm, mostly affecting young people. Due to its high metastatic potential, it is often associated with rapid progression and poor prognosis. In addition, OS cell is characterized by a marked chemoresistance that is responsible for disease relapse in most patients. Therefore, the elucidation of molecular mechanisms underlying resistance to chemotherapy and the identification of new molecular targets for effective treatment of OS patients is fundamental.The enzyme nicotinamide N-methyltransferase (NNMT) catalyzes the N-methylation of nicotinamide and other structural analogs, playing a crucial role in the biotransformation and detoxification of drugs and xenobiotics. Although enzyme overexpression was reported in wide variety of solid tumors, the effect induced by such dysregulation in cancer cell phenotype was partly clarified. Several studies demonstrated that NNMT is able to promote cell proliferation and resistance to chemotherapy. The aim of this study was to explore the potential involvement of NNMT in osteosarcoma.Immunohistochemical analyses have been performed to evaluate NNMT expression in tumor and adjacent healthy tissue of selected formalin-fixed and paraffin-embedded samples from OS patients. To investigate the role played by the enzyme in OS cell metabolism, human OS cell lines have been transfected with plasmid vectors coding short harpin RNAs targeting NNMT mRNA. Efficiency of enzyme knockdown has been assessed by Real-Time PCR, Western blot and catalytic activity assay. The impact of NNMT silencing on cell viability and response to chemotherapeutic treatment was also explored.Results showed that OS samples display a significantly higher NNMT expression compared with that detected in controls. Preliminary results suggest that NNMT knockdown in OS cell lines is associated to a decrease of cell proliferation and migration in vitro, as well as enhanced sensitivity to chemotherapeutic drugs.Data obtained show that the enzyme may represent an interesting tool for OS, thus highlighting its promising role both as diagnostic marker and therapeutic target.

EP037
STIMA eGFR CON FORMULA CKD-EPI: CONFRONTO TRA LE EQUAZIONI CON E SENZA ETNIA

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Introduzione
La creatinina sierica richiesta per valutare la capacità filtrante renale non è un indicatore precoce della perdita di funzionalità in quanto i reni hanno una riserva funzionale ampia, è un parametro con basso indice di individualità e inoltre la massa muscolare, l'apporto dietetico il genere e l'etnia rappresentano variabili che modificano il valore diagnostico dell'esame. Tuttavia, nonostante queste limitazioni le linee guida internazionali della KDIGO raccomandano l'inserimento nel referto di laboratorio della stima del valore della velocità di filtrazione glomerulare (eGFR). Nel nostro laboratorio per valutare l'eGFR attualmente utilizziamo la formula CKD-EPI che tiene conto del valore della creatinina sierica, del sesso, dell'età e dell'etnia. In considerazione del fatto che al nostro centro afferiscono pazienti di etnie diverse e che è ora disponibile una equazione CKD-EPI 21 modificata, eticamente più corretta, che non tiene conto del terribile termine "razza", abbiamo confrontato i risultati eGFR ottenuti con questa nuova equazione rispetto all'equazione attualmente in uso tenendo conto di diversi gruppi etnici.

Materiali e metodi
Sono stati valutati in totale 4704 pazienti esterni afferenti al nostro centro prelievi e appartenenti a quattro etnie diverse (3251 italiani, 1131 sud americani, 215 africani, 125 nord americani). I valori di creatinina sierica sono stati misurati su ADVIA 1800 (Siemens) con metodo enzimatico. La correlazione è stata eseguita con il programma Excel.

Risultati
I risultati evidenziano le seguenti correlazioni tra le due equazioni: per i pazienti italiani Y=0,98+3,9 R^2=0,99, sud americani Y=0,93+7,7 R^2=0,99, nord americani Y=0,98+3,4 R^2=0,99 e africani Y=0,84+4,2; R^2=0,99.

Conclusioni
Dal confronto tra le due equazioni si evidenzia un ottima correlazione per i pazienti italiani, sud americani, nord americani e buona per i pazienti africani confermando la bontà della nuova equazione che oltre ad essere eticamente corretta è sicuramente più pratica dal punto di vista amministrativo. Al fine di evitare errate classificazioni risulta fondamentale il dosaggio della creatinina con metodi enzimatici che presentano una migliore correlazione con il metodo di riferimento e non mostrano interferenze aspecifiche.

EP038
COULD A COMBINATION OF BLOOD MARKERS BE USED IN PLACE OF SUPAR FOR ANAKINRA PRESCRIPTION IN COVID-19 PATIENTS?

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Background and aim. The SAVE-MORE study showed that the early start of treatment with the IL-1α/β inhibitor anakinra, guided by suPAR (Soluble Urokinase-Type Plasminogen Activator Receptor) ≥6ng/mL, in patients with moderate or severe COVID-19, significantly reduced the risk of worse clinical outcome at day 28. With press release 665 of 28/09/2021, AIFA has approved the inclusion of anakinra in the 648/96 list for the treatment of hospitalized adults with COVID-19 and suPAR ≥6ng/mL. However, suPAR methods are not widely available, which hinders the prescription and clinical use of anakinra. Aim of this study was to identify a panel of biochemical tests as a surrogate marker of suPAR positivity (≥6ng/mL).
Methods. The study included 456 (median (IQR) age: 75 ys (60-83); M:F 54:46%) hospitalized patients in the Infectious Disease Unit (n=124) and Medical ICU (n=332) of the Maggiore Policlinico Hospital of Milan with molecular diagnosis of COVID-19. suPAR was measured at admission by suPARnostic TurbiLatex kit (Vendor: ViroGates A/S, Denmark; Italian distributor: B.S.N. Srl) on Roche Cobas c702.
Results. Median suPAR was 7.6ng/mL (4.8-10.8), with 63% of patients displaying suPAR ≥6ng/mL. At the univariate logistic regression analysis, suPAR was found to be associated with age ($p<0.001$), WBC ($p=0.002$), #NE ($p<0.001$), Hb ($p<0.001$), CREA ($p<0.001$), LDH ($p=0.005$), FERR ($p=0.006$), CRP ($p<0.001$), Fib ($p=0.005$), DD ($p<0.001$), but not with sex ($p=0.943$), #LY ($p=0.444$), #MO ($p=0.233$), PLT ($p=0.064$), ALT ($p=0.238$), TBIL ($p=0.534$), TnT ($p=0.153$) and TSH ($p=0.970$). However, at the multivariate analysis, only age ($p<0.001$), Hb ($p=0.043$), CREA ($p<0.001$), LDH ($p=0.021$), CRP ($p=0.005$) and DD ($p=0.004$) were found as independent predictors of suPAR positivity. Percentage of correct classification (< vs ≥ 6ng/mL) and AUC of the multivariate model were 75.1% and 0.83 (95%CI 0.80-0.87).
Conclusions. suPAR is independently associated with age, Hb, CREA, LDH, CRP and DD. Due to the moderate % of correct classification of the multivariate model (75%), we conclude that this combination of blood markers cannot be used as a surrogate of suPAR for anakinra prescription. Further clinical validation is needed to assess a possible role of the model in predicting COVID-19 severity and mortality.

EP039

Evaluation of Anti-SARS-CoV-2 IgG before and after vaccination in subjects with and without previous COVID-19 disease.

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BACKGROUND-AIM
Although more than two years have passed since the beginning of the SARS-CoV-2 pandemic, the interest of Public Health in the development and administration of effective anti-COVID-19 vaccines continues.We aimed to test the antibody response to SARS-CoV-2 vaccination in patients with and without previous infection.
METHODS From June 2021 to November 2021, we recruited 203 patients who were going to receive vaccination against SARS-CoV-2: 123 women (60.6%) with a median age of 44 years (IQR: 33-56) and 80 men (39.4%) with a median age of 43 years (IQR 32-53); 78 patients reported previous SARS-CoV-2 infection (41 women, 37 men). 74 out of 203 were healthy subjects, 84 reported mild to medium allergic history and 45 other diseases. 97.4% of subjects received BioNTech/Pfizer vaccination and, according to Ministerial Dispositions, patients with previous SARS-CoV-2 infection received a single dose (group 1), other patients received two (group 2). After 3 months, 98 subjects received a third dose (57 BioNTech/Pfizer and 41 half a dose of Spikevax-Moderna).The antibody response to vaccination was measured on blood samples collected before vaccination (T0), 10 days after the first dose of vaccination (T10), 15 (T15), 90 (T90) and 180 (T180) days after the second or only vaccination. Samples were tested using Access SARS-CoV-2 IgG (1st IS) on Access UniCelDxI 800 (Beckman Coulter s.r.l.).
RESULTS The comparison between median concentrations in our groups showed a statistically significant difference ($p<0.001$) at T0, T15 and T90, but not at T180 ($p=0.713$). At T0 and T90 the SARS-CoV-2 IgG concentration was higher in group 1, while at T15 it was higher in group 2. At T90 the antibody titer dropped in all patients, but the decrease was higher in group 1. 77 SARS-CoV-2 infections occurred after vaccination (4.2% between T15 and T90, 95.7% between T90 and T180).
CONCLUSIONS We confirm that the antibody titer is significantly associated with a having had previous SARS-CoV-2 infection, but not with age and sex. The probability of contracting the infection after vaccination increases after three months from primary vaccination, confirming the efficacy of vaccination as a preventive measure against SARS-CoV-2 infections and the need of booster administrations.

EP040

POINT OF CARE MANAGEMENT : UN PROBLEMA DI GOVERNO CLINICO - PROGETTO DEL LABORATORIO

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POINT OF CARE MANAGEMENT: UN PROBLEMA DI GOVERNO CLINICO – PROGETTO DEL LABORATORIO

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La rete POCT della Azienda ASST Settelaghi, coordinata e controllata dal laboratorio, è presente nell'Ospedale di Varese e nei 5 Presidi periferici e si sta implementando nelle Case di Comunità. Il numero di analisi gestito con i POCT è sempre più elevato e rappresenta una parte importante dell' attività. L'installazione dei POCT crea nuove necessità nei laboratori che devono controllare strumenti delocalizzati e utilizzati da personale non specializzato. La nostra rete di POCT attuale è costituita da 42 emogasanalizzatori, 120 glucometri, 2 tromboelastografi, 6 POCT per ACT (tempo di coagulazione attivato)e 3 POCT per la presepsina (in fase di installazione).E' necessario creare una Struttura di controllo aziendale costituita da:1) Comitato direttivo multidisciplinare (CDM) formato da dirigenti e tecnici di laboratorio, un rappresentante del comparto infermieristico, uno della ingegneria clinica e uno della direzione medica per valutare esigenze, priorità e strategie.2) Gruppo operativo (GO) costituito da 2 dirigenti di laboratorio con funzioni di coordinamento e 2 tecnici specializzati con funzioni operative.Il Punto chiave è il controllo delle attività attraverso i programmi gestionali e la formazione del personale. Emogasanalizzatori (RAPIDPoint 500 Siemens): sono gestiti da remoto con l'utilizzo dello specifico Software Rapidcom. Glucometri (Stat Strip Nova Biomedical): sono controllati con il software Novanet. Tromboelastografi (Haemonetics): controllati da remoto da un software gestionale (TEG Manager). POCT per ACT(Hemochron Werfen): non ancora collegati in rete . Il personale utilizzatore viene abilitato dopo il superamento di un corso Fad organizzato dal laboratorio in collaborazione con l'ufficio infermieristico. Sono state inserite fino ad ora più di 2000 password personali. E' stata creata una mail aziendale dedicata ai POCT per la segnalazione di tutte le problematiche alla quale risponde il personale dedicato. In conclusione il Laboratorio, per garantire la sicurezza degli esami eseguiti su i POCT, deve costruire una struttura organizzativa capace di coinvolgere ed interagire con personale e competenze anche al di fuori della propria unità.

EP041

Modello organizzativo per la gestione dei POCT aziendali nell'AORN S.Anna e S. Sebastiano di Caserta

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Con il termine "Point of Care Testing" (POCT) si definiscono in generale tutte le analisi eseguite al di fuori del Laboratorio analisi, ovvero "decentrate". Questo decentramento può essere mantenuto all'interno dell'ambito ospedaliero oppure esteso al di fuori di questo ambito e può non richiedere spazi strutturati permanenti.Mediante il Decreto Dirigenziale n.145 del 21.04.2021 (DDRC 145/21), la Regione Campania, al fine di assicurare una migliore di qualità di cura ed assistenza, ha finalmente normato i POCT inquadrandoli come una problematica di Governo Clinico e prevedendo valutazioni in termini di efficacia clinica, gestione del rischio e formazione continua degli operatori coinvolti.Recependo tale Decreto, l'AORN Caserta è stata tra le prime AA.OO. sanitarie regionali a darsi un modello organizzativo per la gestione dei POCT adattando le indicazioni del DDRC 145/21 alla propria complessa realtà che include dispositivi decentrati in 35 UU.OO.raggruppate in 6 dipartimenti clinico assistenziali. A tale scopo si è provveduto ad insediare un Comitato Multidisciplinare Permanente POCT (CMPP), presieduto dal Direttore di Medicina di Laboratorio e di cui fanno parte il Direttore Sanitario Aziendale, il Coordinatore infermieristico, il Direttore della Farmacia e il Direttore della Ingegneria clinica, nonché un Comitato Esecutivo POCT (CEP), costituito dal POCT manager, con funzioni di coordinamento, dal POCT Consultant, con funzioni di verifica dei controlli di qualità, e dai rappresenti clinici ed infermieristici dei diversi dipartimenti aziendali. A questi ultimi fanno riferimento i referenti POCT di reparto (RIRP) e tutto il personale clinico ed infermieristico coinvolto nella gestione delle analisi decentrate.Dal lavoro sinora svolto dai due Comitati, è stata elaborata la mappa dei POCT Aziendali già attivi (15) e di quelli da attivare (88) e il cronoprogramma degli opportuni provvedimenti per l'adeguamento di quanto previsto dal DDRC 145/21 in termini di formazione e connettività.

EP042

Predictive Medicine in breast cancer patients: the identification of variants in genes different from BRCA1 and BRCA2

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Breast cancer (BC) is the most common cancer in the female population. The majority of breast cancers are sporadic, which means that they appear in the most common cases after 45 years of age in people without other cases of cancer in their families. In 20-30% of cases, BC presents familial and clinical characteristics that are highly suggestive for predisposition, which in about half of these cases is confirmed by the presence of a pathogenic variation in the BRCA genes. Patients that are carriers of these variants can benefit from highly specific prevention and medical-surgical treatments. However, BRCA genes explain only about 25% of suspected cases of genetic predisposition. The remaining about 75% of patients, in the absence of a confirmed genetic cause, cannot enjoy the benefits resulting from the presence of known genes variant. At the same time, very often, these patients are not treated as sporadic tumors in the presence of a genetic predisposition phenotype. In particular, the absence of pathogenic variants in the BRCA genes has suggested the possible association of other disease-genes in cases of breast cancer and the possibility of extending the genetic analysis to a multi-gene panel, beyond the BRCA genes (1-3). One-hundred patients affected by early breast cancer and/or with other different cases of neoplasia in their families, were enrolled in the study herein. The samples were subjected to molecular analysis using a multigene panel of 58 genes, including the BRCA1 and BRCA2 genes. The panel is completely customized in our laboratory and contains 50bp in the exonic boundaries of each gene and 3' and 5' UTRs gene regions. We identified 26 patients that carry pathogenic mutations, and 30 different variants. Furthermore, 4 patients showed double variants and 1 presented three different pathogenic/possibly pathogenic variants. Five different patients carry a variant in the BRCA genes (5 out of 26 = 19%), after BRCA genes the most affected genes are CHEK2, APC and FANCC. The data obtained confirm the importance of extending genetic analysis to a larger number of putative predisposing genes as well as extending the use of multi-gene panels to a larger population of patients than those defined by current guidelines; also evaluating the possibility of offering onco-genetic counseling to all patients who are diagnosed with breast cancer.

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EP043

DIAGNOSI EMOMETRICA DI LABORATORIO IN UN CASO DI LAL T.

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INTRUZIONE Paziente uomo (38 aa) giunge in Pronto Soccorso per febbre ricorrente , astenia con terapia cortisonica in atto. All'esame emocromocitometrico si riscontrano i seguenti dati: WBC 25.470 µ /L N : 25 L : 25 M : 50 PLT 113.000 µ/L HGB 6.1 gr / dl MATERIALI E METODI Sullo scattergram WDF è evidente un cluster di distribuzione cellulare anomalo che occupa l'area dei linfociti fluorescenti. Si esegue uno striscio di sangue periferico . L'esame morfologico mette in evidenza un quadro piuttosto complesso che pone il sospetto diagnostivo di LAL : elementi di grandi dimensioni con elevato rapporto nucelo/citoplasma, sottile rima citoplasmatica basofila priva di granulazioni, nucleo a cromatina dispersa con singolo nucleolo. Si procede quindi con la valutazione citofluorimetrica che descrive aspirato midollare ipercellulare (791.000 per microlitro), presenza di una popolazione blastica linfoide pari all' 82 % della cellularita' globale caratterizzata da espressione alta di CD7, CD5 e CD1a, insieme con livelli medi di espressione di CD8 e livelli bassi di espressione di CD10, CD99, con espressione parziale di CD4, CD34, CD2, sCD3, cCD3. Quota linfoide residua normale pari al 2%. Quadro immunofenotipico compatibile con Leucemia Linfoblastica T.RISULTATI Valutando i risultati del paziente in esame, si può osservare come le più avanzate ed innovative tecnologie di analisi ad oggi disponibili in campo ematologico, garantiscano un continuo progredire della ricerca e dello sviluppo garantendo così la scelta dei metodi più idonei in grado di assicurare analisi accurate ed informazioni utili nella diagnostica ematologica. Tre ore dopo il ricovero in Pronto Soccorso , la diagnosi di laboratorio concorreva ad offrire al paziente la tempestiva diagnosi di LAL T da parte dell' U.O.C. di Ematologia.

EP044

The fundamental role of synergy between laboratory professionals and clinicians to minimize pseudohypokalemia cases in subjects affected by hyperleukocytosis

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Hypokalemia is one of the most common electrolytes disorder characterized by a low serum potassium concentration (< 3.5 mmol/L). A potassium level lower than 2.5 mmol/L is defined as severe hypokalemia and represents a potentially life-threatening condition. Here, we reported a case of pseudohypokalemia in a subject admitted to the emergency department of ASST Monza with a diagnosis of a de novo acute myeloid leukemia (WBC = 199.77 x 109/ L). Potassium abnormalities are common in subjects affected by hematological malignancies, and the recognition of pseudohyperkalemia/pseudohypokalemia is essential to prevent erroneous patient's treatments. Hyperleukocytosis could lead to an in vitro spurious reduction of potassium due to the increased potassium uptake by the leukemic blast cells when there is a delay between sample collection and analysis. During the first three days after admission, 14 potassium measurements were requested (8 on serum samples performed with indirect potentiometry, IP, and 6 on whole blood performed with direct potentiometry, DP). In six cases a potassium value below 2.8 mmol/L was detected, and in 5 of them the analysis was carried out with IP. In the 4 cases in which potassium measurements were performed in the same time using both IP and DP, three IP results were below 2.5 mmol/L, whereas DP results were greater than 3.0 mmol/L. We concluded that these pseudohypokalemia cases were due to the delay between serum sample collection and analysis. In our case the delay in the definitive recognition of pseudohypokalemia was primarily due to the lack of communication among medical and laboratory staff when repeated discrepancy between potassium results on serum (IP) and whole blood (DP) were noted. In the presence of a high number of leukocytes, the blood sample must be quickly sent to the laboratory and promptly analyzed, in order to exclude pseudohypokalemia. We suggest that the use of DP using a blood gas analyzer might be an aid to exclude false values obtained with IP, since Point of Care Testing (POCT) is immediately performed. Moreover, the use of specific flags alarm set to the Laboratory Information System (LIS) could help laboratory personnel to recognize samples with altered potassium values caused by hyperleukocytosis.

EP045

DIAGNOSI EMOMETRICA DI LINFOMA

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Introduzione: Lo scopo di questo lavoro è porre l'attenzione sull'importante ruolo che ad oggi riveste la Medicina di Laboratorio quale componente fondamentale dei processi assistenziali.Materiali e Metodi: Maggio 2022, paziente uomo (aa 82). Viene eseguito un emocromo di routine su richiesta del reparto di appartenenza (OBI) dopo essere stato valutato in Pronto Soccorso per febbricola e dispnea. L'osservazione dei dati, corredata dalla valutazione degli scattergram di disposizione cellulare, pone il sospetto diagnostico di disordine linfoproliferativo.

Risultati. La valutazione morfologica e citofluorimetrica confermano il sospetto diagnostico. Presenza di cellule linfomatose , cromatina compatta con nucleoli di grandi dimensioni , citoplasma delimitato ma dai contorni indistinti e lievemente basofilo. Quadro immunofenotipico compatibile con disordine linfoproliferativo B tipo LNH CD19 + CD20+ (a) FMC7+ CD5- CD200- CD23- CD10- CD103- e restrizione clonale per le catene leggere sK delle Ig.Conclusioni: La maggior parte (80-85%) dei linfomi non-Hodgkin deriva da linfociti B; coinvolgendo sia cellule progenitrici che cellule mature . Lo stadio della differenziazione dei linfociti a cui si verifica l'evento oncogenico determina la presentazione e l'esito della malattia. La maggior parte dei linfomi è nodale con un coinvolgimento variabile del midollo osseo e del sangue periferico. La Medicina di laboratorio è fondamentale nell' identificazione precoce del rischio, nella diagnosi e nella scelta di trattamenti più personalizzati efficaci e spesso meno invasivi . Da un punto di vista concettuale, l'essenza della Medicina di Laboratorio è quella di dare risposta al quesito clinico, esplicito od inespresso, esprimendo con pienezza la potenziale visione olistica del paziente .

EP046

L'USO DIAGNOSTICO DEL SEMPLICE ESAME EMOCROMOCITOMETRICO CON L'IMPIEGO DI TECNOLOGIE IN FLUORESCENZA IN UN CASO DI LEUCEMIA ACUTA MIELOIDE.

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INTRODUZIONE. Paziente uomo (63 aa), esegue un emocromo di controllo per sospetta infezione virale.MATERIALI E METODI. L' esame emocromocitometrico evidenzia un'importante anemia , piastrinopenia accompagnate da un dato di globuli bianchi che induce ad ulteriori approfondimenti . L'osservazione della distribuzione dei cluster cellulari e relativi indici di posizionamento suggeriscono un'urgente valutazione morfologica.
WBC : 23.220/µL N : 18 L : 25 M : 57 HGB :9.1 g/L PLT : 22.000 µL MCV: 102.3 fL

L'osservazione microscopica eseguita su striscio di sangue periferico confermava il sospetto diagnostico posto dagli scattergram di distribuzione cellulare. Sono presenti blasti di natura mieloide pari al 55% della cellularità totale, marcata anisopoichilocitosi. L'analisi citofluorimetrica eseguita su midollo osseo evidenzia la presenza di una popolazione blastica mieloide CD33+ + CD56++ CD117++ CD38+ CD13+/- CD11C+/- DR- CD15- CD14- CD19- CD64- CD 34- pari al 67% della cellularità globale. CONCLUSIONI Il caso posto in esame suggerisce quanto un esame di laboratorio correttamente interpretato ed approfondito possa essere illuminante nella diagnosi precoce di patologie altamente invasive e complesse. L'utilizzo di strumentazioni di ultima generazione, accompagnate dall'approfondimento morfologico e da esami specialistici garantiscono un continuo progredire della ricerca e dello sviluppo indirizzando così la scelta terapeutica del clinico verso i metodi più funzionali di diagnostica e terapia ematologica.

EP047

IL PIVKA COME BIOMARCATORE PRECOCE PER LA DIAGNOSI DI CARCINOMA EPATOCELLULARE NEI SOGGETTI AD ELEVATO RISCHIO

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Il carcinoma epatocellulare (HCC) è uno dei tumori maligni più comuni con elevati tassi di mortalità ed è essenziale, quindi, ricercare nuovi biomarcatori per migliorarne l'accuratezza della diagnosi precoce.Scopo di questo studio è stato valutare il valore diagnostico della protrombina indotta da carenza di vitamina K o antagonista-II (PIVKA-II) come potenziale biomarcatore e valutarne la performance in combinazione con l'α-fetoproteina (AFP), marker tumorale di routine nei pazienti ad elevato rischio di HCC. Al tal fine sono stati analizzati i livelli sierici di PIVKA e AFP in 37 pazienti di cui 29 maschi (78%) e 8 femmine (22%), con età mediana di 66 anni (range 44-78), afferenti, nel periodo Gennaio-Dicembre 2021, all'UOSD Fisiopatologia epatica (SATTE) dell'AORN ''Sant'Anna e San Sebastiano'' di Caserta. Di questi 37 pazienti, 26 (70.3%) non presentavano diagnosi di HCC che invece era presente nei restanti 11 (29.7 %). Nel gruppo dei pazienti HCC, il 90.9% (10/11) mostravano valori di PIVKA superiori al cut off e il 36.3% (4/11) valori di AFP alterati, mentre nel gruppo dei pazienti senza HCC tali valori risultavano rispettivamente: pari al 26,9% (7/26) per PIVKA e 0% per AFP.La performance diagnostica del PIVKA è stata calcolata mediante l'area sottesa alla curva ROC (AUC), scegliendo il valore di cut off ottimale $>$ 59 mAU/mL, ottenendo una sensibilità del 90.9 e una specificità del 80.8. In maniera similare sono stati calcolati: valore predittivo positivo (PPV) pari al 66.7, il valore predittivo negativo (NPV) pari al 95.5, il rapporto di verosimiglianza del risultato positivo (LR+) pari al 4.7, e quello del risultato negativo (LR-) pari al 0.1. Sono state poi messe a confronto le AUC dell'AFP e del PIVKA, con un valore più elevato di AUC per il PIVKA, differenza risultata statisticamente significativa (p=0.0094).In conclusione lo studio, pur presentando lo svantaggio della ridotta numerosità campionaria, rileva altresì come livelli elevati di PIVKA correlino con la presenza di HCC e, a confronto con l'AFP, come il dosaggio del PIVKA risulti avere una maggiore sensibilità, candidandolo come marcatore di screening.

EP048

A new LC-MS/MS screening method for the determination of a group of 739 illicit drugs, and other compounds in biological matrices

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Nowadays it is increasingly important from a pharmacological, toxicological, and clinical point of view to have rapid screening tests available for the analysis of many compounds in a short time. Here, we discuss a rapid screening procedure in LC–MS/MS for the qualitative evaluation of 739 compounds in biological samples (blood, post–mortem blood, and urine) including New Psychoactive Substances (NPSs) and other illicit substances. Blood samples were protein precipitated while urine samples were subjected to glucuronidase enzymatic reaction to hydrolyse any metabolites present. Chromatographic separation was carried out using a Restek Allure PFP Propyl (5 μm,60Å,50×2.1 mm) column in gradient elution mode. The deuterated internal standards were d9-methadone and d3-monohydroxycarbazepine. Mobile phases are M1: 2mM NH#HCO_2 +0.2% CH2O2; and M2: acetonitrile +2mM NH#HCO_2 +0.2% CH2O2. The method results in a rapid elution gradient and a change in flow rate during the run. The initial conditions are 90% M1 and 10% M2. Method includes two separate analyses in positive and negative ionization mode, without changing the instrumental parameters and run time was 18 min. Analyses were conducted on a ABSciex API 4500 QTrap instrument in MRM mode on 697 specific transitions. Thanks to the instrumental configuration the conventional analysis proceeds until the signal exceeds a threshold value, the mass spectrometer automatically begins to monitor the specific MRM transition and the entire fragmentation spectrum to obtain a comparable data with the mass spectra present in the database. Following the "match" process, the correct identification of the compound is then provided following the retention time and the MS/MS fragmentation spectrum. Our method is simple with minimal sample handling and immediate comparison of the acquired MS/MS spectra with spectra in the database. This allowed to obtain the correct identification of the compound. This screening method was tested on 150 samples; all the samples were also analysed with confirmation test, to check the obtained screening results. The comparison gives 100% correct identification of the positives and the type of substance. "Polyconsumers" were also identified.

EP049

Light Chain Escape: a diagnostic challenge

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Descritta per la prima volta da Hobbs nel 1971, la Light Chain Escape (LCE) rappresenta una forma di evoluzione di mieloma multiplo (MM) caratterizzata dalla elevata secrezione di catene leggere libere monoclonali non accompagnata dal concomitante incremento della componente monoclonale (CM) intatta originaria. È un evento raro (<2.5% dei casi di MM) caratterizzato da uno shift clonale midollare verosimilmente dovuto alla pressione selettiva dei regimi terapeutici, allo sviluppo di resistenze e a cambiamenti del microambiente midollare. Il caso considerato è riferito ad una paziente (79 anni, caucasica), esordita con MGUS IgG-Kappa (11 g/L; gammaglobuline 21%), nota al nostro centro dal 2004. Durante il monitoraggio annuale si osservava una riduzione lineare della MGUS di circa 1 g/L all'anno, contestuale alla diminuzione delle gammaglobuline. A settembre 2021 compariva per la prima volta proteinuria di Bence Jones di tipo Kappa in tracce (proteinuria totale 0.12 g/24h). A marzo 2022 la CM originaria in zona gammaglobulinica non era più visibile all'elettroforesi, mentre era comparsa una lieve alterazione morfologica in zona beta2globulinica e le gammaglobuline si erano ridotte al 5%. L'Immunofissazione sierica rivelava la scomparsa della CM IgG-Kappa e la presenza di catene leggere libere monoclonali di tipo Kappa in zona beta2globulinica. La misura delle catene leggere libere sieriche (FLC) risultava pari a Kappa 7379 mg/L, Lambda 6 mg/L, ratio K/L 1230. La paziente eseguiva biopsia osteomidollare e TAC in aprile, che rivelavano infiltrato plasmacellulare pari al 80% e microalterazioni litiche ossee diffuse: veniva posta diagnosi di mieloma micromolecolare Kappa, stadio 3A (Durie e Salmon). A maggio 2022 la proteinuria totale era incrementata a 1.14 g/24h e le catene leggere libere Kappa risultavano pari a 10955 mg/L, ratio K/L 2489, creatinina sierica 0.57 mg/dL. CONCLUSIONI: La misura delle FLC, unitamente agli altri esami ematochimici previsti nel monitoraggio, può essere utile, non solo nel MM ma anche nelle MGUS, alla diagnosi precoce di LCE-MM, e può aiutare a prevenire complicanze d'organo (50% di LCE presenta insufficienza renale alla diagnosi), permettendo interventi terapeutici precoci, migliorando morbilità e mortalità di questi pazienti.

EP050

L'importanza dello studio metabolico per il rischio nefrolitiasico in pazienti affetti da cistinuria

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La terapia del Paziente cistinurico prevede l'assunzione di circa 3,0-3,5 litri di acqua, l'alcalinizzazione delle urine con bicarbonato di sodio o con citrato di potassio, una dieta iposodica ed una riduzione del contenuto di proteine animali. In assenza di miglioramenti è prevista la terapia con chelante. Dopo aver seguito la terapia per circa 6-12 mesi, si effettua lo studio metabolico delle urine. Quest'ultimo prevede il dosaggio di alcuni analiti nelle urine delle 24h (calcio, sodio, potassio, magnesio, cloro, fosfato, solfato, citrato, ossalato, ammonio, urea, creatinina, proteine, cistina e determinazione potenziometrica - ditta Hanna), nelle urine della mattina (calcio e creatinina) e calcolo delle supersaturazioni renali per calcio ossalato, calcio fosfato, acido urico e cistina. Ossalurie e citraturie si dosano con reagenti della ditta LTA su Viva E Siemens così come cistinurie e solfaturie (metodiche fotometriche non esistenti in commercio). Gli altri analiti urinari si dosano con strumentazione Cobas 6000 della ditta Roche. Dal 2018 al 2021 sono stati eseguiti per tali pazienti 74 studi metabolici: circa il 30% ha evidenziato una tendenza alla formazione di calcoli di calcio fosfato, accompagnate da quasi il 70% di ipercalciuria moderata e solamente il 20% di iperfosfaturie, in assenza di ipercalciuria. Di questi, la maggior parte dei campioni (oltre il 60%) presenta un pH superiore a 6,5. Solamente il 20% dei campioni cistinurici presenta una diuresi ottimale, superiore ai 3,5 litri; il 50% era compreso tra 2,0 e 3,5 litri mentre il rimanente 30% non raggiunge l'escrezione di 2 litri. La presenza di ipercalciuria e lieve iperossaluria, a pH inferiore a 6, manifesta la contemporanea possibilità, stimata a circa il 4%, di avere calcoli di cistina e di ossalato di calcio. La mancanza di inibitori di calcolosi calcica è preponderante per il potassio (circa il 70%), meno per il citrato (circa il 40%) e bassa per il magnesio (meno del 10%). Oltre il 50% dei campioni urinari testati presenta una ipersolfaturia (aumento delle proteine animali) e più del 60% una ipernatriuria. I dati presentati evidenziano che il monitoraggio dei Pazienti affetti da cistinuria, non può prescindere da un controllo urinario più specifico e mirato principalmente nell'evitare la contemporanea precipitazione di calcoli di cistina e calcio fosfato. L'effettuare uno studio metabolico, rispetto ad un semplice dosaggio di cistinuria, può sicuramente dare una migliore rappresentazione della terapia in atto visto che è possibile avere informazioni sullo stato di idratazione, di alcalinizzazione, sull'assunzione degli inibitori della calcolosi calcica, del sale e delle proteine di origine animale.

EP051

Simultaneous determination of phytocannabinoids in oily based preparations by a new fast LC-MS/MS method

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Medical cannabis is always more prescribed by doctors for an increasingly number of different diseases. Today, the use of cannabis as a medicine has not been rigorously tested mainly due to restrictions related to its production and use. This has led, over time, to a still limited clinical research on the cannabis safety profile and efficacy. Nowadays the pharmacist prepares, in compliance with the rules of good manufacturing practice (GMP), galenic formulations based on cannabis. We report an optimized LC-MS/MS method for the simultaneous determination of tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and tetrahydrocannabivarin (THCV) in oily based preparations. This method required a sample processing optimization with two subsequent dilutions: isopropanol is the solvents selected for the first (dilution factor 1:10) and methanol for the second dilution/extraction step. The extraction step required a dilution factor of 1:100. Added deuterated internal standard were THC-D3, CBD-D3, and CBN-D3.Injection volume was 10 µL. The chromatographic column, an Hypersil Gold PFP (50×2.1 mm, 1.9 µm) was regulated at 40°C.The LC-MS/MS method has been set with an initial composition of 95 %:5%, v: v, M1:M2 for 0.2 min, followed by a linear gradient from 95 % to 25 % (M1) in 7.8 min. Then the M1 percentage was decreased to 0% in 0.1 min. The conditions 0%:100 % (v: v) were maintained for 2 min, followed by a system reconditioning for 5 min. Flow rate was 0.4 mL/min. Multiple Reaction Monitoring (MRM) scan type with collision energy set at 10 V was used. Our method met all requirements in terms of linearity, limit of detections and quantifications (LODs and LOQs), accuracy (precision and trueness, both intra and interday), and matrix effects. Such methodology showed high selectivity, and no matrix interfering signals and then the method was fully validated.This procedure represents a powerful tool for routine analyses of seven phytocannabinoids in less than 9 minutes.

EP052

Circulating histones as common mediators in classical and COVID-19 Sepsis: novel triggers for monocyte and MDW alterations

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Background: Histones (i.e., positively charged nuclear proteins) are key components in chromatin functions that under physiological conditions contribute to DNA packaging and regulate gene expression, but they are significantly mobilized in blood and body fluids during cell and tissue injuries in several pathological processes. Histones mediate both inflammatory pathways and coagulative cascade, crucially linked to the severity and mortality of many human pathologies (e.g., thrombosis, sepsis, COVID-19). SARS-CoV-2 and sepsis infections share common laboratory biomarkers, such as Monocyte Distribution Width (MDW), that is mainly linked to the heterogeneity of monocyte volume; these modifications, upon massive inflammatory activation, predict multiorgan dysfunction and increased mortality rate in several pathological conditions.
No data are available on the roles of histones as MDW modifiers.
Methods: Comparison of MDW index was undertaken by hematology analyzer UniCell DxH900 Hematology Analyzer (Beckman Coulter) on whole blood samples from patients with COVID-19 and Sepsis. The impact of histones on the MDW characteristics was assessed by the in vitro time-dependent treatment of healthy control whole blood with histones and histones+lipopolysaccharide.
Results: We demonstrated the breadth of early, persistent, and significant increase of MDW index in whole blood from healthy subject treated in vitro with histones, highlighting changes similar to those found in vivo in classic and viral sepsis patients. The findings of MDW changes are confirmed by digital microscopy of blood smears, highlighting the histone-induced modifications of cell volume, cytoplasmic vacuolization, and nuclear structure alterations of the circulating monocytes.
Conclusions: Histones contribute to the pronounced and persistent monocyte alterations observed in classical and viral sepsis. Assessment of the biological impact of circulating histone released during COVID-19 and sepsis on monocytes should be considered as key factor modulating both thrombosis and inflammatory processes, as well as the importance of neutralization of their cytotoxic and procoagulant activities by several commercially available drugs (e.g., heparins and heparinoids).

EP053

Introducing a clinical question framework in EQA reports

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EQAs programs are generally structured according to Laboratory Medicine subspecialties such as: hematology, clinical chemistry and the likes. Although this structure may be coherent with laboratory organization and with analytical platforms, it totally lacks a connection with clinical questions. Diagnostic Clinical reasoning , on the contrary, is based on the integration of information deriving from different sources. Nowadays both clinical guide lines and international regulations such as ISO 15189 encourage professionals to take responsibility for the whole process: from clinical question to clinical outcome. In an attempt to follow this this inspiring approach, the Centro Regionale Verifica Esterna Qualità (Careggi Firenze) produced a “sepsis EQA report”. Sepsis was the chosen topic, since Regione Toscana promoted the adoption of a sepsis protocol (1) containing, along with Emergency Department orders to identify potentially septic patients and to perform initial management, a list of blood tests We verify that the relevant analytes were evaluated by means of 7 different EQAS programs: Hematology, Clinical Chemistry, Coagulation, Specific Proteins, Procalcitonin, Cardiac markers and Blood gas analysis. The rational that allowed us to produce an objective summary report, is the following: all EQAs schemes request quantitative data, our statistical approach is based on ISO 13528 and Analytical Performance Specification are defined. To provide, at a glance, an overview of global analytical quality, we gathered all the sepsis protocol tests in just one report and we added an intuitive evaluation based on traffic light code (red, yellow, green), referring to the statistical evaluation customarily performed. The quarterly reports have been sent to Tuscany clinical laboratories located in acute-care hospitals. The report has been released in 2020 and 2021 and it is presently ongoing for the 2022 EQA cycle. This sort of cross view allowed us to identify potential pitfalls such as the lack of shared POCT management and the need of a more stringent evaluation of some measurand in our EQA schemes. Critical analytes resulted to be Procalcitonin and Lactate. This approach, where cross-functional data are shown and can be shared between different professionals working in team to a specific clinical question, is well suited for clinical pathways and may promote a better knowledge of tests use and tests limitations.1)Gestione della Sepsi e dello Shock Settico, Identificazione e Trattamento - (PDTA). http://www.regione.toscana.it/documents/10180/601731/PERCORSO+SEPSI+GRC+TOSCANA_2016.pdf

EP054

Pharmacogenetic analysis of DPYD gene SNPs: retroprospective evaluation of fluoropyrimidines induced toxicity in oncologic patients

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BACKGROUND: Pharmacogenetics in oncology plays a key role in in the comprehension of individual variability of response to chemoterapeutic drugs. Fluoropirimidine metabolism rate limiting step is catalysed by DPYD gene product; this gene is characterized by several polymorphisms, some of which are associated to enzyme activity reduction and therefore to an increased risk of adverse drug reactions (ADRs) development. Fluoropyrimidine drugs submission is therefore strictly ruled by national and international recommendations, which suggest pre-treatment evaluation of four variants. AOU Careggi’s Clinical and Molecular Biochemistry SOD provides a routine clinical pathway aimed to the execution of these analyses.
OBJECTIVES: comprehensive assessment on the state of the art of the local pharmacogenetics approach and retroprospective analysis of a selected cohort of 296 patients (data collected from 2016 to 2019), to elaborate the relationship between post-treatment toxicity and allelic distributions of 10 SNPs (four of which included in the recommendations).
MATHERIALS AND METHODS: Analysis of 9 DPYD SNPs was carried out with Sequenom Mass Array system, which is based on a MALDI-TOF approach. Intronic variants c.1129-5923G>C was evaluated in 296 patients using Real-Time PCR genotyping approach. CTCAE (Common Terminology Criteria for Adverse Events) was used for toxic effects classification.
RESULTS AND CONCLUSIONS: The Pharmacogenetics clinical pathway carried out in the laboratory has shown an increase of 51% of requests compared to five years earlier, and has involved not only AOU Careggi, but also other medical facilities all over Tuscany. The retroprospective analysis has allowed a preliminary evaluation of the correlation between genotype and toxicity. This analysis highlighted at least three important issues: the role of c.2194G>A variant, which is controversial among national and international recommendations; the role of c.85T>C ; the selection of 23 patients who have shown high grade toxicity in the absence of polymorphic variants, as candidates of further investigation on even larger panels of both DPYD and new markers (TYMS, ABCC2) potentially associated.

EP055

Mutational analysis of plasma cell-free DNA in NSCLC patients undergoing immunotherapy: preliminary data on the CORELAB project

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Background: New targeted therapies are changing the treatment of cancer, reducing toxic effects, and improving efficacy. The use of immunotherapy was recently introduced in non-small cell lung cancer (NSCLC) treatment and the research of specific predictive biomarkers is actively pursued. The CORELAB (New prediCtive biOmaRkers of activity and Efficacy of immune check point inhibitors in advanced non-small cell Lung cArcinoma) project, funded by Regione Toscana, has the purpose to monitor lung cancer patient during immunotherapy to identify new predictive biomarkers, comparing tissue and liquid biopsy.
Objectives: Mutational analysis of plasma circulating cell-free DNA (cfDNA) as liquid biopsy for monitoring NSCLC patients treated with immunotherapy.
Materials and methods: Blood samples were collected for each patient before immunotherapy and after 2, 4, 6 and 12 months or until disease progression. cfDNA was isolated from plasma and the mutational status was evaluated by NGS sequencing using a panel of 11 NSCLC related genes.
Results: NGS can reach high levels of sensitivity and specificity to detect low allelic fraction mutations in cfDNA. The percentage of cfDNA samples with mutations in NSCLC driver genes was in line with literature. After 2 months of treatment, a reduction of allelic fraction of mutations previously detected at the basal time was observed. A complete longitudinal monitoring was performed for a small cohort of patients highlighting different trends in mutated-genes' allelic fraction during treatment or disease progression.
Conclusion: cfDNA was confirmed an easily accessible material for monitoring the mutational status of the tumor over time and could represent a useful prognostic or predictive biomarker in NSCLC patients treated with immunotherapy. Mutational status and allelic fraction variation data, collected during immunotherapy, will be correlated with clinical parameters of treatment response, disease progression, overall survival to assess the actual potential of cfDNA as predictive biomarker.

EP056

Altered expression of the HOXA2 gene promotes breast tumorigenesis and predicts survival in breast cancer patients

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Accumulating evidence suggests that genetic and epigenetic biomarkers can play a role in the detection and monitoring of breast cancer (BC). Deregulation of the expression and methylation of the Homeobox A2 (HOXA2) gene is a potential biomarker in various cancers. However, the direct involvement of HOXA2 in breast tumorigenesis remains unknown. In the attempt to address this issue, we carried out high-throughput RNA sequencing and DNA methylation array studies of 43 laser-microdissected breast tissue samples, and in silico analyses using The Cancer Genome Atlas database. We first estimated the diagnostic potential of the HOXA2 gene using receiver operator curves, and its prognostic value using the Kaplan-Meier Plotter database as well as conventional immunohistochemistry (n=96). Then, to determine the role of HOXA2 in breast carcinogenesis, we performed loss- and gain-of function in vitro assays. Lastly, we asked whether altered HOXA2 expression is related to a mechanism of DNA-methylation via epigenetic reprogramming based on DNA demethylating treatment. In these experiments HOXA2 resulted in significantly hypermethylated and downregulated in human BC tissues. Moreover, low expression levels of HOXA2 were associated with more aggressive BC types, and therefore with an unfavorable survival.

Functional analyses revealed that low abundance of HOXA2 significantly enhanced proliferation, migration and invasion in BC cell lines. In contrast, forced HOXA2 expression remarkably inhibited migration and invasion, as well as proliferation, and promoted cell death in vitro. Finally, epigenetic reprogramming significantly reverted HOXA2 silencing in BC, thereby reducing cell proliferation. HOXA2 results as a novel tumor suppressor gene whose downregulation, also induced by its promoter hypermethylation, is implicated in BC progression and predicts a poor prognosis in BC patients.

EP057

VALIDATION OF REFERENCE INTERVAL OF GLYCATED ALBUMIN IN HEALTHY CAUCASIAN PREGNANT WOMEN

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Background and aim. Glycated albumin (GA) emerged as a useful biomarker for diagnosing and monitoring gestational diabetes mellitus (GDM). The establishment of reference intervals (RI) is mandatory before introducing it in clinical practice. The aim of this study was to validate a previously calculated RI of GA in healthy caucasian pregnant women.Methods. The study included 71 healthy pregnant women, median age (IQR) 32 ys (28-35), subgrouped into first (n=8), second (n=11) and third (n=52) trimesters of pregnancy. Exclusion criteria were the same as the original work (age < 18 years; fasting plasma glucose (FPG) ≥ 92 mg/dL; altered glycemia values during OGTT; pre-existing diabetes, endocrine, hepatic, or renal diseases). GA was measured on plasma by an enzymatic method (IL Werfen, Germany). Results. Median (IQR) GA levels in the trimester groups were, respectively, 11.4% (10.8-13.3), 12.4% (11.5-13.6%) and 12.0% (10.9-12.6). No significant differences were found between groups (KW p=0.336). GA was not associated with age, BMI, gestational age, or any glucose measurement during OGTT. When applying the upper limits (UL) of the reference interval (1st trim: 15.72%, 2nd trim: 15.49, 3rd trim: 14.57%), 2/71 (2.8%) subjects were outside the UL (1 subj at 2nd trim with 15.80% and 1 subj at 3rd trim with 14.60%). However, both subjects resulted being inside the RI when considering the limit of the 90%CI of the UL, respectively 15.92% for the 2nd trim and 15.01% for the 3rd trim)Conclusions. Previously calculated RI for GA in healthy caucasian pregnant women was confirmed using a validation cohort of 71 subjects. Unlike the original work, no difference was found between pregnancy trimesters, probably due to lower statistical power.

EP058

Biomarkers of synaptic dysfunction to discriminate Alzheimer's disease from other neurological disorders

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Background. The post-synaptic protein Neurogranin (Ng) and presynaptic protein α-Synuclein (α-Syn) have recently emerged as biomarkers of Alzheimer's Disease (AD). The aim of this study is to investigate the role of Cerebrospinal Fluid (CSF) Ng and α-Syn in AD patients as potential biomarkers of synaptic dysfunction. Methods. We measured CSF Ng and α-Syn concentrations in a cohort consisting of patients with AD (n=44), no-AD Neurodegenerative Disorders (NADD, n=17), and no Degenerative Disorders (NDD, n=37) by commercial-available ELISA kits. Results. CSF Ng levels were significantly higher in AD than in NADD and NDD, while we did not find any statistical difference in CSF α-Syn levels among groups. We also found that CSF Ng levels correlated positively with diagnostic delay and negatively with Mini Mental State Examination scores. Moreover, Aβ42/Ng and Ng/α-Syn ratio showed a statistically significant difference among groups and discriminated AD patients from NADD and NDD controls. Finally, we found that APOEε4 carriers had higher Ng levels than non-carriers. Conclusions. Our results support the possible role of Ng as a biomarker for AD and the Aβ 42/Ng ratio as a reliable index of synaptic dysfunction/degeneration to discriminate AD from other neurological conditions.

EP059

An European collaborative study on 476 patients with AA amyloidosis: identification and validation of survival and renal staging systems

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Soluble biomarkers have been largely used in staging systems for prognostication in immunoglobulin light chain (AL) transthyretin (ATTR) amyloidosis, but not in amyloidosis reactive to chronic inflammation (AA). The aim of the study was to identify prognostic biomarkers and to propose and validate survival and renal staging systems for AA amyloidosis. We collected data from 476 newly diagnosed patients with AA amyloidosis: 233 were diagnosed in Pavia (testing cohort) and 243 in Heidelberg (validation cohort). Multivariable analysis was performed to identify prognostic factors for overall survival (OS) and renal survival (RS) in the Pavia cohort and cut-offs of continuous variables were identified by ROC analysis predicting death or dialysis at 24 months. Since the cut-offs of b-type natriuretic peptide (BNP) and amino-terminal proBNP (NT-proBNP) were not clinically significant, we elected using their upper reference limits. No differences were observed between the two cohorts in biomarkers concentration. After a median follow-up of 3.8 years, 109 patients died with a median OS of 13 years. Multivariable analysis identified serum albumin (cut-off: 30 g/L; Hazard ratio [HR] 2.81, P=0.017), estimated glomerular filtration rate (eGFR, cut-off: 45 mL/min x 1.73 m2; HR 3.47, P=0.047) and elevated BNP/NT-proBNP (cut-off: 100 ng/L and 332 ng/L; HR 10.19, P <0.001) as risk factors for OS. We proposed a 3 stages survival staging system (stage I: 0 risk factors, stage II: 1-2 risk factors; stage III: 3 risk factors) that discriminated between patients with significantly different OS both in the testing and the validation cohorts. Progression to end-stage renal failure occurred in 120 patients and median RS was 9.4 years. Multivariable analysis identified eGFR (cut-off: 35 mL/min x 1.73 m2; HR 3.69, P<0.001) and 24h-proteinuria (cut-off: 3 g/24h; HR 2.36, P=0.006) as risk factors for RS. Renal staging system was built assigning patients with 0 risk factors to stage I, 1 risk factor to stage II and 2 risk factors to stage III and discriminated between patients

with a higher risk of dialysis both in the testing and the validation cohorts. This study for the first time establishes and validates powerful biomarker-based staging systems for OS and RS in AA amyloidosis.

EP060

ANTI-NUCLEOCAPSIDE IgG ANTIBODIES: A POTENTIAL MARKER FOR A MORE ACCURATE EPIDEMIOLOGICAL MONITORING OF SARS-COV-2 INFECTION

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On March 11th, 2020, the World Health Organization (WHO) declared the pandemic status of CoronaVirus Disease-19 (COVID-19) caused by SARS-CoV-2. Viral nucleic acid detection using molecular testing is the gold standard to diagnose SARS-CoV-2 infection. Asymptomatic subjects or with mild symptoms may not be subjected to molecular diagnostic tests with important repercussions on epidemiological estimate. The overall evolution especially of post-pandemic dynamics, will be probably obtained by the detection of immune response. Humoral immune response against the nucleocapsid (N) protein is related to SARS-CoV-2 infection. The detection of these antibodies could discriminate cases with from cases without history of COVID-19 for a more accurate evaluation of population exposed to SARS-CoV-2. In this retrospective study, we evaluated four subclasses of SARS-CoV-2 IgG (anti-S1, anti-S2, anti-RBD, anti-N) in n.200 blood samples (February-March 2022) of health workers of Azienda Sanitaria Provinciale (ASP) di Ragusa, already vaccinated against Covid-19 in the period February-March 2022. A statistically significant association between SARS-CoV-2 infection and seropositivity to anti-N IgG (p-value <0.0001) was found. In order to evaluate the SARS-CoV-2 anti-N antibodies titer trend, the cases with a positive history for COVID-19 were classified as follows: cases with infection within three months and cases with infection after three months from serological test.No statistically association (p-value= 0.075) was found between seropositivity to anti-N IgG and SARS-CoV-2 infection within and after three months. We observed that approximately 30% of cases with a history for COVID-19 was seronegative for anti-N IgG within three months from infection.In post-pandemic period the evaluation of anti-N IgG in a time interval of less than three months could identify asymptomatic population exposed to SARS-CoV-2. Although our findings should be validated with a larger cohort, these results suggest that anti-N IgG might be used as a marker of early infection and assure a more accurate epidemiological estimate.

EP061

Role of the immature platelet fraction (IPF) in the diagnosis of thrombocytopenia in childhood.

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IPF % is a parameter used to identify the relative percentage of immature fraction of total platelet population, actually only a few group of hematology analyzers provides this value. In 2016, a new generation of instruments, supplied by a technology of flow cytometry coupled to fluorescence, allowed to measure, with an improved accuracy, the total platelet amount and its immature proportion.The aim of this work was to assess the IPF% role in the differential diagnosis of thrombocytopenia in pediatric setting. The study was focused on a group of patients admitted to the Onco-hematology Unit of Pediatric Hospital G. Salesi (Ancona, Italy). A retrospective analysis of clinical data about all registered cases (n.284) of thrombocytopenia, shows as the IPF% value was reported for only 46 patients; in children with acute and chronic immune thrombocytopenic purpura (ITP) the IPF reached a mean value of 12 % instead, in congenital disease, the parameter was significantly higher. The observed congenital cases are rare disorders: Bernard-Soulier syndrome, MYH9 related disease and Sitosterolemia associated thrombocytopenia. The analyzed data shows how, in genetic diseases, IPF% may be greater than 40%, especially in children affected by MYH9 mutation. Published data confirms what we observed in acute and chronic ITP such as in those affected by genetic disease(1,2). It's clear how IPF values are significantly different comparing the two groups, according to the underlying mechanisms that characterize the etiology of the two conditions (acquired and congenital). Infact, in genetic disease, the released platelets are few and in an immature stage. Our emerging findings support the idea that, IPF% according to other parameters, may support the suspect of genetic illness. A further study of the IPF% and total platelet count trend during the hospitalizion, in a small group of chronic ITP patients, highlights as the two analyzed parameters result to be in a significant inverse linear relationship. On this evidence, the immature platelet fraction may represent an useful tool for the follow up of patients pharmacologically treated and for the prevention of the fall in platelet count.

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EP062

Prevalence of alcohol and other psychotropic substances in injured and suspected impaired drivers in Friuli Venezia Giulia

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Studies documenting the prevalence of polydrug use among drivers suspected of being under their influence or involved in road accidents in Italy are limited. In this analysis, 166 drivers of both sexes (female 30% and male 70%) were considered. The average age was 44+/-19 years (min 15 ÷ max 88) and the subjects were predominantly (70% of cases) 50 years or younger. Blood Alcohol Concentration (BCA) was determined by headspace gas chromatography with flame ionization detection (HS-GC-FID) and urinary ethyl glucuronide (EtG) metabolite, a biomarker of recent alcohol consumption, was assessed by immuno-enzymatic assay. To estimate recent use of other psychoactive drugs, urine samples were processed with IVD-CE-certified MassTox® Drugs kit (Chromsystems) and analysed by Ultra-High Performance Liquid Chromatography-tandem mass spectrometry (UHPLC Shimadzu HPLC Nexera X2, Citrine QTRAP MD Sciex). The assay allows the simultaneous and quantitative detection of 108 psychoactive substance and their metabolites.Although BCA was not required for 15% of the patient, alcohol was the most commonly detected substance, present in 31/166 (18.7 %) of the drivers. Urinary positivity for EtG was found in 48.2 % of the drivers and 83.9% of the samples with a positive result for BCA were also positive for EtG. Among the 166 enrolled drivers, 96 (57.8%) resulted positive for at least one drug and/or EtG in urine sample, excluding the positivity for administrated drugs in accordance with the information provided by the Emergency Department. One or more psychoactive drugs were found in 33/166 (19.9 %) of urine samples. In these records, cannabis was found in 39.4 % of the subject tested positive, benzodiazepines in 27.3%, cocaine in 24.2% and morphine in 12.1%. Positivity for CNS stimulants (MDMA and MDA) and hallucinogens (ketamine and LSD) was also evidenced, although in lower percentage (<10%). A significant percentage of the cases (33%) resulted positive for BCA and at least one psychoactive substances.Our preliminary data suggest to date in Friuli Venezia Giulia a significant percentage of injured drivers are under the influence of alcohol or have recently used drugs of abuse. Further studies will allow us to extend the analysis to a larger group of subjects.

EP063

ESTIMATION OF THE REFERENCE INTERVAL FOR GROWTH HORMONE IN NEWBORNS BY A PREVIOUSLY VALIDATED ANALYTICAL METHOD USING DRIED BLOOD SPOTS

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Background and aim. Severe deficiency of GH (GHD) of the newborn is a rare but potentially life-threatening disease. GH evaluation using dried blood spots (DBS) may offer several advantages: small sample volume, easier transportation and storage, reduced costs, allowing centralization and method standardization. Aim of the study was to estimate the reference interval for GH in newborns by a previously validated analytical method using dried blood spots.

Methods. GH reference interval was estimated in 812 healthy newborns (M:F 48:52%) attending the Neonatology Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan in the period July-October 2021. Heel-prick samples, spotted onto Guthrie cards (LTA Srl) and dried at RT, were stored at -20°C until analysis. Briefly, GH was extracted using 250 µL of PBS 1X. Samples were incubated at RT on an orbital shaker for 16 hours and then centrifuged at 12500 rpm for 1 min. GH in supernatants (SN) was measured by Immulite 2000 (Siemens Healthineers). Reference limits for GH deficiency was estimated at percentiles 2.5th and 5.0th by the Harrell–Davis bootstrap method, with 90%CI calculated by the bias-corrected and accelerated bootstrap method (BCa), using 5000 bootstrap replicates.

Results. Precision was 11.1% at 1 µg/L (LOQ) and between 2.4% and 6.1% in the range 1.5-50 µg/L. All GH measurements required 21 analytical sessions (6 months): CV% for the 21 calibration curve slopes, low (3 µg/L) or high (10 µg/L) controls were, respectively, 6.9%, 14% and 6.5%.Median (IQR) GH levels were 18.2 µg/L (12.1-25.2 µg/L). Reference limits for GH deficiency, estimated at percentiles 2.5th and 5.0th, were, respectively, 5.9 µg/L (90%CI 5.2-6.4) and 7.1 µg/L (90%CI 6.6-7.4). GH levels were not associated with sex, birth weight or height standard deviation scores, gestational age, type of delivery or mother's variables (age, smoking habit, gestational diabetes).

Conclusions. To our knowledge, this is the largest monocentric study combining DBS samples and GH measured by an automatic immunoassay analyzer. The reference limits estimated in this study are in accordance with previous published works using ELISA and may help confirming the clinical suspicion of neonatal GHD.

EP064

EVALUATION OF GROWTH HORMONE LEVELS IN PRETERM NEONATES USING DRIED BLOOD SPOTS

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Background and aim. Congenital growth hormone deficiency (cGHD) is a rare but life-threatening condition whose diagnosis is challenging, both in healthy neonates and in preterm ones. We recently estimated GH reference interval (RI) in 812 healthy newborns (HN) using dried blood spot samples and a previously validated method. Aim of this study was to provide values for random GH in preterm newborns (PN).

Methods. GH was evaluated in 78 PN at the Neonatal Intensive Care Unit of Ospedale Maggiore Policlinico (Milan). GH measurement was performed at 48 hs after birth (GH1). In 41 out of 78 PN a second GH determination (GH2, at 15 days) was also available.

Results. Median (IQR) GH1 values were 14.9 μg/L (9.8-21.1). No gender differences were found (p=0.089). The percentile 5th (3.4 μg/L) calculated in PN was lower than the lower limit of the RI estimated in HN (percentile 5.0th: 7.1 μg/L, 90%CI 6.6-7.4). All 4 PN with GH <3.4 μg/L required an emergency C-section before the 34th week of gestational age (GA). Decreasing GH was associated with increasing invasiveness of ventilation (no: 20.1, CPAP/biphasic: 15.1, high flows: 12.4, invasive: 5.4; overall KW p=0.026). GH was significantly lower in PN needing invasive ventilation than in not ventilated ones (Bonferroni's correction p=0.048). A low-to-moderate correlation was found between GH levels and GA (rho=0.295, p=0.009). No association was, instead, evident with maternal age (p=0.072), smoke (p=0.138), Medically Assisted Procreation (p=0.172), parity (p=0.520) or other neonate variables, including jaundice (p=0.276) and SDS auxological parameters (all p>0.05). Considering PN with both GH determinations, GH1 levels were significantly higher than GH2 (14.4 vs 9.8 μg/L, respectively, p=0.018). GH decreased in most neonates (26/41=63%). Interestingly, GA was higher in PN with GH1>GH2 than in those with GH2>GH1 (median 33 vs 30 weeks, p=0.024).

Conclusions. Our data show that PN have lower GH than HN. GH in PN is associated with ventilation and GA. The latter finding, along with the association between lower GA and increase in GH2 levels, could be explained by an incomplete maturity at birth of the somatotrophic axis in very PN, with the subsequent GH increase reflecting an extra-uterine maturation of the axis itself.

EP065

UN CASO DI CARCINOMATOSI MENINGEA: L'IMPORTANZA DELL'ESAME CITOLOGICO DEL LIQUOR NEL LABORATORIO DI PATOLOGIA CLINICA.

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SCOPO. Vogliamo qui presentare un caso di carcinomatosi meningea rilevata all'esame citologico del liquor.
CASO CLINICO/METODI. Una Paziente di 56 anni è stata ricoverata nel reparto di neurologia del P.O. S. Filippo Neri per un deficit visivo all'occhio sinistro. La Paziente aveva una anamnesi positiva per neoplasia mammaria. In corso di ricovero è stato eseguito l'esame chimico-fisico del liquor. L'esame evidenziava: aspetto opalescente, xantocromico, glucosio 60 mg/dL (ratio glicorrachia/ glicemia: 65%) e proteine 262 mg/dL (Roche Cobas c702); 40 cellule/µL (Sysmex XN-10), di cui leucociti 13/ µL; il citogramma mostrava circa il 60% di cellule ad alta fluorescenza (TC-HF). È stata eseguita la revisione microscopica del sedimento dopo citocentrifugazione e colorazione May Grumwald-Giemsa. L'esame citologico ha evidenziato, oltre ad eritrociti e rari neutrofili, la presenza di cellule abnormi di grandi dimensioni, polimorfe, con grandi vacuoli, talora binucleate, isolate e a gruppi. I dati sono stati confermati su un secondo prelievo di liquor dopo una settimana (che presentava una cellularità maggiore). La Paziente è stata quindi sottoposta a risonanza magnetica del SNC che ha permesso di porre diagnosi di carcinomatosi meningea.
DISCUSSIONE. Grazie alla tecnologia in automazione è possibile superare i limiti dei conteggi manuali, gravati da imprecisione e variabilità inter-operatore; resta fondamentale la revisione microscopica, in particolare nei casi in cui il citogramma strumentale segnala la presenza di cellule "non-leucociti" ad alta fluorescenza, che necessitano di approfondimento.
CONCLUSIONI. Questo caso clinico dimostra l'importanza dell'esame citologico del liquor e del rapporto del Laboratorio con il clinico: la conoscenza del quesito clinico può permettere l'esecuzione tempestiva degli opportuni esami di approfondimento. Un vantaggio nell'eseguire l'esame citologico presso il Laboratorio di Patologia Clinica risiede nella rapidità di risposta rispetto all'esame citologico convenzionale eseguito nei servizi di Citopatologia; nonostante non possa sostituire quest'ultimo nella diagnosi definitiva, permette una risposta che può orientare decisioni cliniche in tempi più contenuti.

EP066

EMOGLOBINA J-BANGKOK RILEVATA IN ELETTROFORESI CAPILLARE: CASO CLINICO

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SCOPO. Vogliamo qui presentare un caso di Emoglobina J-Bangkok rilevata in un Paziente italiano che ha eseguito presso un Centro Prelievi della ASL Roma 1 un prelievo per la ricerca delle Emoglobine patologiche.
MATERIALI E METODI. La ricerca delle emoglobine patologiche è eseguita nel nostro Laboratorio con metodica capillare (Hemoglobine Sebia Capillarys 3 TERA, Sebia, Francia). Il campione del Paziente in esame è stato analizzato anche con metodica HbA1c (Hemoglobin(e) Sebia Capillarys 3 TERA, Sebia, Francia) ed è stato eseguito l'esame emocromocitometrico con conteggio dei reticolociti su Sysmex XN-10 (Sysmex, Giappone). Per il 2° livello è stata eseguita l'estrazione del DNA genomico da leucociti di sangue periferico mediante Kit QIAamp® DSP DNA Blood Qiagen) e il sequenziamento diretto (strumento 3500 DNA Analyzer Applied Biosystem) dei frammenti corrispondenti all'esone e alle giunzioni introne-esone del gene HBB (NM 000518.5) e la Reverse Dot Blot hybridization (RDB) (a-Globin StripAssay, ViennaLab Diagnostics GmbH CE-IVD).
RISULTATI. All'analisi con la metodica Hemoglobin(e), si osservava la presenza di una variante emoglobinica che migrava nella zona Z12 in percentuale pari al 51,8%; l'emoglobina A2 era 2,7% e la emoglobina A 45,5% con morfologia dei picchi normale. Il Paziente aveva una emoglobina di 14,2 g/dL, un MCV di 91,4 fl e un MCH di 31,3 pg/mL; i reticolociti erano 0,78%. L'analisi con metodica HbA1c presentava un picco anomalo tra la HbA1c e la HbA0, e uno sdoppiamento del picco della HbA1c. L'analisi molecolare ha rilevato la presenza della emoglobina J-Bangkok in eterozigosi: HBB β56 [D7] Gly -> Asp.
DISCUSSIONE. Nella nostra esperienza la metodica capillare consente di rilevare numerose varianti emoglobiniche; questa variante appare di particolare interesse in quanto non è presente nel database delle emoglobine patologiche del software. Abbiamo segnalato la variante alla Ditta produttrice per integrare il database. Sono necessari ulteriori studi per verificare se la presenza della emoglobina J-Bangkok possa interferire nella misurazione della HbA1c. L'HbJ-Bangkok è una variante emoglobinica con fenotipo ematologico normale, tuttavia potrebbe modificare il fenotipo ematologico se associata ad altri difetti emoglobinici.

EP067

Adaptation of the statistical algorithm for processing the results of the EQA programs in accordance with the standard UNI CEI EN ISO/IEC 17043:2010

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Introduction. The Regional Reference Center for the Quality of the Laboratories of the Lombardy Region (Center) guarantees External Quality assessment (EQA) programs, some of them with quantitative results. The corresponding results are processed in reports and contain set of statistical parameters in order to evaluate the laboratories performance. These parameters are calculated by peer groups of methods and through a self-developed algorithm on its own web platform (www.qualitalaboratorilombardia.it).In order to comply with the international standard ISO 17043 which defines the general requirements for interlaboratory evaluation tests, the Center has updated the statistical algorithm and performed the appropriate technical validation, before using it.
Methods. The new algorithm, applied when the number of laboratories (N) for peer groups is N >7, includes 1) identification of the outliers according to Hampel method and, if not applicable, according to methods of Grubbs and Tukey; 2) calculation of the robust standard deviation through the robust estimator Qn; 3) calculation of the robust mean (assigned value) using the robust Hampel estimator; 4) calculation of the measurement uncertainty 5) comparison with acceptance limits based on the "state-of-the-art" model. The algorithm was first implemented in the R v. 4.0.3 Language and validated by comparing the results with those obtained using additional R packages or commercial software. The R code was then translated into the PHP language to allow integration into the Center's web platform. Measurement of free T3 (Roche homogeneous group, 63 results), free T4 (Beckman Coulter Access, 48), TSH (Abbott Alinity, 15) from sample 7 of cycle 2021 of Hormones and Tumor Markers A EQA scheme were used for comparison.
Results. Results obtained with the R language and with PHP were comparable and agreed at least to the second decimal place. The data anlysis is returned as a pdf file or as an interactive HTML report in which selective visualization of all peer groups is possibile.
Conclusions. The self-developed statistical algorithm implemented by Center allows the calculation of robust and correct statistical parameters and complies with the requirements of the international standard ISO 17043.

EP068

Evaluation of Prostate health index (PHI) as a biomarker for prostate cancer

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Background. Multi-parametric magnetic resonance imaging (mpMRI) represents the gold standard for identifying patients at high risk of prostate cancer (PCa) eligible for prostate biopsy. However, it has several drawbacks. The aim of this study was to assess the diagnostic performance of prostate health index (PHI) to detect patients at high risk of PCa, defined according to a PI-RADS score >3. Methods. We enrolled all consecutive patients with suspected PCa who underwent a mpMRI of the prostate evaluated using the PI-RADS criteria. PSA parameters, including total PSA, free PSA, and p2PSA were tested by a Beckman Coulter DxI 800 Immunoassay System (Beckman Coulter, Taiwan Inc.) to obtain PHI (PHI = (p2PSA/free PSA) × √PSA). Results. The study population consisted of 110 patients with a median (IQR) age 66 (60-71) ys. In the whole sample, median (IQR) total PSA, fPSA, PSA ratio and PHI were, respectively, 5.87 ng/mL (3.90-8.74), 0.94 ng/mL (0.60-1.36), 16% (12-23), and 47 (32-64). Patients were classified according to PI-RADS into three groups: <3 (47%), =3 (14%) and >3 (39%). An association was found between PI-RADS groups and decreasing PSA ratio (<3 vs =3 vs >3: 18.9 vs 16.1 vs 12.8%; overall KW p<0.001) as well as increasing PHI values (40.0 vs 45.7 and 58.9; overall KW p<0.001). At the post-hoc tests, only a difference (<3 vs >3) for both PSA ratio (p<0.001) and PHI (p<0.001) was found. The association between PHI and PI-RADS remained even after PHI was partitioned in 0-20 vs 21-39 vs ³ 40 classes (Fisher test p=0.016). No association was instead evident between PI-RADS and total PSA. The AUCs of PSA ratio and PHI to classify patients with PI-RADS <3 vs >3 were, respectively, 0.77 (95%CI 0.67-0.87) and 0.75 (95%CI 0.64-0.85). Conclusions. PHI could represent a reliable biomarker for detecting patients at high risk of PCa, who should undergo prostate biopsy.

EP069

Bone morphogenetic protein-2 and -4 and its receptors mRNA expression in an in-vitro model of vascular calcification.

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Vascular calcification (VC) is a complex ectopic calcification process considered the most important hallmark of atherosclerosis. The onset and progression of VC are similar to bone formation, thus based on the bone-vascular axis theory. Different studies have highlighted the role of bone morphogenetic proteins (BMP)- a group of at least 30 proteins belonging to the TGF-β superfamily that act by binding to a heterodimeric complex of transmembrane receptors (BMPR)- in VC. We aimed to study the involvement of BMP system (BMP-2/4, BMPR1a/1b, and BMPR2) expression in an advanced in-vitro model able to simulate the biological environment of the vascular wall by assessing the ability of phosphates mixture to induce the osteoblastic switch of Human Coronary Artery Smooth Muscle cells (HCASMCs), a key event in VC. The HCASMCs were cultured in a double-flow bioreactor (LiveBox2, IVTech Srl, IT), allowing static and/or dynamic conditions. The induction of HCASMCs calcification was obtained by supplementing DMEM HG with 1.9 mM phosphates solution (NaH2PO4/ Na2HPO4) for 7 days. After the incubation period, HCASMCs viability and calcium quantification were tested. Real Time-PCR of the BMP system was performed at the end of each experiment. The analysis of cell viability demonstrated that calcifying media significantly decreased HCASMCs viability in static conditions. Real Time-PCR of the BMP system in HCASMCs revealed a marked increase in the phosphate-induced calcification of both BMP-2 and BMP-4. In particular, we observed a higher amount of BMP2 transcript (p=0.0096), as expected, paralleled by mRNA expression increase of the receptors BMPR1a (p=0.0023) and BMPR1b transcripts as compared to controls. BMPR2 remained in a steady state in the experimental setting. The roles of the BMPs in osteogenesis are well documented, however, their involvement in VC is more complex and less defined. Our studies provide new pieces of evidence on how osteotropic factors, such as BMP-2 in synergy with its receptors, are modulated in VC caused by increased phosphate uptake and on their involvement in the osteogenic phenotype of HCASMCs.

EP070

Low-density Lipoprotein cholesterol (LDL-C), Homocysteine (HCY) and C-Reactive Protein (CRP): clinical significance in NSTEMI and STEMI patients differentiated by gender.

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OBJECTIVE. Hyperhomocysteinemia has emerged in recent years as an important factor for cardiovascular risk stratification and the definition ischemic patients' conditions associated with other markers (LDL-C and CRP). On top of ECG diagnosis, the dosage of parameters such as HCY and LDL-C strengthen the prognosis of STEMI and NSTEMI conditions.METHODS. LDL-C, HCY and CRP were analyzed in 587 patients admitted in the Cardiology Division of S. Andrea Hospital in Vercelli, from March 2021 to March 2022: 102 patients (73 men and 29 women) belonged to the NSTEMI group, 161 (117 men and 44 women) to the STEMI group and 324 patients with non-ischemic cardiac diseases (212 men and 112 women) were considered as control group (OTHER). Statistical analysis was carried out to assess the significance of the three markers analyzed by Wilcoxon's non-parametric and ANOVA tests.RESULTS. In the overall population the percentages of pathologic values for LDL-C was 40%, for HCY and CRP were respectively 62% and 66%. Significance of LDL-C using Wilcoxon test resulted of $p<0.0001$ for STEMI vs OTHER and of $p= 0.0055$ for NSTEMI vs OTHER whereas the returned p-values of the total population for LDL-C were $p <0.0001$ with ANOVA. HCY produced only a significant p-value (0.0251) in the Wilcoxon test when STEMI was compared with OTHER group. Evaluation of CRP values had no statistical significance. Gender stratification showed a significant difference compared to the total population. In females, p-values for LDL-C and CRP for all three conditions and both tests showed no significance, while HCY had significance in the comparison between STEMI and OTHER with a p-value of 0.028. Otherwise, the male population confirmed the statistical trend for the LDL-C values (ANOVA and Wilcoxon tests). Unlike the total and female populations, the significance for HCY values was absent. CRP showed a mildly significant difference ($p = 0.0481$) when comparing the STEMI and OTHER populations.CONCLUSION. The cardiovascular risk markers we evaluated (LDL-C, HCY, CRP) could have different impact in males and females with ischemic cardiac conditions (STEMI and NSTEMI). Further analysis should focus on the role of HCY in females and LDL-C in males.

EP071

Serological diagnosis of Idiopathic inflammatory myopathies as a support to clinical characterization

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The idiopathic inflammatory myopathies (IIM) are rare, heterogeneous, systemic autoimmune disorders, characterized by inflammation of skeletal muscle and multi-organ involvement, classified in several subgroups based on clinical and serological characteristics.
In our autoimmunology hub, we routinely test the IIM samples performing both first (ANA) and second level (IB) tests. We use the Euroline immunoblot (IB) method which allows a multiparametric screening and a semiquantitative measurement for 16 autoantibodies, including myositis specific antibodies (MSA: SRP, EJ, OJ, Mi-2α, Mi-2β, TIF1-γ, MDA5, NXP2, SAE1, PL-12, PL-7, Jo-1) and myositis associated antibodies (MAA: Ku, PM/Scl-75 and PM/Scl-100).
We aim to verify the role of specific IIM diagnostic as a tool to classify patients in more homogeneous serological profiles.
We evaluated the trend of ANA vs IIM requests in the 2018-2022 period showing that, beside a quite stable number of ANA tests prescriptions, we observed an increased request for specific IIM diagnostic and a consequent elevated antigens positivity detection rate with various antibodies profile.
Our analysis evidenced the following ANA vs IIM IB request proportion: 4920 (30.5% positive) ANA vs 10 IIM in 2018, 4973 (33.1% pos) vs 18 IIM in 2019. Starting from 2020, we observed an increasing trend of IIM requests: despite a slight decrease of ANA tests (n=3640, 29.7% pos), 21 IIM IB were requested. The increase was more evident in 2021 (5186 ANA, 26.9% pos vs 40 IB) and in the first semester of 2022 (2526 ANA, 28.3% pos vs 57 IB) Focusing on 2022, 21 out of 57 IB (36,8%) were reported as positive: 13 of them had single reactivity to MSA/MAA, and 9 showed multiple reactivity to 2(n=7), 3 (n=1), or 4 (n=1) antigens.
IB antigen specific profiles identified three major serologic subgroups; 3 MAA (PM/Scl-75 and PM/Scl-100), 3 MSA/ antisyntetase-related (EJ, OJ, Jo-1, PL-7, PL-12) and 13 MSA dermatomyositis related (Mi-2α, Mi-2β, TIF1-γ, MDA5).
The reason for the increased rate of laboratory IB requests and associated number of detected cases will be further investigated to ascertain if this is due to an increased awareness of the available diagnostic tools to classify patients in homogeneous group.

EP072

HEAD-TO-HEAD COMPARISON OF PHI AND PROCLARIX FOR THE IDENTIFICATION OF CLINICALLY SIGNIFICANT PROSTATE CANCER

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Prostate Cancer (PCa) is the most frequently diagnosed cancer in men and the second leading cause of men cancer related deaths.
Widespread use of PSA led to high rate of overdiagnosis and overtreatment. New diagnostic tools are needed to identify clinically significant PCa (csPCa) and choose a personalized treatment. In the recent years, several PCa biomarkers have been proposed with a clear tendency towards the use of panels biomarkers or combination of biomarkers and clinical variables. Among them, there were Prostate Health Index (PHI), based on a methematical combination of the molecular forms of PSA, and the most recent Proclarix, an index score based on the evaluation two glycoproteins (thrombospondin-1 and catepsin D) total and free PSA and the age of the patient. The aim of our study was to perform a head-to-head comparison of Proclarix and PHI.
Methods
Before prostate biopsy (minimum 16 cores), 345 subjects were enrolled, and blood specimens were collected. Whole blood was allowed to clot before serum was separated by centrifugation.Serum aliquots were stored at -80°C until sample were processed. Specimens were analyzed in blinded fashion for PSA, fPSA and p2PSA by Acces2 Immunoassay system analyzer (Beckman Coulte, Brea, CA, USA) calibrated against the WHO standard for PSA and fPSA. Thrombospondin-1 and Catepsin D were measured using the CE-marked Proclarix kit (Proteomedix). Comparison of AUC and performance at predefined cut-offs was performed to predict csPCa. Decision curve analysis was used to compare clinical benefits.
ResultsROC curve showed that Proclarix and PHI had a similar performance for predicting csPCa (AUC 0,78 vs 0,74, p=0,369). However, PHI had a higher specifity and the cut off of 40. Decision curve analysis showed that the combination of Proclarix and PHI had a highest clinical benefit.
Conclusion
In this study, Proclarix and PHI showed similar performance of csPCa. The combination of the two tests had the best performance avoiding 50% of unneeded biopsies, missing a very low percentage of csPCa.

EP073

Use of an anti-TCRB-1 Mab (JOVI-1) in multiparametric flow cytometry: analysis of a case of paucicellular T-LGLL (T-cell large granular lymphocytic leukaemia).

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Background

T-LGLL diagnosis was often challenging due to the lack of T cell clonality assays and the occurrence of morphologic and immunophenotypic similarities between malignant and reactive T-cells. Recently, the restricted expression of one of the two TRBC segments has been considered a potential marker of clonality in TCR alpha/beta positive T cells since these cells can mount only one of them, in a way conceptually akin to light chain expression in B lymphocytes.The recent availability of a Mab specific for one of the two segments (Mab JOVI-1 specific for TRBC-1) makes a rapid assessment of T-clonality by flow cytometry feasible; the unavailability of an analog TRBC-2 specific Mab does not constitute an issue, being both a homogeneous expression and a homogeneous lack of TRBC-1 considered as equivalent for diagnostic purposes.

Case presentation

Here we present the use of an anti-TRBC-1 Mab (JOVI-1, Becton Dickinson©) in a case of T-LGLL with a low number of LGL in peripheral blood.An 81-year-old patient with persistent and worsening neutropenia (WBC 4.00 x109/L, neutrophil granulocytes 0.88 x109/L lymphocytes 2.14 x109/L) was observed. All investigations aimed to point out autoimmune or dysmetabolic conditions or chronic infections were negative, except for the presence of surgical hypothyroidism. A lymphocyte study was also performed (Canto II™, Becton Dickinson©) and was focused on the expression of CD3, CD4, CD8, CD5, CD7, CD2, CD27, CD26, CD56, CD57, TRBC-1, and TCR gamma/delta.The multiparametric analysis spotted the existence of a minor CD8+ subset (0.730 x109/L) homogeneously displaying CD2+ CD3+ CD4- CD5dim CD7+ CD16- CD56+ CD57+ phenotype. The labeling with JOVI-1 Mab showed bimodal distribution of TRBC-1 both in CD4+ and CD8+ T lymphocytes considered as a whole, but revealed a selective TRBC-1 absence in the CD8+ CD56+ minor subset. The data was suggestive of clonality and supported diagnosis of T-LGLL, consistent with the clinical status of the patient.

Conclusion

This case report is an example of how TRBC-1 analysis is a game-changer in the investigation of T clonality, susceptible to represent a new diagnostic tool, especially used in a multiparametric approach and particularly in the case of low numerosity of the suspicious population.

EP074

IL TAT IN LABORATORIO E L'IMPORTANZA DELL'ANALISI DELLE SOTTOFASI DEL PROCESSO

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Introduzione

Il turnaround time (TAT) è critico in emergenza per l'outcome del paziente e vari esami necessitano di un TAT che rientri nei limiti adeguati per applicare gli algoritmi diagnostici proposti da Linee Guida; un esempio è la Troponina (Tn).

La stessa definizione di TAT deve essere ben chiara, perché se la prima definizione secondo Lundberg era il circolo brain-to-brain, frequentemente è stata scomposta in sottofasi pre, intra e post laboratorio.

Per la fase intralaboratorio esistono studi che hanno valutato il tempo analitico (TA), che va dall'arrivo del campione al termine dell'analisi e il tempo totale (TL) dall'arrivo alla disponibilità del dato al clinico, comprese le fasi di validazione tecnica e clinica.

Materiali e metodi

Scopo di questo studio è stato quello di rivalutare l'assetto organizzativo delle apparecchiature analitiche presenti in laboratorio, per vagliare nuovi modelli organizzativi che migliorassero il TA.

E' stata quindi eseguita un'analisi dettagliata della configurazione dei sistemi analitici in uso rispetto al carico di lavoro, alle tipologie di IVD, alla loro stabilità on-board, producendo un progetto riorganizzativo.

Risultati

Attraverso l'elaborazione dei dati e le successive simulazioni è stato selezionato un progetto che ha rimodulato le analisi di biochimica e immunometria eseguite sui tre sistemi analitici.

Da aprile 2022 è stato dedicato un sistema analitico alle richieste urgenti, un sistema alle richieste in routine e il terzo in mirroring alle urgenze e a parte della routine. Sui due sistemi per la routine, esami e carichi di lavoro sono stati distribuiti secondo criteri di efficienza.

A fine maggio sono stati valutati i TAT dell'esame Tn, rilevando che rispetto allo stesso mese dell'anno precedente il TA si è ridotto (90% in 52 minuti vs 58); il TL è aumentato da 81,3 a 87,3 minuti, il TL del solo Pronto Soccorso è diminuito da 57,7 a 53,5 minuti.

Un'altra elaborazione ha suddiviso il tempo TL distinguendo il 90° percentile con validazione in middleware e l'arrivo del dato in LIS e quello comprendente la validazione in LIS. I risultati ottenuti su tutte le Tn eseguite (1795/1950), rispetto al 2021 sono stati rispettivamente 81,0/78,0 e 81,3/87,2 minuti.

Conclusioni

I risultati ottenuti sulla Tn attestano che la riorganizzazione dei sistemi analitici è stato efficace riducendo di 6 minuti il 90° percentile del TA; questo risultato però non si è proiettato su tutti i TL.

Lo scorporo della fase intralaboratorio nei suoi step ha rilevato che le maggiori criticità sono concentrate nel trattamento del dato analitico.

Grazie a questo studio è stato attivato un piano di miglioramento che prevede l'analisi di tutte le fasi di trattamento del dato per ricercare ed implementare nuove modalità di trattamento del dato, mediante l'implementazione nei software di migliori criteri di validazione automatica.
Tutto ciò dimostra come l'efficienza di un processo si costruisce anche attraverso l'analisi critica delle sue singole sottofasi.

EP075

CONFRONTO TRA DUE SISTEMI AUTOMATIZZATI PER LO SCREENING DI CAMPIONI DA SOTTOPORRE A URINOCOLTURA

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Introduzione
L'urinocoltura è il gold standard per la diagnosi delle infezioni del tratto urinario ma richiede grossi carichi di lavoro con tempi di refertazione di 24 ore per i campioni negativi e di 48 ore per quelli positivi. La gestione clinica richiede tempi rapidi per impostare terapie mirate evitando l'utilizzo di antibiotici ad ampio spettro. Scopo dello studio è valutare le performance diagnostiche di due sistemi automatizzati per lo screening delle batteriurie rispetto al gold standard. L'obiettivo dello screening è quello di evitare colture non necessarie individuando i campioni negativi con test rapidi, economici, facili da eseguire, sensibili e con un elevato valore predittivo negativo.

Materiali e metodi
137 campioni urinari (da mitto intermedio, da catetere e da sacchetto) sono stati sottoposti a urinocoltura e contestualmente processati con il sistema in Light Scattering Alfred 60 (Alifax) che utilizza vials contenenti brodo eugonico e con il sistema UF-5000 (Sysmex) che prevede il conteggio in citofluorimetria di batteri, leucociti e cellule epiteliali (EC). Sono stati considerati positivi i campioni che su Alfred 60 raggiungevano un valore di 5000 UFC/mL e su UF-5000 conteggi batterici >58/µL per maschi, >46/µL per femmine e leucociti >10/µL (sono stati esclusi dal confronto i campioni con EC >30/µL indicative di contaminazione e con conducibilità <7 mS/cm indicativa di campione diluito). I dati di entrambi gli strumenti sono stati comparati con i risultati dell'urinocoltura a 18/24 ore e con il programma Excel sono stati calcolati i rispettivi valori di sensibilità (SS), specificità (SP), valore predittivo positivo (VPP) e valore predittivo negativo (VPN); è stata fatta altresì una valutazione dei turn around time (TAT).

Risultati
UF-5000 ha evidenziato valori di SS e VPN leggermente inferiori rispetto ad Alfred (83,9 vs 88,7 e 86,7 vs 90,0), mentre i valori di SP e VPP sono risultati superiori per UF-5000 (86,7 vs 84,0 e 83,9 vs 82,1). La diagnosi di negatività è stata ottenuta dopo 2 minuti con UF-5000, dopo 3 ore e mezza con Alfred 60 e dopo 18/24 ore con esame colturale.

Conclusioni
Dal confronto emergono i vantaggi legati all'utilizzo dei due sistemi automatizzati per lo screening delle batteriurie che mostrano sensibilità e valore predittivo negativo accettabili per un test di screening con il vantaggio della notevole rapidità di esecuzione.

EP076

NEW AND INSIDIOUS INTERFERENTS IN PROTEIN ELECTROPHORESIS

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Introduction: New drugs used to treat plasma cell disease, termed therapeutic monoclonal antibodies (tmAb), are capable of interfering with current electrophoretic separative techniques both during and for several weeks after administration.
The first drug approved by the Food And Drug Administration (FDA) for multiple myeloma is daratumumab a human IgG1/κ tmAb against the plasma cell surface antigen CD38.
The risk of interference depends on: the patient's (pc's) original Monoclonal Component (CM) isotype, compliance with therapy, and treatment phase. In post-treatment, the therapeutic monoclonal band should not be detectable more than 3-6 months after the last administration.
The interference of these drugs is of particular relevance for pts with a CM IgG kappa or free kappa light chain only. In these pts, co-migration of the therapeutic antibody and malignant CM may hinder proper evaluation of the pt. The pc could be considered to have a very good partial response (VGPR) to therapy rather than a patient in complete response (CR).
Materials and Methods: Hydrashift 2/4 daratumumab (Sebia) is the only FDA-approved reagent for recognizing interference that can be performed on the HYDRASYS 2 semiautomated gel platform (Sebia).
This assay is based on the "daratumumab-specific immunofixation electrophoresis reflex assay" (DIRA), which uses an anti-daratumumumab antibody to form an immucomplex and thus displace the migration of daratumumab from the CM of the pc.
Results: with the DIRA-test, proper separation of the drug occurs, which is observed in the anodic position in the α region in agarose gels. The band shift allows us to check whether the original CM of the pc is still present, usually in the cathodic position in the β-γ region.
In serum, the drug is detectable up to concentrations of 0.1 g/L.
Conclusions: The DIRA test proves to be effective but needs close collaboration with the clinician, who must inform the laboratory so that appropriate diagnostic testing is done to avoid false positives. The test is specific only for daratumumab, and new tmAbs have been evaluated in recent years so more assays will be needed to evaluate the presence of these drugs.

EP077

Hb Alessandria a Novel Variant of β-globin Chains Detected by Capillary Electrophoresis

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HbA1c biochemical marker routinely used in the management of diabetes mellitus may also give the possibility of detecting asymptomatic or silent Hb variants that would otherwise not be found. A 68-year-old woman had come to the laboratory to measure of HbA1c. Peripheral blood samples were collected in tubes with K3EDTA for measurement of Hb A1c and erythrocyte parameters and then for genetic DNA analysis. A blood sample was also collected to assess oxygen affinity. We initially measured Hb A1c by capillary electrophoresis (CE) (Capillarys 3 Tera with Capi3 HbA1c kit; Sebia). The system detected the presence of an Hb variant, then failed to release a calculated Hb A1c value and reported the presence of an "atypical profile". Next, with the intention of obtaining a measured value of Hb A1c, we used high-performance liquid chromatography (HPLC) with the VARIANT IITM Dual Kit program (Bio-Rad Laboratories). In this case, the instrument indicated an Hb A1c measurement but showed no Hb variant. To confirm the presence of Hb variant, we also performed analysis by CE (Capillarys 3 Tera with Hemoglobin(e) kit). Abnormal Hb was observed in "zone (F)," well separated from Hb A. Erythrocyte parameters were measured using an hematology analyzer (ADVIA 2120 Hematology System; Siemens): the patient appeared to be clinically normal without erythrocytosis or other hematologic abnormalities. A reduced p50 for oxygen corresponding to 23.9 mmHg (n.r.: 25.5-30.8) was measured in the patient using an ABL90 FlexPlus blood gas analyzer (Radiometer Medical). The result obtained thus showed a slightly decreased affinity of hemoglobin for O2. Stability test at 37°C to isopropyl alcohol was normal. Sequencing of the beta-globin gene revealed a heterozygous missense mutation of codon 37 (TGG>TTG). This new variant was called Hb Alessandria and included in HbVar with 3267 as id number.The importance of using CE or HPLC methods in the diagnosis and monitoring of diabetes mellitus in populations with a high prevalence of Hb defects is confirmed by the occasional finding of this new variant. Moreover the high heterogeneity of globin defects may require examinations with instruments based on different separative principles in the search for greater accuracy, sensitivity, and specificity.

EP078

Are polymorphisms of ACE1, ACE2, IFTM3 and TMPRSS2 genes associated with susceptibility and severity of SARS-CoV-2 infection? A systematic review and meta-analysis

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Background:Some human polymorphisms of angiotensin-converting enzyme 1 (ACE1), ACE2, Type 2 transmembrane serine proteases (TMPRSS2) and interferon-induced transmembrane protein 3 (IFITM3) genes may have an effect on the susceptibility to SARS-CoV-2 infection and increase the risk to develop severe COVID-19.We conducted a systematic review of current evidence to investigate the association of genetic variants of these genes with the susceptibility to virus infection and patient prognosis.
Methods:We systematically searched Medline, Embase and The Cochrane Library for articles published until May 2022, and included observational studies covering genetic association of ACE1, ACE2, TMPRSS2 and IFITM3 with COVID-19 susceptibility or prognosis.The study selection and review process were performed by 2 authors independently.We pooled data as convenient in meta-analyses using the Mantel–Haenszel Method (RevMan5.4).Odds ratio(OR) values and 95% confidence intervals(CI) were calculated. Heterogeneity among studies was tested using I^2 statistic tests. In presence of substantial heterogeneity (I^2>50%),the random-effects model was used to analyse the data.
Results:The literature search identified 1,968 references.Thirty-three studies (19 on ACE1, 7 on ACE2, 5 on TMPRSS2 and 5 on IFITM3),enrolling 4,067 COVID-19 patients, met the inclusion criteria and were included. ACE1 rs4646994 and rs1799752, ACE2 rs2285666, TMPRSS2 rs12329760 and IFTM3 rs12252 were identifies as common polymorphisms.Our meta-analyses showed an association between genetic polymorphisms and susceptibility to SARS-CoV-2 infection for IFTM3 rs12252 TT (OR 0.56; 95%CI 0.39-0.79, p<0.0001, I2=0%) and CT (OR 1.64;95%CI 1.15-2.33, p<0.0001, I2=0%) genotypes and for ACE1 rs4646994 DI (OR 0.57;95%CI 0.34-0.96; p=0.03, I2=.92%).Likewise, meta-analyses uncovered that both ACE1 rs4646994 DD (OR 1.26;95%CI 1.03-1.53, p=0.02, I2=48%) and rs12252 IFITM3 CC (OR 2.26;95%CI 1.05-4.89, p=0.04, I2=0%) genotypes carriers had a significantly increased risk of developing severe COVID-19.
Discussion:These results provide a critical evaluation of genetic polymorphisms as predictors in SARS-CoV-2 infection.ACE1 DD and IFITM3 CC polymorphisms would lead to a genetic predisposition for severe lung injury in patients with COVID-19.

EP079

VERIFICA DELLA PRECISIONE DEI GAS EMATICI pH, pO2 e pCO2 SUGLI EMOGASANALIZZATORI RAPIDPOINT500 (SIEMENS HEALTHCARE) NEL LABORATORIO SMEL2 DELL'ASST PAPA GIOVANNI XXIII DI BERGAMO ATTRAVERSO I CONTROLLI DI QUALITA'

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Il Laboratorio SMeL2 dell'ASST Papa Giovanni XXIII di Bergamo, certificato ISO 9001:2015, ha verificato la precisione (protocollo CLSI EP 15-A3) di pH, pCO2 e pO2 sui due emogasanalizzatori RAPIDPoint500 (Siemens Healthcare; M1 e M2) presenti, collegati al software RAPIDComm 7.0 necessario per la gestione remota del POC Manager, usando Controlli di Qualità (CQI) del fornitore su tre Livelli (L1, L2, L3). Sono stati analizzati 5 replicati di ciascun Livello di CQI per 5 giorni (06-10/06/2022), una volta al giorno, elaborati dal software MedComp 1.0. La ripetibilità totale (DS Laboratorio=DSL) è risultata inferiore al Valore di Verifica (VV) ottenuto sulla base della ripetibilità dichiarata dal produttore per ciascun analita. Per M1: a) pH L1, DSL=0,002 e VV=0,008; b) pH L2, DSL=0,002 e VV=0,007; c) pH L3, DSL=0,002 e VV=0,006; d) pCO2 L1, DSL=0,836 e VV=9,386; e) pCO2 L2, DSL=0,568 e VV=6,030; f) pCO2 L3, DSL=0,670 e VV=2,768; g) pO2 L1, DSL=0,686 e VV=4,185; h) pO2 L2, DSL=1,055 e VV=3,711; i) pO2 L3, DSL=1,340 e VV=3,751. Per M2: l) pH L1, DSL=0,003 e VV=0,010; m) pH L2, DSL=0,004 e VV=0,008; n) pH L3, DSL=0,004 e VV=0,006; o) pCO2 L1, DSL=2,251 e VV=10,035; p) pCO2 L2, DSL=1,248 e VV=6,549; q) pCO2 L3, DSL=0,649 e VV=2,654; r) pO2 L1, DSL=0,783 e VV=4,105; s) pO2 L2, DSL=0,584 e VV=3,563; t) pO2 L3, DSL=0,927 e VV=3,696. Il test di Grubbs ha verificato l'assenza di dati estremi. Inoltre, in base al database di Westgard i traguardi per pCO2 sono ottimali se CVa (CV Analitico)=1,20, desiderabili se è 2,40 e minimi se è 3,60, quindi deriva che: in M1, CVa=1,3 per L1 e 1,8 per L2, quindi desiderabile, mentre è CVa=3,5 per L3, quindi minimo; in M2, il CVa è 2,6 per L1, 3,4 per L2 e 3,2 per L3, quindi minimi. Dal medesimo database, i traguardi per pH sono ottimali se CVa=0,88 e si rileva che: in M1, CVa=0,0 sempre, quindi ottimale; in M2 0,07<CVa<0,09, ottimale. Inoltre il Laboratorio usa da tre mesi i CQI Liquichek Blood Gas Plus EGL (Bio-Rad) con Unity Real Time e il CV cumulativo per pCO2 in M1 è: CVa=4,17 per L1, minimo, mentre è 2,56 per L2, desiderabile; in M2, CVa è desiderabile in L1 (2,99) e L2 (2,87). Per pH, in M1 CVa=0,09 in L1 e L2; in M2 è 0,08 in L1 e 0,07 in L2, quindi sempre ottimali. Pertanto, la valutazione delle prestazioni è soddisfacente.

EP080

VERIFICA DELLA PRECISIONE DEL GLUCOMETRO "MASTER" ACCU-CHEK INFORM II (ROCHE) NEL LABORATORIO SMEL2 DELL'ASST PAPA GIOVANNI XXIII DI BERGAMO ATTRAVERSO CONTROLLI DI QUALITA' DI PARTE TERZA

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UOC SMeL2 Analisi Chimico Cliniche ASST Papa Giovanni XXIII, Bergamo (BG)

Il Laboratorio SMeL2 dell'ASST Papa Giovanni XXIII di Bergamo, certificato ISO 9001:2015, ha glucometri Accu-Chek Inform II (Roche), che sfruttano la Glucosio Deidrogenasi con aspirazione capillare frontale del campione, hanno intervallo di misura 10–600 mg/dL, due Livelli di Controllo liquidi (CQI), Strisce di misura Plasma calibrate esenti da interferenza da maltosio, compensano l'ematocrito nell'intervallo 10-65%, richiedono 0,6 μl di campione (capillare, venoso, arterioso, neonatale) e un tempo di misura di 5 secondi. In Laboratorio c'è un glucometro "Master" configurato per garantire il passaggio d'informazioni wi-fi tra i glucometri aziendali ed il software-POC dedicato Cobas IT1000 (Roche), necessario per la connettività e la gestione remota del POC Manager. La precisione dichiarata dal fornitore è già stata verificata usando le soluzioni acquose dei CQI sui Livelli Low (30-60 mg/dL) e High (261-353 mg/dL) (protocollo CLSI EP 15-A3), ma è stata ripetuta usando i CQI Meter Trax Control (Bio-Rad) in uso da quest'anno per i Livelli Low (40-106 mg/dL) e Medium (120-232 mg/dL), stabili 31 giorni a 2-25°C dall'apertura, gestiti dal software Unity Real Time. Il Fornitore definisce accettabile la performance (ISO 15197:2013) per concentrazioni di glucosio: pari o inferiori a 100 mg/dL, una Deviazione Standard (DS) pari o inferiore a 5 mg/dL; superiori a 100 mg/dL, un Coefficiente di Variazione (CV) pari o inferiore al 5%. Sono stati analizzati 5 replicati di ciascun Livello di CQI per 5 giorni (13-17/06/2022), una volta al giorno e i risultati sono stati elaborati dal software MedComp 1.0. La ripetibilità totale è risultata inferiore al Valore di Verifica (VV) ottenuto sulla base della ripetibilità dichiarata dal produttore: per il CQI Low, DS Laboratorio=1,383 verso VV=6,721; per il CQI Medium, DS Laboratorio=3,450 verso VV=11,157. Il test di Grubbs ha verificato l'assenza di dati estremi per il CQI Low ma non per il Medium: però, poiché la ripetibilità ottenuta sperimentalmente è compatibile con quella dichiarata dal produttore, non è stato necessario ripetere quest'ultima valutazione. Inoltre, il CVa (CV Analitico) delle serie è 1,96 per CQI Low e 2,10 per CQI Medium, incontrando il traguardo di qualità Desiderabile (2.8) dal database di Westgard.

EP081

A novel GCK Large Genomic Rearrangement (LGR) in a Ukrainian patient with Maturity-Onset Diabetes of the Young type 2 (MODY2) detected by Clinical Exome Sequencing (CES)

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Background: Maturity-onset diabetes of the young (MODY; OMIM # 606391) is a rare form of diabetes, non-autoimmune and non-insulin dependency, with autosomal dominant inheritance, typically diagnosed before 25 years of age. To date, pathogenic variants in 14 genes have been reported to cause the MODY phenotype. GCK (NG_00847.2), encoding the glucokinase enzyme, was the first MODY gene to be identified. Heterozygous inactivating variants in this gene cause a subtype of MODY, subtype glucokinase (GCK-MODY), an autosomal dominant mild fasting hyperglycemia. This subtype is present at birth but is often subclinical and only detected later in life (MODY2; OMIM#125851). To date, more of 620 pathogenic GCK variants, distributed throughout the whole gene sequence, have been reported. Partial or whole gene deletions have been identified in a small number of MODY2 patients and have been shown to be a rare cause of GCK-MODY in different populations. Aim: To report the result of the molecular analysis performed in a Ukrainian patient with clinical diagnosis of MODY. Methods: The clinical exome was captured with the Clinical Exome Solution®# kit (SOPHiA Genetics) and the sequencing performed with the NextSeq 500 system (Illumina). We designed a virtual panel of 14 genes out of those included in the capture kit and associated with MODY. Bioinformatics analysis for the detection of single-nucleotide variants (SNVs), small insertions/deletions (indels) and Copy Number Variation (CNVs) was performed using the SOPHiA DDM platform (SOPHiA Genetics). The SALSA MLPA kit for MODY (P241-E1 MODY Mix 1; MRC-Holland, Amsterdam, The Netherlands) was used for relative quantification of all GCK exons. Results: No pathogenic sequence variants were detected in all 14 genes; however, NGS CNV analysis was able to identify a large deletion involving the last three exons of the GCK gene. This result was confirmed by MLPA technique. Conclusion: At the best of our knowledge, the novel rearrangement, associated to a clinical condition of MODY2, has never been reported before. In addition, our result show that the NGS presents a comprehensive approach for analysing patients with suspected MODY and provided a successful differential diagnosis of MODY subtypes.

EP082

EVALUATION OF THE ANALYTICAL PERFORMANCES OF THE NEW DIASYS PROCALCITONIN ASSAY ON THE ROCHE COBAS ANALYZER

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Background and aim. Procalcitonin (PCT) is a biomarker widely used to detect sepsis and for antibiotic stewardship. In this study, we evaluated the analytical performances of the new DiaSys PCT method on Roche Cobas c702 analyzer (PCT-DS) and compared it with the Elecsys BRAHMS PCT (PCT-EL) on Roche Cobas e801 and with BRAHMS PCT-sensitive Kryptor (PCT-BK).
Methods. The new DiaSys PCT-DS (DiaSys Diagnostic Systems GmbH, Germany) method is a particle enhanced immunoturbidimetric test, with a minimum reportable result of 0.16 μg/L. Linearity was tested according to CLSI EP06-A (2 curves, 0-30 μg/L and 0-150 μg/L, 11 solutions/curve, 3 replicates, single run); LOD and LOQ were checked according to CLSI EP17 A2 (10 pools, 2 replicates, 5 days); imprecision was assessed following CLSI EP05-A3 (6 concentrations, 20 days, 2 runs, 2 replicates) and method comparison was performed on 152 serum samples.
Results. Linearity (goal <5%) was verified up to 80 μg/L. LOD and LOQ were 0.18 and 0.20 μg/L. Repeatability and within-laboratory imprecisions ranged, respectively, from 1.95% (at 9.7 μg/L) to 8.60% (at 0.34 μg/L) and from 6.29% (at 12.7 μg/L) to 12.48% (at 9.7 μg/L). For the method comparison study we obtained PCT-BK (x) vs PCT-DS (y): slope 1.02 (95%CI 0.97-1.07), intercept 0.08 (95%CI 0.06-0.11); PCT-EL (x) vs PCT-DS (y): slope 1.20 (95%CI 1.14-1.25), intercept 0.08 (95%CI 0.05-0.11); PCT-BK (x) vs PCT-EL (y): slope 0.83 (95%CI 0.81-0.86), intercept 0.01 (95%CI 0.00-0.02). In the range 0-2.0 μg/L Bland-Altman analysis has shown the following biases: PCT-BK vs PCT-DS -31% (95%CI -41% to -23%), PCT-EL vs PCT-DS -47% (95%CI -55% to -40%) and PCT-BK vs PCT-EL 18% (95%CI 13% to 22%). Using cut-offs at 0.25 and 0.5 μg/L, the overall agreements between PCT-BK and PCT-DS were 133/152 (Cohen's k=0.58, 95%CI 0.40-0.72) and 140/152 (0.84, 95%CI 0.75-0.93), between PCT-EL and PCT-DS 127/152 (0.50, 95%CI 0.33-0.65) and 139/152 (0.83, 95%CI 0.74-0.92), between PCT-BK and PCT-EL 146/152 (0.90, 95%CI 0.81-0.97) and 147/152 (0.93, 95%CI 0.88-0.99).
Conclusions. The new DiaSys PCT method showed good analytical performances and good overall agreement with other PCT methods investigated. Agreement with PCT-EL and PCT-BK was lower for PCT levels <0.5 μg/L.

EP083

Appropriatezza diagnostica in neonatologia: emoglobina glicata o albumina glicata?

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Alla nascita, l'emoglobina fetale (HbF) costituisce circa il 70-90% dell'emoglobina totale presente nei globuli rossi. La sintesi di HbF continua dopo la nascita ma diminuisce gradualmente sino a costituire meno dell'8% di tutta l'emoglobina a sei mesi di vita. Una bambina di un mese di vita, nata da madre con diabete gestazionale, giunge nella nostra struttura per la valutazione della concentrazione di emoglobina glicata (HbA1c). La determinazione di HbA1C deve essere valutata con cautela in tutte le situazioni in cui la sopravvivenza degli eritrociti è ridotta. Questa condizione si verifica non solo in emoglobinopatie, anemie, emorragie, trasfusioni, splenectomia ma anche in neonati in cui l'età media degli eritrociti è inferiore a tre mesi e il passaggio da HbF ad HbA non è ancora giunto al naturale completamento. La quantificazione di HbA1c, eseguita con il metodo della cromatografia liquida ad alta prestazione (HPLC) a scambio ionico (Bio-Rad VARIANT™ II HbA2 /HbA1c Dual Program) ha prodotto un tracciato anomalo: assenza delle frazioni HbA1c e HbF e presenza della frazione HbA1c labile (La1c) con un valore del 55.0%. La determinazione dell'assetto emoglobinico in elettroforesi capillare (Capillarys 3 TERA, Sebia-Lisses, France) ha evidenziato nel tracciato la presenza di HbA (31%), HbF (68.6%) e HbA2 (0.4%), in percentuali coerenti con l'età della bambina. La presenza in elevata concentrazione di HbF e LA1c non ha permesso, pertanto, di ottenere, con la strumentazione presente in laboratorio, la quantificazione di HbA1c. La valutazione dell'appropriatezza degli esami richiesti e la ricerca del test maggiormente efficace per rispondere al quesito del clinico è uno dei compiti primari della medicina di laboratorio.Il suggerimento al clinico, in questo caso, è stato quello di richiedere il dosaggio di albumina glicata (GA), test che fornisce una indicazione dei livelli glicemici nel corso dei 20-30 giorni precedenti al prelievo, età della neonata.

EP084

Could mutations of Insulin Receptor gene be associated with severe obesity?

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Insulin resistance (IR) syndrome is a rare disorder characterized by the inability of insulin to stimulate glucose disposal. The major cause is a combination of genetic predisposition and environmental factors. Insulin receptor (INSR) gene mutations constitute a heterogeneous and less common cause of IR. Over 150 INSR genetic variants have been identified resulting in a spectrum of insulin resistance syndromes of various severity including: Donohue syndrome, Rabson-Mendenhall Syndrome and type A insulin resistance syndrome.
We report a case of familiar insulin resistance type A. The proband, second of five siblings, with a strong familiar history for obesity and type 2 diabetes, came to our attention at the age of 16 for a severe obesity and extremely high levels of insulin (801.6 mcU/l). She started a metformin therapy with scarce success on IR and weight control. A more detailed clinical evaluation revealed hypertension, polycystic ovarian syndrome, acanthosis nigricans, signs of hyperandrogenism, hypertrichosis and acne. Considering the severe phenotype and the extremely high levels of insulin, to rule out the presence of IR syndromes, we searched for mutation involving INSR gene using Next Generation Sequencing. The patient resulted heterozygous for the variant in exon 13 (c.2682+1G>A) of INSR. The analysis was extended to all members of her family, obese and normo-weight, and it resulted present only in proband's mother and in two brothers, all obese. This result was unexpected because most cases of type A IR present a lean phenotype and the obesity is usually considered an exclusion criterion to investigate INSR mutations. Although most cases of IR due to mutations in the INSR hesitate in impaired glucose metabolism, it is important to screen these patients for diabetes and prediabetes and, in case of negative screening, to establish a follow up.
In conclusion, even if Insulin receptor signaling defects play a minor role in the etiology of IR, they should always be considered in the diagnostic flowchart also in obese patients. In our case the correct diagnosis has been fundamental for the young patient both to identify a tailored therapy, changing the unsuccessful metformin therapy with Lyraglutide, and also to identify other mutated family members.

EP085

New systemic immune inflammation indexes associated with overall survival in glioblastoma

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Background. The Systemic immunity and inflammation indexes SI derived from blood cells may potentially integrate the impact of biomarkers derived from tumor tissue, gaining increasing attention in the field of clinical oncology.

Materials and methods. Glioblastoma (GBM) is the most common and most aggressive malignant primary brain tumor in adults, resulting in dire prognoses and mortality rates overlapping the incidence. So we tested 12 different SI using blood tests from patients with isocitrate dehydrogenase 1 and 2 wild-type glioblastomas, treated with radio-chemotherapy.Hematological parameters (the total white cell count W, neutrophils N, lymphocytes L, monocytes M and platelets P count) were obtained using a Sysmex XE-2100 (Sysmex, Kobe, Japan) automated blood analyzer and the related reagents, used strictly in accordance with the manufacturer's instructions.We tested 2 old (1-2) and 10 new SI:
1) SII (Systemic immune-inflammation index) = N × P/L
2) PLR (Platelet-lymphocyte ratio)= P/L
3) PW/L = P × W/L
4) NPW/L = N × P × W/L
5) NPM/L = N × P × M/L
6) NPMW/L = N × P × M × W/L
7) NPM/LW = N × P × M/(L × W)
8) NP/LM = N × P/(L × M)
9) NP/(L + M) = N × P/(L + M)
10) NPW/LM = N × P × W/(L × M)
11) NP/WLM = N × P/(W × (L + M))
12) NPW/(L + M) = N × P × W/(L + M)

Results. A total of 77 patients, comprising 43 males and 34 females, with a median age of 64 years (age range 26–84), who were treated between October 2010 and July 2020, were included in the present analysis (approved by a local ethics committee). The primary endpoint was their overall survival. In the univariate Cox regression analysis, all the indexes except two (3-12) showed a statistically significant impact on Overall Survival. In the multivariate Cox regression analysis, NPW/LM and NPM/L maintained their statistically significant impact value.

Conclusions. This univariate analysis confirms the potential of systemic inflammation indexes in patients with glioblastoma, while the multivariate analysis verifies the prognostic value of NPW/LM and NPM/L.

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EP086

Comparing volumetric absorptive microsampling Mitra® with dried plasma spot for quantification of triazoles in plasma

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Background & Aims: Recently, growing attention has emerged for micropsampling techniques as alternative to dried blood samples (DBS) for therapeutic drug monitoring (TDM). Volumetric Absorptive Microsampling (VAMS), called Mitra® (Neoteryx), are devices consisting of porous absorbent tips that allow collection of few blood volumes (# 10 µl) overcoming the hematocrit effect (1). So far, Mitra® have been used in several pharmacokinetic studies (2-5). Here, we have compared plasma concentrations of three antifungal agents (Voriconazole, Posaconazole and Isavuconazole) to those obtained with dried plasma spot (DPS) and Mitra® sampling. Methods: 50 samples of plasma, DPS and Mitra® were obtained from pediatric patients hospitalized in our Centre. Mitra® were collected by dipping tips into whole blood before centrifugation for plasma recovery. Antifungal concentrations were measured using a validated LC-MS/MS kit (MassTox® Antimycotic Drugs/EXTENDED) provided by Chromsystems (Chromsystems Instruments & Chemicals). Results: Positive correlation was found for Posaconazole plasma vs DPS (Spearman r=0.80, p<0.001). Bland-Altman test gave a bias of 0.13. A similar result was obtained for Posaconazole plasma vs Mitra® (Spearman r=0.83, p<0.001) while bias was -0.22. Correlation of Voriconazole plasma vs DPS produced a Spearman r of 0.94 (p<0.001) and a bias of 0.15. A positive correlation was observed for Voriconazole plasma vs Mitra® (r=0.92, p<0.001) with a bias of -0.36. Isavuconazole correlation of DPS vs plasma and plasma vs Mitra® revealed a Spearman r= 0.93 (p<0.001) and r= 0.88 (p<0.001), respectively. A bias of -0.33 and -0.16 were observed for DPS and Mitra®, respectively. Conclusions: Our results demonstrate that DPS and Mitra® could be used as alternative to conventional blood sampling for TDM of antifungals. Mitra® do not require plasma recovery and are suitable for pediatric and neonatal patients for whom ethical and physiological concerns limit the collection of large blood volumes. Moreover, as soon collected, Mitra® can be stored and shipped to bioanalytical laboratory facilitating remote TDM. However, in the next future a further validation of Mitra® results will be needed by measuring drugs' levels after heel or finger prick blood sampling. REFERENCES:1) P. Denniff, N. Spooner, Volumetric absorptive microsampling: a driedsample collection technique for quantitative bioanalysis, Anal. Chem. 86(2014) 8489–8495, http://dx.doi.org/10.1021/ac5022562.2) Sciberras D, Otoul C, Lurquin F, Smeraglia J, Lappert A, De Bruyn S, Jaap van Lier J. A pharmacokinetic study of radiprodil oral suspension in healthy adults comparing conventional venous blood sampling with two microsampling techniques. Pharmacol Res Perspect. 2019 Jan 28; 7(1):e00459. doi:

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EP087

The importance of measuring free digoxin serum levels after digoxin poisoning

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Background & Aims: Digoxin is a cardioactive drug with a narrow therapeutic window. Pediatric digoxin poisoning can result from accidental intake, renal impairment and reduced drug metabolism. Severe intoxication is presented as dangerous dysarrhythmias and requires prompt medical intervention. First line of treatment is based on intravenous administration of digoxin-specific antibody fragments (e.g. DigiFab®) according to digoxin levels at the steady state or to the amount of digoxin ingested. Here, we present a Case of digoxin intoxication highlighting the importance of measuring serum levels of free digoxin in order to facilitate therapeutic management.
Methods: A 54-days-old male affected by paroxysmal supraventricular tachycardia (SVT), was treated with atrial stimulation, amiodarone, propranolol and digoxin per os (Lanoxin®). Serum levels of digoxin were measured using an electrochemiluminescence immunoassay (Elecsys Digoxin, Roche Diagnostics). In order to separate and measure free digoxin fraction, serum samples were ultra-filtrated using Centrifree® devices (Merck Millipore) (1).
Results: During maintenance therapy, an incorrect oral administration of digoxin (500 mcg) caused bradycardia, hyperkalemia (6.65 mEq) and hypermagnesemia (2.25 mEq). Analysis of total digoxin levels was performed one hour later and revealed a concentration of 21.1 ng/mL (therapeutic range 0.6-1.2 ng/mL, laboratory alert > 2 ng/mL), therefore a vial of 40 mg of digoxin-specific antidote was quickly administered. After 12 hours, concentrations of total and free digoxin were 14.90 and 0.66 ng/mL, respectively. In the following days, levels were observed in the therapeutic range and no further administration of DigiFab was required.
Conclusion: In this Case report, measurement of free digoxin levels did not change the therapeutic approach, however, we believe that measuring unbound digoxin could be helpful not only in presence of renal impairment or concomitant medications that can affect digoxin pharmacokinetic, but also to guarantee an appropriate antidotic treatment. Moreover, determination of free digoxin fraction ensures that plasma concentrations of pharmacologically active drug will fall within the therapeutic range in patients with an ongoing SVT medical condition.
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EP088

Implementation of rotational thromboelastometry in patients undergoing cardiac surgery: health outcomes.

Laboratorio de Bioquímica Clínica

Background: Perioperative coagulopathy and postoperative bleeding are themost common complications in patients undergoing cardiac surgery, especially when the cardiovascular surgery is associated with cardiopulmonary bypass (CPB).In this context, some studies suggest that implementation of viscoelastic point-of-care tests (POCT), such as rotational thromboelastometry (ROTEM , in conjunction with a specific algorithm for coagulation management, allow for better control of hemostatic pathology.
Methods: Retrospective cohort study including 675 patients who underwent cardiac surgery with cardiopulmonary bypass. The incidence of clinical postoperative complications were analyzed before and after ROTEM® implementation.
Results: Following viscoelastic testing and the implementation of a specific algorithm for coagulation management, the incidence of any allogeneic blood transfusion decreased (41.4% vs 31.9%, p=0.026) during the perioperative and postoperative period (26.5% vs 19.2%, p=0.061).In addition, significant reductions were detected in the incidence of heart disease (57.7% vs 55.8%, p=0.275; statistically significant reductions were detected in the incidenceof postoperative pericarditis (3.6% vs 1.2%, p=0.043), postoperative renal failure (1.6% vs 3.2%, p=0.435), postoperative sepsis (1.2% vs 0.9%), p=0.337) and postoperative hematologic complications
(postoperative bleeding (9.5% vs 5.3%, p=0.037), surgical reexploration (6.0% vs 2.9%, p=0.035).and length of Intensive Care Unit (ICU) stay (6.0 days vs 5.3 days, p=0.026). Finally, we observed a statistically significant decrease in the lengths of Intensive Care Unit (ICU) stay (6.0 days vs 5.1 days, p=0.026), after implementation of the POCT system and the specific algorithm for coagulation management. There were no statistically significant group differences with respect to total hospital stay (16.7 days vs 13.5 days, p=0.076). In-hospital mortality associated with cardiac surgery also did not change (4.5% vs 2.4%, p=0.132).
Conclusion: The monitoring of hemostasis by ROTEM® in cardiac surgery, was associated with decreased incidence of allogeneic blood transfusion, clinical postoperative complications and lengths of hospital and ICU stay.

EP089

Comparison the efficacy of manual and ultrasonic ablators system instrumentation for the periodontal treatment through Infrared-ATR Spectroscopy.

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Introduction Periodontitis is a multifactorial disease associated with dysbiotic plaque biofilms that affects the supporting tissues of the teeth. The gold standard for the treatment of inflammatory periodontitis is hand instrumentation with curettes, alternatively can be used ultrasonic ablators systems. The purpose of this study is to compare therapeutic effectiveness of the ultrasonic versus manual instrumentation. Correlations were effectuated between infrared spectra of gingival crevicular fluid (GCF) and clinical parameters, before and after treatment. Material and Methods Three absorbent paper cones were used to collect fluid without blood and saliva contamination from 16 patients. The perio-paper strip was then placed into a sterile 1.5 mL eppendorf containing 40 µL extracting solution (CH3OH/ H2O – 50/50). The tube was vortexed for 30 s and centrifuged (15 min, 1500 gr). The infrared spectroscopic analysis was performed with Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) technique. 5 µL of sample was pipetted onto diamond crystal of ATR accessory and sample was dried using a slow nitrogen flow. Univariate analysis (Student t-test) was performed to compare the difference in the relative intensities of specific bands to identify significant results. Results and Discussion An exploratory analysis was performed to identify potential significant spectral differences in the “fingerprint area” (1800–900 cm-1). The absorbance of spectra related to vibrations of functional groups of nucleic acids (1240, 1087 cm-1 PO2- symmetric and asymmetric stretch) and protein (1542 cm-1) before and after treatment are statistically significant ($p < 0.05$). We can see that gingival crevicular fluid DNA and protein concentrations a decreased in T1 group. The mean gingival crevicular fluid spectra of the manual instrumentation and ultrasonic ablation system groups after clinic intervention appears similar. This result suggests a decrease in inflammation confirming a good therapeutic efficacy with both clinical tools. Spectral analysis could be used for the evaluation of therapy by monitoring specific bands for the assessment of the inflammatory state of periodontal pockets.

EP090

Evidence of the presence of antinuclear antibodies (ANA) in healthcare workers after mRNA based anti-SARS CoV-2 vaccines

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Introduction: Evidence from clinical trials strongly supports the safety and efficacy of the different COVID-19 vaccines. Indeed, the risk to develop a severe form of the disease, possibly leading to death, it is highly decreased in fully vaccinated individuals. Nowadays, vaccines effects and their possible ability to stimulate an autoimmune reaction are still poorly understood. The aim of this study was to check the development and /or persistence of antinuclear antibodies (ANA) in healthcare workers (HCPs) after mRNA based anti-SARS CoV-2 vaccines.
Methods: In this study, 77 HCPs were considered (60 females and 17 males, age range 26-67 years, median age 48) without any history of COVID-19 infection. All the subjects were vaccinated with 2 doses of BioNtech/Pfizer BNT162b2 mRNA. Furthermore, half of them received a third dose of the same vaccine, whereas the other half of Moderna (Spikevax). Blood Samples were collected before the inoculation of the vaccine (T0), at 3 (T1) and 12 months (T2) after the first dose. Therefore, at T1 all the subjects received two doses of vaccine and at T2 three doses. ANA presence was evaluated using indirect immunofluorescence on Hep-2 cells (EUROIMMUN test kit) at dilutions: 1:80, 1:160, 1:320, 1:640. Fisher and Wilcoxon statistical tests were performed using GraphPad Prism 9 Software.
Results: Among 77 subjects enrolled, at T0 25 were positive for ANA (23 maintained this positivity also at T1 and T2) and 52 were negative. At T1, 46/52 remained negative, whereas 6/52 became ANA positive (5 maintained this positivity also at T2). At T2, 30/46 were still negative, instead 16/46 became ANA positive. In addition, from T1 to T2, it has been observed a statistically significant increase of ANA presence. Conclusion: Our results suggest that mRNA based anti-SARS CoV-2 vaccines seem to induce the onset of de novo ANA in 22/77 (28,57%) of subjects and that the percentage of positivity seems to directly correlate to the number of vaccine expositions: 6/77 (7,79%) after 2 doses; and 16/77 (20,78%) after 3 doses.

EP091

Validazione di un Metodo RUO per la determinazione degli anticorpi anti SARS-CoV-2 IgG N e S1-RBD in matrice salivare

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Introduzione. Dall'anno 2020 il mondo è stato costretto ad affrontare la prima pandemia dell'era contemporanea, ovvero l'infezione da SARS-CoV-2. A due anni di distanza dall'insorgenza, l'attenzione scientifica è ancora molto alta per l'elevato tasso di contagio dovuto al continuo mutare di questo virus. La determinazione degli anticorpi specifici (Ab) può essere utile in pazienti immunocompromessi e nei casi di MIS-C. Nei pazienti pediatrici in terapia immunomodulante, la determinazione degli Ab in matrice salivare può essere di aiuto nel monitoraggio del titolo Ab post-vaccinazione ed, eventualmente, nella scelta delle strategie vaccinali. Scopo dello Studio. Validare un metodo commerciale ELISA RUO (RayBio COVID19 N e S1 RBD) per la determinazione degli Ab anti SARS-CoV-2 IgG N e S1-RBD in matrice salivare. Materiali e Metodi. La saliva di 47 pazienti pediatrici è stata raccolta utilizzando Salivette (ditta SARSTEDT ref. 51.1534), e in seguito centrifugata entro 3 ore dalla raccolta a 4000 giri per 5 minuti. Le prove di precisione sono state eseguite su 6 campioni ripetuti in quadruplicato e le prove di linearità (diluizioni seriali intero, 1/2,1/4,1/8,1/16,1/32,1/64) in duplicato. I risultati degli Ab salivari sono stati confrontati con gli Ab anti SARS-CoV-2 IgG S-RBD eseguiti su Maglumi 2000 Plus (SNIBE, Shenzen,Cina). Risultati. La ripetibilità, espressa come coefficiente di variazione (CV) varia dal 20.0% (ad un livello 4.26 kAU/L ± 0.84 kAU/ L) al 3.8% (ad un livello di 2.33 kAU/L ± 0.088 kAU/L).

I test di diluizione eseguiti evidenziano una linearità, seppur si discostano dal valore atteso per un range che va da +66% a – 9.9%. La correlazione tra IgG salivari e sieriche è risultata soddisfacente (Spearman $r = 0.979$, $p < 0.001$), sebbene tra i due metodi sembra essere presente una differenza di un fattore 100. Conclusioni. Il sistema per la determinazione degli Ab salivari testato presenta buone caratteristiche di precisione, mentre la linearità è meno soddisfacente soprattutto a valori di Ab elevati. Ulteriori indagini sono in corso per verificare la cinetica post-vaccinazione e post-infezione degli Ab salivari.

EP092

Surfactant protein D (SP-D) as biomarker of SARS-COV-2 infection

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Beside lowering the surface tension at air-liquid interface in the alveoli, the pulmonary surfactant has a pivotal role in triggering the elimination of pathogens or any hazardous materials introduced with breathing. Among the components of the pulmonary surfactant, surfactant protein-D (SP-D) is a low abundant (0.6%) hydrophilic protein that is able to promote pathogens clearance binding highly conserved glycosidic residues on their surface. SP-D also cooperates in the maintenance of lung homeostasis by directly modulating immune system activity. Previous investigations on acute respiratory distress syndrome (ARDS) patients demonstrated a significant increment of SP-D serum level compared with healthy donors. Since in physiological condition SP-D is not permeable to alveoli-capillary membrane and poorly express by other tissues, this enhancement is likely due to an impairment of the pulmonary barrier caused by prolonged inflammation. In view of the above, the present work aims to investigate SP-D as diagnostic and/or prognostic marker for COVID-19. In particular, a retrospective study on a relatively large cohort of patients of Hospital Pio XI of Desio (i.e., 79 mild cases plus 123 severe cases) was conducted to assess differences of the hematic levels of this biomarker among COVID-19 patients and healthy donors and if SP-D serum levels resulted a risk factor for disease severity and mortality. The performed analyses, using an Anova-Mixed model, showed a significant difference in the mean of log SP-D between COVID-19 patients and healthy donors: 150 ng/mL was identified as threshold value to best discriminate the mentioned groups. Significant differences were also found between dead vs survived patients, as well among severe vs non-severe cases. In all cases, SP-D serum levels presented significantly higher values for COVID-19 patients, dead and severe cases.Moreover, further analysis conducted with Logistic Mixed models, highlighted that SP-D, in a model with Age, C-reactive protein and cancer status, resulted the strongest significant risk factor of mortality (model predictive accuracy, AUC=0.826), and in a lesser extent for risk of severity.The overall data suggest that SP-D can be a predictive marker of COVID-19 disease and its outcome.

EP093

Next Generation Laboratories: Workflow Optimization.

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The optimization of the laboratory workflow, the strategic use of human resources and the reduction of analytical errors have always been major challenges for diagnostic laboratories. Since its inception, the introduction of automation in the laboratory has had the aim of responding to the increasingly evident need to reduce or, where possible, eliminate the multiple manual procedures connected to the execution of the analyzes. In the laboratory of Centro Medico Santagostino we used the Abbott GLP System. At the beginning of the day, the instruments connected to the automation chain are initialized. Maintenance, calibrations and checks are carried out so that they are ready to receive and massively process the samples. Samples placed in the Bulk Input Module are checked in, put into single-sample automatic cars, then carried to the centrifuges, and sorted when required. The operator can follow the path of the samples through 2 software, managed by the middleware (AMS): Track Workflow Manager (TWM), which calculates the route of the sample based on the order received from the middleware, and, Track Sample Management (TSM), which manages the cars according to the route. The samples sent to the instruments are analyzed and the results transmitted to the Laboratory Information System (LIS). The implementation of rules in the middleware allows the automatic verification of test results and the execution of reflex tests, rerun or any storage of samples not suitable for processing. At the end of the processing, the tubes are capped and stored, recording the position in the archive. Through the use of GLP we are able to manage about 600 samples per day including serological, hematological, molecular biological and microbiological investigations. The automation, well implemented with IT systems, allows adequate control of the process, facilitates the detection of critical issues, optimizes resources and improves the standardization of procedures and, through validation rules and algorithms supported by the middleware, allows for better reliability of the result. The reorganization of the processes based on the GLP system made it possible to optimize the activities of human resources in all sectors.

EP094

Two years of pandemic from SARS-Cov-2: role and opportunity of the Bergamo ASST Papa Giovanni XXIII research biobank

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BACKGROUND - AIM

The Biobank of ASST Papa Giovanni XXIII of Bergamo, established in 2014 and certified UNI EN ISO 9001:2015 is a non-profit service unit aimed at collecting, preparing and storing biological material. During the COVID-19 pandemic, the Biobank collected and stored biological samples from patients with SARS-Cov-2 to conduct and start research projects, collections that today represent one of the most important heritage available to the scientific community.

MATERIALS AND METHODS

The unit consists of three rooms with electronic access control: a room containing 32 mechanical freezers with the remote alarm control “Spylog”; a cryobank with 10 tanks of liquid nitrogen managed by the alarm system “Cryoability”; a laboratory for the treatment of pre-preservation of biological samples equipped with “Freezerworks” software that guarantees anonymization and traceability.

RESULTS

Since the beginning of the COVID-19 pandemic the Biobank has registered a significant increase in activities by carrying out the biobanking of 22012 samples from 8354 patients. Currently it has 13800 of serum/plasma aliquots; 4357 of whole blood aliquots; 2318 of respiratory samples (nasopharyngeal swabs and lavages/broncho-alveolar aspirates); 1247 aliquots of materials such as swabs, fluids, tissue and biopsies; 290 aliquots of other material (liquor, stool, urine, saliva, sputum, eluate);The samples, collected with the active participation of clinicians and researchers, have now been used to start several clinical, diagnostic and research projects.

CONCLUSIONS

Biological Banks represent an indispensable source of resources for diagnosis and research. Biological collections, today, increasingly need to be organized and structured according to common and shared rules, both at local and national as well as, above all, at international level. Building on these primary goals, the ASST Papa Giovanni XXIII Research Biobank during the Covid-19 pandemic period, continued to carry out its work unceasingly, collecting biological material according to established international criteria achieving the objective of recognition by the Italian Node of the European Research Infrastructure of Biobanks and Biomolecular Resources (BBMRI-ERIC).

EP095

Personalized therapy: the crucial role of the DPYD c.2194G>A (V732I) allele in the treatment of colorectal cancer patients candidates for treatment with fluoropyrimidines

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5-Fluorouracil (5FU) is a chemotherapeutic agent belonging to the class of antimetabolite drugs, which exert a toxic action causing death of neoplastic cells. 5FU is mostly used as a standard treatment for colorectal cancer; the development of toxicity phenomena was related to the partial or complete deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD), limiting factor of the catabolism of fluoropyrimidines. Only 3-5% of 5-FU is converted to an active metabolite, while 85% of the drug is inactivated by DPD to 5-fluoro-dihydrouracil (5-FDHU). A reduced enzymatic activity of the DPD can be the cause for the presence of adverse drug reactions and toxicity in the patient, with multiorgan involvement, which can sometimes lead to death. The variants of the DPYD gene recommended by the AIOM guidelines are:DPYD*2A (IVS14+1G>A, c.1905+1G>A); DPYD*13 (c.1679T>G); DPYD c.2846A>T, D949V; DPYD c.1236G>A (HapB3); DPYD c.2194G>A (V732I).Patients with complete DPD deficiency are at high risk of life-threatening or fatal toxicity and should not be treated with fluoropyrimidines, but this is a rare condition; while patients with partial deficiency should be treated with a reduced dose of the drug. Before starting treatment it's crucial to determine the genetic profile of the patients candidates to therapy with fluoropyrimidines.
In our cohort of the 300 samples analyzed by Real Time PCR, 225 (77%) are wild type, none are homozygous mutated. The heterozygosity found for each variant are: DPYD*2A (IVS14+1G>A, c.1905+1G>A): 2%DPYD*13 (c.1679T>G): 0.2%DPYD c.2846A>T: 1%DPYD c.1236G>A (HapB3): 3%DPYD c.2194G>A: 17%The mutated DPYD c.2194G>A (V732I) allele, in heterozygous form, is significantly represented in the population examined: considering the 15% reduction in drug administration imposed by this genotype, molecular profiling is essential before starting therapy with 5FU.

EP096

EARLY REPORT ON DIAGNOSTIC IMPLEMENTATION OF A FULLY AUTOMATED IMMUNOASSAY FOR PLASMA BIOMARKERS OF ALZHEIMER'S DISEASE

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Phosphorylated tau at threonine 181 (pTau), Amyloid-β1-42 (AB 1-42), Amyloid-β1-40 (AB 1-40) are established CSF biomarkers for Alzheimer's disease mirroring amyloid and tau pathology as identified by gold standard imaging techniques. Blood-based biomarkers gained from recent improvements in the analytical methods the perspective of an implementation in screening programs and serial monitoring of new therapies [1].
In this preliminary study 7 patients (4 M/3 F, aged 58-73) were enrolled from Regional Brain Aging Center of University of Padova. Each patient underwent lumbar puncture as part of a diagnostic workup for cognitive decline, including testing for Alzheimer's Disease CSF biomarkers.
pTau, AB 1-42 and AB 1-40 were tested in CSF with a fully automated electrochemiluminescent immunoassay (Lumipulse G1200 by Fujirebio, Japan) and in plasma with a research method by the same manufacturer, with measuring ranges: 0.05-60 ng/L pTau, 0.10-1000 ng/L AB 1-42, 0.10-5000 ng/L AB 1-40. Blood was collected in K2 EDTA tubes, centrifuged at 4000 rpm for 5 minutes within 3 hours from collection and tested either immediately or after storage at -80°C.
Correlation between plasma and CSF biomarkers was evaluated using Spearman coefficients (Analyse-it Software). Clinical agreement through diagnostic accuracy was defined with cut-offs of < 8.1 for AB 1-42/pTau ratio and < 0.069 for AB 1-42/1-40 ratio established for CSF testing at our laboratory and calculated as (true positives+true negatives)/patients*100.
Plasma concentrations were included in the measuring ranges for each assay. Spearman's coefficients highlight a significant correlation between plasma and CSF AB1-42/pTau ratio ($r = 0.83$, $p < 0.05$); for pTau, AB 1-42 and AB 1-42/1-40 ratio, the positive correlation did not reach statistical power ($r = 0.71$, 0.60 and 0.64 respectively). Diagnostic accuracy was 86% for plasma AB1-42/pTau ratio and 71% for AB 1-42/1-40 ratio.
These preliminary results reinforce the potential of plasma biomarkers and need further confirmation to accomplish diagnostic implementation.
[1] Teunissen, C. et al., 2022. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. Lancet Neurol, Jan, 21(1), pp. 66-77.

EP097

APPLICATION OF ANA REFLEX ALGORITHM IN ROUTINE CLINICAL PRACTICE: OUR EXPERIENCE AND RESULTS

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Background and aim.The introduction in clinical practice of Reflex test for the detection of anti-nuclear antibodies (ANA), the ANA-Reflex, has proven to be a good strategy to improve efficacy in the diagnosis of autoimmune rheumatic diseases. Accordingly, in presence of positive ANA, it is advisable to perform test for antibodies against the extractable nuclear antigens (ENA). However, ANA-Reflex is not yet juridically implemented in all regions of Italy and this translates into an inappropriate laboratory testing.The aim of our study was to evaluate the advantage of application of ANA Reflex algorithm in the clinical laboratory of University Hospital Policlinico of Palermo. Moreover, due the high frequency of false positivity of ANA, we also evaluated the ideal serum dilution for screening anti-ENA antibodies. Methods. The study included 5310 ANA tests ordered to our Laboratory in the period May 2018 – May 2022. The presence and titers of serum ANA were assayed on HEp-2000 slides (Immunoconcept, Sacramento, CA) at a starting serum dilution of 1:80, and positive samples were further diluted to a final titer of 1:1280. Serum samples positive for ANA at dilution 1:160 were further processed to detect anti-ENA, using immunoblotting assay (EUROIMMUN, Lu #beck, Germany). Results. Among 5310 ANA screening tests performed, 1389 (26%) anti-ENA was not performed by application of ANA Reflex while 1765 (33%) anti-ENA tests were not requested by clinicians. In 2156 (41%) a subsequent anti-ENA test was performed. ENA were positive in 661 (31%) out of 2156 subjects. Considering 1:160 as ANA cut-off (<=1:160 vs >160), Se, Sp, PPV and NPV for ENA positivity were, respectively, 60%, 71%, 55% and 75%; considering, instead, 1:320 as ANA cut-off (<=1:320 vs >320), Se, Sp, PPV and NPV for ENA positivity were, respectively, 49%, 86%, 68% and 74%. Conclusions. Our results, suggest that ANA Reflex is a good model to improve appropriateness in autoimmune diagnostics. Thus, application of ANA Reflex on the national level is at this point imperative for harmonization of results. Finally, we assessed that ANA positive at a dilution 1:160 is the best cut-off for screening anti-ENA.

EP098

PTH: old analytic problems

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Introduction

PTH II generation assays (G2) cross-react with 7–84 PTH fragments while PTH III generation (G3) do not detect 7–84 PTH but measure, in addition to 1–84 PTH, a post-translational form called amino-PTH, overproduced in many patients with parathyroid carcinomas. Guidelines for the diagnosis of primary hyperparathyroidism (PHPT) and KDIGO guidelines, emphasize that G2 and G3 assays have similar clinical values for the diagnosis of PHPT and for the follow-up of mineral and bone disorders related to chronic kidney disease (CKD). Many clinical laboratories worldwide use G3. We describe our experience with the G3 assay in a cohort of hyperparathyroidism (HPT) and CKD patients in transition from G2.

Methods

PTH was determined in 148 serum samples from 56 CKD patients and 92 patients from Endocrinology Unit, 43 with HPT. Samples were analyzed by 2 chemiluminescence immunoassays (DiaSorin, Stillwater, MN, USA): LIAISON N-TACT PTH GEN II (15-88 pg/mL) and LIAISON 1-84 PTH Assay III (6.5-36.8 pg/mL). Statistical analysis was performed with MedCalc Software Ltd. Receiver operating characteristic (ROC) analysis was performed only for patients from Endocrinology Unit to determine absolute cut-off levels with better accuracy.

Results

Mean values±SD were 354.4±391.5 and 108.6±127.1 pg/mL (R^2=0.979 p<0.01) for CKD patients and 112.4±107.5 and 41.4±33.8 pg/mL (R^2=0.8485 p<0.01) for other patients using G2 and G3 respectively. Both methods showed agreement of 99.9% for CKD patients (2 positive for G2 only) and 94.6% for other patients (2 affected correctly diagnosed by G2, 3 health of which 1 correctly diagnosed by G2 and 2 by G3). ROC curve analysis performed for G2 and G3 showed an AUC=88% (p<0.0001). Based on these curves the most accurate G2 cut-off was 78.2 pg/mL (sensitivity 88%; specificity 82%) while for G3 was 32.4 pg/mL (sensitivity and specificity of 83%). FN results were observed in 12 patients and FP results in 5 patients.

Discussion

G3 mean concentrations were one-third compared to G2, in line with the different reference range. Clinical values of results were similar with G3 and G2 for all patients confirming guidelines. The adoption of G3 is related to VEQ results (CV 60% G2 LIAISON few users). To avoid the FNs, the cut-off could be reduced in HPT patients.

EP099

L'utilizzo del rapporto albuminuria/creatininuria come marcatore per la diagnosi e la valutazione della risposta d'organo nell'amiloidosi renale AL

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Il seguente caso riguarda un uomo di 70 anni. Nell'estate 2021, sono comparsi edemi declivi e schiuma nelle urine. Gli esami di laboratorio hanno mostrato proteinuria 4,02 g/24h e creatinina 0,85 mg/dL (velocità di filtrazione glomerulare stimata [eGFR] 89 mL/min). Come ulteriori accertamenti sono stati eseguiti una valutazione dell'emoglobina glicata, risultata nella norma (5,7%; v.r. <6.1%), l'immunofissazione del siero che ha rilevato una componente monoclonale IgGlambda e l'immunofissazione delle urine che non ha rilevato proteinuria di Bence Jones. Il paziente è stato inviato presso il Centro Amiloidosi di Pavia nel sospetto di amiloidosi renale. Il paziente non era riuscito a portare in visita la raccolta delle urine delle 24 ore per motivi logistici (comune di residenza a >1000 Km da Pavia). Pertanto, è stato valutato il rapporto albuminuria/creatininuria (UACR) sul primo spot del mattino, che è risultato 4500 mg/g (v.r. <30 mg/g). L'immunofissazione del siero ha confermato la componente monoclonale IgGlambda, la concentrazione delle catene leggere libere lambda nel siero era 125 mg/L, con rapporto kappa/lambda 0,20 (v.r. 0,26 1,65), la creatinina era 0,91 mg/dL (eGFR 85 mL/min), la fosfatasi alcalina, il frammento ammino terminale del propeptide natriuretico di tipo B (NT proBNP) e la troponina I sono risultati nei limiti di riferimento. La colorazione con rosso Congo su grasso periombelicale ha dato esito positivo e l'analisi ultrastrutturale ha consentito di concludere per amiloidosi AL (lambda). È stata iniziata una terapia con bortezomib, melphalan e desametasone. Dopo due cicli, il quadro ematologico era di remissione completa (RC), UACR era 2200 mg/g e la creatinina 0,92 mg/dL (eGFR 84 mL/min). Dopo un totale di quattro cicli, la RC era mantenuta, in presenza di un'ulteriore riduzione di UACR (1050 mg/g) con eGFR stabile (84 mL/min). Il paziente ha inoltre eseguito una quantificazione della proteinuria delle 24 ore in un laboratorio vicino al domicilio, che è risultata 1,10 g/24h, confermando il miglioramento del danno renale. Data la risposta ematologica e renale, la terapia è stata sospesa. UACR è un marcatore utile ed affidabile nella diagnosi e valutazione della risposta renale nell'amiloidosi AL.

EP100

Identificazione di un clone plasmacellulare mediante Next Generation Flow Cytometry su sangue midollare in un caso di amiloidosi AL cardiaca in assenza di componenti monoclonali e con rapporto delle catene leggere libere sieriche nella norma

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Il caso è quello di un uomo di 82 anni con una storia clinica di fibrillazione atriale. Nel marzo 2022 ha eseguito una visita cardiologica di controllo e l'ecocardiogramma ha mostrato ipertrofia diffusa del ventricolo sinistro, in un quadro sospetto per amiloidosi cardiaca. È stata eseguita una scintigrafia ossea con bifosfonati, che ha mostrato un quadro non compatibile con amiloidosi da transtiretina (ATTR). Nel maggio 2022 gli esami di laboratorio eseguiti presso il Centro Amiloidosi di Pavia hanno mostrato: assenza di componenti monoclonali (CM) all'elettroforesi ed immunofissazione ad alta risoluzione su gel di agarosio, catene leggere libere kappa 25,34 mg/l (3,3-19,4) e lambda 15,84 mg/l (5,7-26,3) con rapporto kappa/lambda di 1,60 (0,26-1,65), proteinuria 0,15 g/24h, creatinina 0,95 mg/dl (0,73-1,18), fosfatasi alcalina 166 UI/L (46-170), NT-proBNP 1250 ng/L (<334) e troponina I 22 ng/L (<47 come 99°percentile della distribuzione). L'agosapirato di grasso periombelicale ha dato esito positivo alla colorazione con rosso Congo e l'immunoistochimica in microscopia elettronica è risultata positiva per catene leggere libere kappa. È stata posta diagnosi di amiloidosi da catene leggere immunoglobuliniche (AL) ad interessamento cardiaco. Data l'assenza di CM e la presenza di un rapporto delle FLC nella norma, è stata eseguita una valutazione midollare per la ricerca del clone plasmacellulare. La biopsia osteomidollare ha rilevato plasmacellule (<5%) non clonali e la citofluorimetria su sangue midollare ha mostrato una piccola popolazione di plasmacellule (0.1%; fenotipo: CD56-/CD19+) in assenza di restrizione clonale. È stata eseguita la ricerca di un clone plasmacellulare mediante Next Generation Flow Cytometry (NGF), una metodica già impiegata nell'amiloidosi AL per la ricerca di malattia minima residua midollare nei pazienti in remissione completa (sensibilità della metodica: 10-6). Questo esame ha rilevato la presenza di 42 plasmacellule (pari allo 0.0004% degli eventi totali) con restrizione clonale kappa e fenotipo aberrante (CD138+, CD38dim, CD56-, CD19-, CD27dim, CD45dim, CD117-, CD81-). L'utilizzo di NGF permette l'identificazione alla diagnosi di piccoli cloni plasmacellulari non rilevabili mediante le metodiche convenzionali nell'amiloidosi AL.

EP101

Il ruolo del laboratorio di biochimica clinica nella diagnosi di un raro caso di gammapatia monoclonale di significato clinico

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Il caso clinico è quello di un uomo di 76 anni con storia di ipertensione arteriosa che nel 2019 ha presentato difficoltà nella deambulazione. È seguito un progressivo decadimento con calo ponderale che ha portato in aprile 2021 a ricovero per accertamenti. Una TC dello scheletro ha mostrato lesioni osteoaddensanti alle coste e alle vertebre e l'elettromiografia ha riscontrato segni di polineuropatia sensitivo-motoria demielinizzante. L'elettroforesi capillare del siero ha rilevato un tracciato nei limiti; la creatinina era 1,02 mg/dL, la fosfatasi alcalina 71 U/L (v.r. <120 U/L), la calcemia 8,22 mg/dL, l'emoglobina 12,2 g/dL (MCV 92 fL), i globuli bianchi 6000/mcL, e le piastrine 550000/mcL. È stato posto il sospetto di sindrome paraneoplastica in neoplasia occulta ma gli accertamenti eseguiti, tra cui una biopsia di una lesione ossea, hanno dato esito negativo. Nel febbraio 2022 il paziente è stato ricoverato per scadimento soggettivo. In questa occasione, l'immunofissazione ha documentato una componente monoclonale IgGlambda. Il paziente è stato inviato presso il nostro Centro per studiare un nesso eziologico tra la gammapatia monoclonale ed il quadro clinico. Il paziente presentava uno stato di marcata ipotrofia muscolare e cachessia e anestesia a calza bilaterale. Gli esami di laboratorio hanno mostrato una componente monoclonale IgGlambda all'immunofissazione del siero, proteinuria di Bence Jones lambda all'immunofissazione urinaria, catene leggere libere lambda 142,42 mg/L (v.r. 5,7–26,3), creatinina 0,95 mg/dL, fosfatasi alcalina 150 U/L (v.r. <170 U/L), calcemia 9,2 mg/dL, NT-proBP 190 ng/L (v.r. <334), emoglobina 13,2 g/dL, leucociti 4500/mcL, piastrine 575000/mcL, VEGF 2053,5 pg/mL (v.r. 62–707). Gli esami endocrinologici hanno mostrato TSH 3,677 mIU/L (v.r. 0,400–4000), FSH 12 IU/L (v.r. 0,7–11,1), LH 9,2 IU/L (v.r. 0,8–7,6), FT4 7,6 pg/mL (v.r. 8–19), FT3 1,55 pg/mL (v.r. 1,8–4,2), GH 3,14 ng/mL (v.r. <3), prolattina 146 ng/mL (v.r. 2,5–17), testosterone totale <0,1 ng/mL (v.r. 2,7–15). Sulla base di questi esami, è stata posta diagnosi di sindrome POEMS. Il laboratorio di biochimica clinica ha un ruolo centrale nello studio e nella diagnosi delle gammapatie monoclonali di significato clinico.

EP102

L' ESAME BIOCHIMICO DEL LIQUIDO PLEURICO: UN MODELLO PER LA REFERTAZIONE

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Il versamento cavitario è una raccolta patologica di liquido nella cavità pleurica, addominale e pericardica dovuta ad una condizione patologica sistemica e/o localizzata, causata da malattie gravi. L'analisi dei liquidi, nella fattispecie quello pleurico, per il clinico rappresenta uno dei principali criteri diagnostici nella valutazione dell'eziopatogenesi del versamento. Recependo l'indagine conoscitiva SiBioc/AIPO relativa alla gestione del processo diagnostico del liquido pleurico, il Settore Biochimica Clinica dell'UOC Patologia Clinica dell'AORN Caserta ha predisposto, in accordo con la UOC Pneumologia, un modello di refertazione specifico per il Liquido Pleurico, concordando un pannello di esami standard e riportando nel referto il calcolo automatico dei rapporti di concentrazione dei diversi analiti. In tal modo si ottengono degli indici analitici che consentono di effettuare una diagnosi differenziale fra essudato, trasudato e altro versamento. L'accettazione del campione in order entry genera un codice univoco specifico e due barcode: uno per il liquido e uno per il campione di siero del paziente. Sul referto vengono riportati oltre alla valutazione macroscopica (aspetto, colore), il pH, LDH, Proteine Totali, Glucosio, Albumina, Colesterolo, Trigliceridi, Amilasi, Bilirubina Totale ed i calcoli automatici dei rapporti di concentrazione di LDH e proteine secondo i criteri di Light. Vengono, altresì riportati i rapporti di concentrazione di Colesterolo, Trigliceridi, Amilasi, Bilirubina e il gradiente di albumina secondo criteri aggiuntivi. In questo modo la distinzione iniziale fra Essudati, Trasudati, e Chilotorace che è di grande aiuto al clinico per orientare la diagnosi differenziale e le cause del versamento, sarà generata automaticamente dal sistema informatico, riducendo sia la tempistica di refertazione che eventuali possibilità di errore.

EP103

Evaluation of the new SENTiFIT® 800 high-throughput analyzer for the quantification of fecal hemoglobin with FOB Gold® reagents

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Background

Colorectal cancer screening programs have been established in many countries in order to reduce incidence and mortality amongst the population. The most used test for this purpose is the Fecal Immunochemical Test (FIT) that identifies occult blood in feces, therefore millions of FIT tests must be analyzed every year in dedicated and clinical labs.

The purpose of this study was to evaluate the performance of the new SENTiFIT® 800, fully automated and high throughput immunoturbidimetric analyzer for the quantification of human hemoglobin in stools using the FOB Gold® Latex Wide reagents. The SENTiFIT® 800 Analyzer is a high-end system dedicated to fecal testing able to process up to 550 test/h, 1 million tests every year samples in total automation and full traceability. It delivers a relevant reduction of hands-on time, better management of resources and decreasing of manual errors. The system is fed by a dedicated sample loader (Rack Handler RH-150) that provides a steady flow of up to 250 samples to the analyzer unit.

Methods

The SENTiFIT® FOB Gold® Latex Wide is a quantitative immunoturbidimetric latex test based on antigen-antibody agglutination reaction detected at 570 nm. The tests were conducted following CLSI Guidelines and Microsoft Excel statistical tool Analyse-it was used.

Results

-Limit of Blank (*) 5.9 ng/mL
-Limit of Detection (*) 10.0 ng/mL
-Limit of Quantitation (*) 17.6 ng/mL
-Intra-assay imprecision 3.6% (47.2 ng/mL), 2.8% (83.5 ng/mL), 2.8% (125.4 ng/mL), 2.4% (293.5 ng/mL), 2.4% (794.7 ng/mL).
-Total imprecision during 20 testing days up to reagent age of 31 days, 3.7% (50.9 ng/mL), 3.1% (84.0 ng/mL), 2.4% (126.5 ng/mL), 2.5% (294.7 ng/mL), 2.4% (785.1 ng/mL).
-On board calibration/reagent stability 30 days with only T=0 calibration
-Linearity (*) up to hemoglobin 1000.0 ng/mL
-Reportable range 18.0 – 1000.0 ng/mL
-Instrument correlation vs SENTiFIT 270 y= -5.75 +0.99 x (Deming fit); r = 0.999
-Sample carryover on low sample 0.1%
-Hook effect not detectable up to 30000 ng/mL
(*) Performed on two reagent batches

Conclusions

SENTiFIT® 800 Analyzer showed excellent analytical performances for occult blood detection confirming it's a reliable instrument for high volume CRC Screening Programs and clinical laboratories.

EP104

APPLICATION OF LABORATORY TESTING FOR THE EVALUATION OF IMMUNE-RESPONSE TO BNT162b2 VACCINE IN HAEMODIALYSIS PATIENTS AND KIDNEY TRANSPLANT RECIPIENTS

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Background: Haemodialysis patients (HD) and kidney transplant recipients (RTx) are vulnerable populations at higher risk for coronavirus disease 2019 (COVID-19) because of their impaired immune status. They were considered a priority for COVID-19 vaccination, despite they were excluded from vaccine trials. We used laboratory testing for a prospective observational study in HD e RTx to evaluate humoral and cellular immunity after the third dose with BNT162b2 m-RNA vaccine and to compare, in both groups, the humoral response after the second and the third vaccine doses.

Methods: Patients were recruited from two Nephrology Unit of Southern Italy. The study was performed in 99 patients divided in two homogeneous groups: RTX (n=49) and HD (n=50). Samples were collected twelve weeks after the vaccination doses with BNT162b2. A chemiluminescent immunoassay (Abbott, US) was used for the quantification of IgG antibodies against the Receptor Binding Domain region of SARS-CoV-2 Spike glycoprotein (anti-RBD IgG) and, simultaneously, a commercial surrogate virus neutralization test (Euroimmun, Germany) was used for the determination of neutralizing antibodies (NAbs). An interferone (IFN)-γ releasing assay (IGRA test) was used for the evaluation of the T-cellular response (Euroimmun, Germany) after the third vaccine dose.

Results: Twelve weeks after the third injection of BNT162b2, the median circulating levels of anti-RBD IgG in HD patients and RTx were 5459.5 AU/mL and 961 AU/mL respectively ($p < 0.001$) and NAbs showed significantly higher values in HD (98%IH) compared with RTx (52% IH). Even the IFN-γ levels, measured for the evaluation of cellular immunity, were significantly higher in HD (684 mU/mL) than RTx (33 mU/mL) and a significant correlation was found between NAbs and IGRA-test values both in HD ($p < 0.001$) and RTx ($p < 0.001$). Finally, we compared values of anti-RBD IgG and NAbs obtained after the second and the third vaccine doses. Both groups showed significantly higher values after the third dose.

Conclusions: A great variability in the humoral and cellular response to anti-COVID-19 vaccination was observed in HD and RTx, with a stronger response in the former group. The third dose was globally effective at reinforcing the humoral response in HD and RTx.

EP105

Evaluation of three chemiluminescence immunoassay for the detection of SARS-CoV-2 antibody after BNT162b2 mRNA COVID-19 vaccine

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Background:Vaccination programs have been highly effective for curbing the spread of SARS-CoV-2 . In this context, serology surveillance of SARS-CoV-2 antibodies represents a useful tool for monitoring of potential protective immunity in the population but the long-term antibody response over time remains an open question.

Methods: We compared the performance of three SARS-CoV-2 antibody serological immunoassays in 600 vaccinated individuals after two and third BNT162b2 mRNA COVID-19 (Comirnaty) vaccine dose.Results: All three methods were able to detect a post-vaccine humoral immune response with good analytical performance obtained from a comparison with serum samples obtained in the pre-Covid-19 era. Median (IQR) anti-RBD IgG, Access SARS-CoV-2 IgG (1st IS) and Access SARS-CoV-2 IgG II levels of the subjects investigated were, respectively, 687 BAU/mL (131-2325), 419 IU/mL (58-1091) and 104 AU/mL (14-274). The analysis of the comparison of the methods showed a correlation between the levels of antibodies of the three methods analized. We also considered the kinetics of subjects with multiple doses and observed that the differences between the absolute decreasing gradients were statistically significant (overall Friedman test p <0.001). Conclusions: All immunoassays considered in the study proved useful in evaluating the antibody response to the vaccine. Our results suggest that postvaccination testing of antibody response is an important and feasible tool for following people after vaccination and selecting individuals who might require a third / fourth dose of vaccine at an earlier time point or subjects who may not need to another dose of vaccine due to previous SARS-CoV-2 infection. Reference: Lo Sasso B. et al. Longitudinal analysis of anti-SARS-CoV-2 S-RBD IgG antibodies before and after the third dose of the BNT162b2 vaccine. Sci Rep. 2022; 12: 8679. Published online 2022 May 23.

EP106

Impact and efficacy of the BNT162b2 mRNA vaccine against SARS-CoV-2 infections by evaluation of IgG S-RBD antibodies

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Background: Vaccines represent the most effective tool to contain SARS-CoV-2 infection as well as monitoring their effectiveness is essential to assess individual protection against the pathogen. Immunosurveillance by evaluating anti-spike protein receptor-binding domain (S-RBD) antibodies represents a useful tool to estimate the long immunity against Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) infection. The aim of this study was to evaluate the kinetics of antibody response in vaccine recipients. Methods: We measured anti-S-RBD IgG levels by indirect chemiluminescence immunoassay on Maglumi 800 (SNIBE, California) in 1013 healthy individuals naïve to SARS-CoV2 infection after two and three COVID-19 vaccine doses. Results: Median (IQR) anti-S-RBD IgG levels at the first measurement (baseline) were 1206 (522-2601) BAU/mL. Females displayed significantly higher median baseline anti-S-RBD IgG levels than males (1407 vs 1091 BAU/mL, p=0.003). No association was found between age and baseline anti-S-RBD IgG levels. Antibody levels gradually decrease to a steady state after four months since the peak, and the decay is independent of age, sex, vaccine doses, and baseline antibodies titer. The third dose induces a high anti-S-RBD IgG reactivity in individuals with previous high response and trigger a moderate-high anti-S-RBD reactivity also in individuals with an initial low-moderate anti-S-RBD IgG response. Thus, a third SARS-CoV-2 vaccine dose is associated with a significant immunological response.Conclusions: Monitoring anti-S-RBD IgG levels as a correlate of protection is helpful for answering important questions about virus neutralization and immunity against SARS-CoV-2. A third SARS-CoV-2 vaccine dose is associated with a significant immunological response. Thus, our results support the efficacy of the vaccine programs and the usefulness of the third dose.

EP107

Antineutrophil Cytoplasmic Antibodies and Covid-19: A Manifestation of Immunological Dysregulation

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Background:Coronavirus disease 2019 (COVID-19) is characterized by different manifestations, including an immune system imbalance. However, the specific mechanism that triggers a dysregulated immune response is not yet completely known.AntiNeutrophil Cytoplasmic Antibodies (ANCAs) are autoantibodies directed against various neutrophil antigens, including MyeloPerOxidase (MPO) and PRoteinase 3 (PR3). In this study, we investigated the potential usefulness ofanti-MPO and anti-PR3 to elucidate whether the infection stimulates autoantibody production and contributes to autoimmunity activation in COVID-19 patients.Methods:We assessedone hundred ten patients hospitalized for COVID-19, 62 (interquartile range [IQR], 52-72) years, admitted to COVID-19 Units at the University Hospital "P. Giaccone" of Palermo, Italy. Hematological, biochemical, and inflammatory parameters were evaluated. ANCA testing (anti-MPO and anti-PR3) was analyzed using a chemiluminescent assay (ACL AcuStar; Instrumentation Laboratory). Results: Laboratory results revealed a reduction in lymphocytes, higher levels of C Reactive Protein (CRP), and IL-6. In the great majority (76%) a moderate decrease in vitamin D levels was found. In addition, a weak increase in serum D-dimer and high-sensitive troponin T (hs-TnT) concentrations were observed in 37% and 51% of patients. Our analysis showed that anti-MPO and anti-PR3 antibodies were present in <2% and <5%, respectively, of study population. Conclusions:It has been known that SARS-CoV-2 can trigger a strong immune response in some individuals. Our results do not show greater activation of autoimmune response given the low rate of ANCAs positivity encountered. However, the study is ongoing for a long-term evaluation of patients.

EP108

suPAR evaluation in patients with SARS-CoV-2 infection: our experience

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Introduction

Elevated soluble urokinase Plasminogen Activator Receptor (suPAR) is a biomarker associated with adverse outcomes. We aimed to investigate the associations among plasma suPAR levels (testing the cut-offs ≤4 and ≥6 ng/mL that supports patient discharge/hospitalisation, respectively) with other biomarkers such as PCR, PCT, IL-6 and with sex, age, discharge/death and WHO disease severity in patients tested positive for SARS-CoV-2.

Methods

We performed an observational cohort study of 99 patients (37 females, 62 males) presenting with COVID-19 symptoms at Department of Infectous and Critical Care of our Hospital in April 2020. Plasma suPAR was measured using suPARnostic kit (Virogates, Denmark), an immunoturbidimetric method on Abbott Alinity i platform. Patients were followed for development of mechanical ventilation, mortality or discharged. Statistical analysis was performed using Principal Component Analysis (PCA) that can be applied to datasets to obtain a simplified model for stratifying patients by reducing the number of variables. PCA weights the variables according to their relative importance. This method, in our case, can aid in determining key variables in management of patients affected by SARS-CoV-2.

Results

The mean age was 58 years; women had a higher concentration average of suPAR (8.9 vs 8.3 ng/mL) but the subdivision by sex did not determine any clustering. All variables showed a positive correlation with disease severity, better with IL-6 and suPAR (IL-6=25.3%, suPAR=24%, age=16.4%, PCT=15.4%, PCR=17.2%), allowing a subdivision of 3 groups (severe/critic: IL-6=227.65 pg/mL, suPAR=9.26 ng/mL; moderate: IL-6=48.1 pg/mL, suPAR=7.35 ng/mL, paucisymptomatic: IL-6=3.7 pg/mL, suPAR=2.78 ng/mL). Combining the variables and discharge/death outcome showed positive correlation although this did not result any clear clustering (n.78 discharged: IL-6=214 pg/mL, suPAR=8.23 ng/mL; n.14 dead: IL-6=286 pg/mL, suPAR=11.31 ng/mL).

Discussion

Our data show that suPAR levels increase as the disease worsens. Statistical analyses demonstrated that suPAR levels are positively correlated with age and IL-6 levels. Therefore, further evaluation of suPAR plasma levels in different symptoms of COVID-19 patients could still provide important indications for early admission and treatment.

EP109

Evaluation of SARS-CoV-2 antibodies dinamics after booster dose

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Introduction
At november 2021 in our public hospital, all employees have been received the III dose of SARS-CoV-2 vaccine with BNT162b2 mRNA. To evaluate the immune response to the vaccine, the antibodies measurement directed against the S protein or, more specifically, against the RBD domain stimulated by vaccination, was performed. The aim of this study is to evaluate SARS-CoV-2 antibody dinamics over 6 months after vaccine (at T0, T1, T2, T3: before III dose, at 1, 3 and 6 months, respectively).
Methods
We collected serum samples from 46 laboratory workers at T0,T1,T2,T3 (between november 2021 and may 2022). Serologic testing for specific SARS-CoV-2 anti-RBD IgG were performed by chemiluminescence method on Alinity Abbott instrument; according to manifacturer, the cut-off is 50 AU/mL. All samples were tested also for anti-N IgG with chemiluminescence assay on Alinity Abbott instrument (cutoff is 1.4 AU/mL) to evaluate subjects affected by COVID-19.
Results
At T0, the mean concentration of anti-S IgG is 581±303 AU/mL for 42 subjects (91%) that received II dosis until to April 2021 whereas the mean concentration is 3963±1386 AU/mL for 4 workers (9%) affected by SARS-CoV-2 between I dosis and november 2021. At T1, the mean concentration of anti-S IgG is 23326± 15905 AU/mL (min/max value 4766 and 69816 AU/mL) without difference among subjects that had the upper concentration at T0. At T2, the mean concentration of anti-S IgG is 27374±27057 AU/mL (min/max value 1038 and 80000 AU/mL) with mean concentration of 60561±22281 AU/mL in 6 subjects affected by SARS-CoV-2 between T1 and T2.At T3, the mean concentration of anti-S IgG is 20610±12403 AU/mL (min/max value 1825 and 39796 AU/mL) with mean concentration of 22525±10121 AU/mL in 5 subjects affected by SARS-CoV-2 between T2 and T3.
Discussion
We found that booster dose of the vaccine triggers robust immune responses in healthy recipients, COVID-19 triggers an earlier and more intense immune response even after 2-3 months from III dose; in all cases, however, antibody titers remain at high levels in COVID-19 recovered patients. Although virus infection among vaccinated subjects is rare, this would seem to promote a more intense immune response after boosting dose, inducing antibody titers significantly higher and likely more durable.

EP110

Monitoring the effects of BNT162B2 vaccine on platelet activation and immunephenotyping by multidisciplinary approaches: The longitudinal CORE study.

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Background:
Vaccination is an effective strategy to prevent symptomatic COVID-19 but rare adverse effects have been reported, including cerebral venous thrombosis. Aim of this study was:
1) to monitor platelet abnormalities after vaccination in subjects receiving two doses of RNA-based BNT162B2;
2) to analyze the link between platelet alpha-granules and the open canalicular system (OCS);
and 3) to measure concentrations of 3 miRNAs known to be associated with platelet activation.
Material and Methods:
Healthy subjects were enrolled among healthcare workers of IRCCS MultiMedica (CORE study). Blood and nasopharyngeal swabs were collected before immunization (T0), and after 24 hours (T1-1st dose), 7 (T2), 14 (T3), 21 (T4-2nd dose) and 28 (T5) days, obtaining plasma, fresh platelets and PBMCs. Hematological parameters were measured by Sysmex analyser (XN 9000), morphology by Cellavision DM 96, and serological tests for the detection of immunoglobulin G (IgG) and M (IgM) by TGS COVID-19 kit (Technogenetisc). The cut-off values to assess immunopositivity after vaccination were in accordance with WHO International Standard 20/136. RNA extraction from nasopharyngeal swab was performed using Janus G3 and Chemagic 360. Real-time RT-PCR Assay was used to detect SARS-CoV-2 virus (Perkin Elmer). qPCR (QuantStudio 6 flex, Applied Biosystems) was used to detect miR-21, miR-125b and miR-185 in plasma. Washed platelets were analyzed by Transmission Electron Microscopy (TEM). Kruskal-Wallis test were performed for comparisons. Flux cytofluorimetry on PBMCs were used in a subgroup of subjects after a 3rd dose, as pilot study.
Results: Forty-six subjects (31.8% men; 68.2% women; mean age 36 years-old) were enrolled. Overall, anti-Sars-Cov-2 IgG concentration peaked between 21-28 days after the 1st dose, whereas IgM did not reach positivity in all cases. Mean hematological counts were in the normal range, with significant decrease of neutrophils over time, relative to T0. Peripheral blood smear showed at T5 13,64% increase of giant platelets, and 39,47% increase in thrombocyte aggregation (ANOVA, p=0.02). Morphological TEM analysis showed that the number of platelet alpha granules decreased at T2 and T3, but increased at T4 and T5. Conversely, OCS determined using canalicular/platelet area (um2) ratio, increased at

T2 and T3, then returned to baseline values. Plasma miR-125b expression values increased at T1 ($p<0.0001$ vs T0), whereas miR-185 slightly increased. No significant differences were found for miR-21. Correlation between these 3 miRNAs were significant (miR-21 vs miR-125b, $r=0.74$, $p<0.0001$; miR-21 vs miR-185, $r=0.9$, $p<0.0001$; miR-185 vs miR-125b, $r=0.76$, $p<0.0001$). All miRNAs returned to baseline values after 28 day. In a subgroup where PBMC were processed the day after the 3rd dose, we observed increased mean frequencies of CD4+producing IFN-g, CD8+, CD8+producing IFN-g, and NK cells-producing IFN-g (54%, 63%, 672%, 665%, respectively).
Conclusions: In healthy subjects, BNT162B2 vaccination transiently induced a variety of abnormalities of platelet activation and expression of miRNAs, that subsided 1 weeks after 2nd dose. This could contribute to explain the mechanism of early prothrombotic abnormalities observed in vaccinated subjects. In addition, 3rd dose could improve the response to SARS-CoV2, inducing increased CD4+-, CD8+- and NK- cells producing IFN-g.
The CORE study was approved by Local Ethical Committee. All participants provided written informed consent.

EP111

Metodo analitico combinato di nuovi biomarcatori nel cancro alla prostata.

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Abstract BackgroundProstate cancer is the most frequent malignant tumor among males (19%), often clinically silent and of difficult prognosis. In clinical diagnoses among the gold standards for PC diagnosis and monitoring are prostate-specific antigen (PSA) testing, digital rectal examination, and prostate needle biopsies. PSA screening has still a large grey area for patients with leads to overdiagnosis Although numerous studies have highlighted the diagnostic and prognostic role as PSA, their measurement often does not allow for to identify of the presence of the disease. However, among the circulating biomarkers, many authors suggest the evaluation of the levels of polyamines, belonging to the arginine and lysine cycle. Among these molecules, agmatine has recently received particular attention, due to its role as a potential inhibitor of polyamines synthesis commonly derived from arginine. Alterations in metabolic enzymes of the polyamine system have been reported to play a role in predisposition to prostate cancer. Related to these are a lot of human miRNA sequences, linked to cancer pathogenesis. MicroRNAs, in prostate cancer (PC), play a relevant role as biomarkers and show a specific profile and have been used as therapeutic targets. Clinical evidence suggests new biomarkers are needed to improve existing diagnostic. The miRNA and polyamines expression profiles from tumor versus normal tissues could be helpful and differ not only between cancerous and non-cancerous tissues but also between different cancer types and subtypes, whose measurement in plasma would seem to provide new diagnostic perspectives.MethodsThree groups of human patients (Tumor, precancerous lesion, and Hyperplasia) were recruited from a cohort of patients with suspected prostate cancer (N = 170). The study investigated whether miR-145, miR-148, and miR-185 and polyamines circulating levels in plasma, could be used as molecular biomarkers, to allow distinguishing between individuals with benign prostatic hyperplasia, precancerous lesion, and prostate cancer. Obtained plasma was tested by an LC-HRMS method and total, RNA was isolated from plasma, and TaqMan MicroRNA assays were used to analyze miR-145, miR-185, and miR-148 expression. Statistics on the receiver operating characteristics curve (ROC), and multivariate analysis were used to examine the predictive value of markers in the discrimination among the three patient groups. Results
Statistical analysis models revealed good discrimination

among three classes of patients using both the levels of the circulating polyamines and miRNAs. AUC above 0.8, sensitivity ranging from 67% to 89%, specificity ranging from 74% to 89% and accuracy from 73 to 86%, considering the validation set, have been achieved. Agmatine plasma levels were measured in prostate cancer patients (39.9 ± 12.06 ng/ml), benign prostatic hypertrophy (77.62 ± 15.05 ng/ml) and borderline patients (53.31 ± 15.27 ng/ml). ROC analysis of the agmatine panel showed an AUC of 0.959 and $P \leq 0.001$ and has demonstrated to be able to distinguish patients concerning the three clinical classifications.Then, differential miRNA expression among patient groups was evaluated. Mirna levels were combined with clinical assessment outcomes, including results from invasive tests and polyamines levels, using multivariate analysis to examine their ability in discriminating among the three patient groups.ConclusionsUsing an LC-HRMS method, it is shown that measuring plasma polyamines profile, and in particular, agmatine plasma levels can distinguish patients with prostate cancer, from benign prostatic hypertrophy and precancerous lesion patients. Furthermore, results suggest that, also, miRNA is a promising molecular tool for clinical management of at-risk patients. Multivariate analysis of the combined data allows the three clusters of patients to be distinguished naturally.

EP112

STUDIO IN UNA POPOLAZIONE DI DONATORI DI SANGUE SOTTOPOSTI A VACCINAZIONE ANTI SARS-COV-2 NELLA UOC SIMT e CPE DELLA ASL ROMA 1

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In seguito allo stato di emergenza sanitaria dovuto alla pandemia da Covid 19 la UOC SIMT e CPE del P.O. S. Spirito della Asl Roma 1 si è confrontata con una complessa realtà riguardante il settore delle donazioni di sangue. Abbiamo preso in considerazione una popolazione di 805 donatori che hanno donato nel secondo semestre del 2021 presso una Unità di raccolta del SIMT e sono state indagate le seguenti variabili: stadio del ciclo vaccinale anti SARS-COV-2, tipologia del vaccino somministrato, reazioni avverse riferite in seguito alla vaccinazione.Tra gli 805 donatori di sangue, 491 maschi e 314 femmine, 703 (87%) sono risultati vaccinati con almeno una dose, mentre solamente 102 (13%) non aveva ancora intrapreso alcun ciclo vaccinale. Tra i donatori vaccinati abbiamo riscontrato che il 25% aveva ricevuto almeno una dose di Astrazeneca/Vaxzevria, il 56% di Pfizer/BionTech, il 9% di Janssen ed il 10% Moderna. Tra i vaccinati, il 96% aveva ricevuto 2 dosi, l'1% solamente la prima ed il 3% la 3°dose. Studiando la progressione percentuale dei non vaccinati da luglio a dicembre, si nota come essa abbia avuto un brusco calo dal 26 % di luglio allo 0% di dicembre, inversamente proporzionale all'aumento percentuale dei vaccinati sia con la 2° che con la 3° dose (rispettivamente 77% e 14% a dicembre 2021). 616 (88%) donatori di sangue hanno riferito di non aver presentato nessuna reazione conseguente alla vaccinazione. 87 (12%) donatori hanno riferito la comparsa di sintomatologia che abbiamo suddiviso in tre livelli: lieve (cefalea, artralgie, brividi con T < 38°) nell' 81% dei casi, pari a 70 donatori grave (malessere generale con brividi scuotenti e febbre > 38'C) nel 18% dei casi, pari a 16 donatori gravissima (reazione allergica ed interessamento del sistema nervoso) registratasi in un solo casoConclusioni Nella popolazione presa in considerazione la percentuale di vaccinati risulta in linea con quanto rilevato nella popolazione nazionale, dimostrando in tal modo l'attenzione del donatore alla propria ed altrui salute. Questo atteggiamento nell'approccio alla donazione è la base per la costruzione di un atteggiamento di fiducia nella scienza e nel personale medico grazie al continuo dialogo proattivo tra donatore e struttura trasfusionale.

EP113

CONFRONTO TRA IL TEST SIEROLOGICO DI SCREENING ESEGUITO SU UN GRUPPO DI DONATORI DI SANGUE PRE E POST VACCINAZIONE COVID 19

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Per valutare l'esposizione al virus COVID 19 dal giugno 2020 i donatori di sangue potevano richiedere di eseguire il test sierologico per la ricerca di anticorpi anti COVID 19 contestualmente alla donazione. Si è valutato l'andamento delle richieste di esecuzione del test, i risultati e l'impatto della vaccinazione SARS-CoV-2 tra i donatori di sangue nelle Strutture Trasfusionali della ASL Roma 1. Il test sierologico è stato effettuato con metodica LIAISON SARS-CoV-2 S1/S2 IgG–DiaSorin (dosaggio delle IgG neutralizzanti contro le subunità S1/S2 della glicoproteina spike, titolo espresso in Arbitrary Unit/ml –AU/ml- valore negativo < 12) fino al febbraio 2021, successivamente con metodica LIAISON SARS-CoV-2 TrimericS IgG (dosaggio IgG vs proteina SPIKE trimerica, titolo espresso in Binding Arbitrary Unit /ml-BAU-WHO- valore negativo < 33.8). In caso di positività all'esame sierologico il donatore era inviato presso le postazioni della Asl Roma 1 per eseguire il tampone rinofaringeo molecolare. Abbiamo diviso il periodo di osservazione in 3 gruppi: 1°) giugno 2020 - dicembre 2020, 2°) gennaio 2021 - giugno 2021, 3°) luglio 2021 - dicembre 2021. Nel 1°periodo su un totale di 6142 donazioni sono stati eseguiti 1061 test sierologici (17% dei donatori totali), di questi 47 sono risultati positivi e 5 con valori borderline, (5% di positivi/donatori testati). Nel 2°periodo su 5701 donazioni sono stati eseguiti 825 test sierologici (14% del totale), con 132 esiti positivi e 17 esiti borderline (18 % di positivi/donatori testati). Nel 3°periodo su 6297 donazioni sono stati eseguiti 47 (1% del totale) test sierologici complessivi con 11 esiti positivi e 5 esiti borderline (34% di positivi/donatori testati). Tra i donatori risultati positivi e sottoposti a tampone rinofaringeo molecolare, soltanto 2 di essi nel 1°periodo di valutazione sono risultati positivi. La nostra casistica indica un progressivo calo delle richieste di test sierologici nella popolazione di donatori con il passare del tempo, dato che diventa rilevante nel 3°periodo in cui l'introduzione della vaccinazione ha portato ad una notevole riduzione del numero di test, a ciò si associa un aumento della percentuale di positività dei test effettuati, come atteso nella popolazione dei non vaccinati.

EP114

La proteinuria delle 24 ore identifica un rapido miglioramento del danno renale in un caso di amiloidosi AA dopo miglioramento del quadro flogistico

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Una donna di 64 anni con storia di infezioni delle vie urinarie (IVU) ricorrenti esegue degli accertamenti per la comparsa di edemi declivi, con il riscontro di proteinuria 6,34 g/24 h (rif. <0,15 g/24 h). La creatinina era 0,92 mg/dL (velocità di filtrazione glomerulare [eGFR] 61 mL/min/1,73 m2) e proteina C reattiva (PCR) 33 mg/L (rif. <5 mg/L). La paziente è stata ricoverata in Nefrologia, dove una biopsia renale ha mostrato depositi di materiale amorfo eosinofilo in sede mesangiale, positivi alla colorazione con rosso Congo. L'analisi immunoistochimica ultrastrutturale aveva mostrato fibrille di amiloide immunoreattive ad anticorpi anti-siero amiloide A (SAA). È stata posta diagnosi di amiloidosi reattiva a flogosi cronica (AA) ad interessamento renale e la paziente è stata valutata presso il Centro amiloidosi. La concertazione di SAA era 95,5 mg/dL (rif. <6,4 mg/dL), la PCR 29,6 mg/dL (rif. <5 mg/L), la proteinuria 6,84 g/24h e la creatininemia 0,83 mg/dL (eGFR 69 mL/min/1,73 m2). L'esame delle urine e l'urinocoltura avevano mostrato la presenza di Eschierichia coli (carica batterica >100000 UFC/mL). Il trattamento dell'amiloidosi AA si basa sull'identificazione e trattamento della causa dell'infiammazione con l'obiettivo di ridurre la concentrazione della SAA per prevenire la progressione della malattia. In assenza di altre cause di flogosi cronica, considerando la storia clinica di IVU ricorrenti e l'episodio infettivo attivo, è stato impostato un trattamento antibiotico con beneficio. Al controllo degli esami dopo 6 mesi, la concentrazione di SAA era 32,7 mg/dL (rif. <6,4 mg/dL), la PCR 15,9 mg/L (rif. <5 mg/L), la proteinuria 4,14 g/24h e la creatinina 0,85 (eGFR 67 mL/min/1,73 m2). Nei mesi seguenti si sono verifictae due IVU, trattate efficacemente con antibiotico. A 12 mesi dalla diagnosi, persisteva modesta flogosi (SAA 14,5 mg/dL, PCR 9 mg/L), in presenza di un ulteriore miglioramento del danno renale (proteinuria 2,4 g/24 h, eGFR 65 mL/min/1,73 m2). Similmente all'amiloidosi da catene leggere immunoglobuliniche (AL), i biomarcatori hanno un ruolo fondamentale nell'amiloidosi AA per monitorare l'andamento della flogosi e del danno renale, che può migliorare contestualmente alla riduzione della concentrazione di SAA.

EP115

Evaluation of SARS-CoV-2 neutralizing antibodies before and after III dose of the vaccine

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Introduction

We all know that the most important task in combating COVID-19 pandemic is to produce enough effective vaccines and the greatest number of vaccinated subjects within a time frame. Nevertheless, the goal of this worldwide effort should be aligned to raise protective level of neutralizing antibodies (NAb) in vaccinees. It is clear that the NAb can block a viral invasion at the initial access to human receptors. Some methods for NAb measurement are commercially available.In this study, we evaluate the immune response of all employees receiving the III dose of BNT162b2 mRNA vaccine at November 2021. Serological NAb determination was performed before the administration of the III dose of vaccine (T0) and then 1 (T1) and 3 months (T2).

Methods

We collected serum samples of 46 laboratory workers at T0, T1 and T2. We determined the concentration of Anti-S IgG with SARS-CoV-2 IgG II Quant kit by chemiluminescence method on Alinity i Abbott (cutoff 50 AU/mL) in all samples and of NAb in 11 laboratory workers at T0,T1,T2 using SARS-CoV-2 Anticorpi Neutralizzanti kit (SGM Italia), an immunoturbidimetric method (high concentration >30 AU/mL, high % inibition >56%) on Alinity i Abbott platform. This method is able to detect NAb which bind specifically to the binding domain of the RBD receptor blocking the human ACE2 receptor. Statistical analysis was performed using MedCalc Software Ltd.

Results

At T0, T1 and T2, the mean±standard deviation concentration for anti-S IgG is 1016±1462, 22239±22480, 41777±30778 AU/mL and for NAb is 33±24, 100±100, 95±12, and the mean inibition percentage (%NAb) is 66±51, 309±105 and 320±164, respectively. Comparison between anti-S IgG and NAb at T0 showed a good correlation (R^2=0.88); the NAb concentration was upper of linearity an all subjects at T1 and T2. Comparison between anti-S IgG and %NAb showed the same trend.

Discussion

The anti-S IgG are significantly reduced after 6 months from II dose of vaccine and increased about 30 times at T1. Concentration become half at T2 in all subjects who not affected by SARS-CoV-2 between T1 and T2. Although all subjects had a high %NAb at T0 that becomes 5 times higher at T1 and remains high at T2, 7 out of 11 subjects have been infected by SARS-CoV-2. Further studies are needed to define better the SARS-CoV-2 neutralizing activity and the suitable routine test to measure them.

EP116

Serum biomarkers of liver fibrosis in metabolic (dysfunction)-associated fatty liver disease (MAFLD): analytical performances, validation study and comparison with liver biopsy stages.

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Background: Fibrosis is a hallmark histologic event of chronic liver diseases, including metabolic (dysfunction)-associated fatty liver disease (MAFLD). Liver fibrosis is characterized by the excessive accumulation and reorganization of the extracellular matrix and drives liver-related complications. The gold standard for fibrosis staging is liver biopsy. Several non-invasive tests have been recently developed in order to limit risks and sampling drawbacks associated with liver biopsy. In this study we evaluated the analytical method performances for the determination of liver fibrosis serum biomarkers and the actual relationship between the biochemical parameters and histologic stages of liver fibrosis in a cohort of patients with MAFLD.

Methods: We collected serum of patients submitted to liver biopsy for suspected MAFLD. Liver fibrosis stages on liver biopsy (from F0 to F4) were defined according to Kleiner scoring system. All sera were analysed by chemiluminescence immunoassays (Maglumi X8 -Snibe Co. Ltd) to detect the following markers: collagen IV (CIV), cholylglycine (CG), hyaluronic acid (HA), laminin (LN) and aminoterminal procollagen type III peptide (PIIIP). Moreover, we evaluated the analytical performance of Maglumi X8. The imprecision and repeatability were assessed by means of high- and low-level pools of serum samples according to the 3x5 protocol.

Results: In this preliminary study, 56 patients were analysed: 12 patients with F0, 19 with F1, 7 with F2, 15 with F3 and 3 with F4. Also, we detected fibrosis biomarkers in serum of 15 healthy volunteers. The imprecision was acceptable for all fibrosis biomarkers (CV for low levels range from 1% for CG to 11% for LN, for high levels range from 5% for CG to 12% for HA) and repeatability was optimal for all serum liver biomarkers (CV range from 2% for CIV to 8% for PIIIP). Overall, our results showed a small increase of liver fibrosis markers in patients with advanced disease (F2+F3+F4) with respect to patients without fibrosis (F0+F1). Furthermore, we observed a significant increase in concentrations of LN in patients with MAFLD with respect to healthy controls (mean 44 ng/mL and 19 ng/ml respectively, p<0.0001) and CIV concentrations between patients with advanced and early stages of fibrosis (median 13 ng/ml vs 11 ng/ml respectively, p=0.02).

Conclusions: Serum biomarkers for liver fibrosis seem to have different diagnostic performances compared to the stages of MAFLD at liver biopsy. If some biomarkers are informative for advanced fibrosis, they do not perform as well in intermediate stages of liver fibrosis.

EP117

Effects of GLP-1 receptor agonists on myokine levels and pro-inflammatory cytokines in patients with Type 2 Diabetes Mellitus.

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Background: Glucagon like peptide-1 (GLP-1) based therapies exerts favorable effects on glycemic control, lipid metabolism, blood pressure and other cardiovascular risk markers in subjects with Type 2 Diabetes Mellitus (T2DM): yet, its effect on the main markers of sarcopenic obesity is still largely unknown. The aim of the current study was to evaluate the changes in circulating serum Irisin and InterLeukin-6 (IL-6), in patients with T2DM after 6 and 12 months of GLP-1 treatment. Methods: Eighty-five patients with T2DM (51 men and 34 women; age: 60.4±9.8 years) inadequately controlled with insulin or other hypoglycaemic drugs were added to dulaglutide (n=44) and liraglutide (n=41) treatment. Anthropometric variables, lipid profile and glycemic parameters were assessed by routine analysis, serum Irisin by enzyme immunoassay kit (EK-067-29; Phoenix Pharmaceuticals, Karlsrue, Germany) and IL-6 concentrations by Elecsys IL-6, ECLIA (Electrochemiluminescence) assay (Roche, Milan, Italy). Results: After 6 months of GLP-1 analogues a significant decrease in BMI ($p<0.001$), Waist Circumference (WC) ($p<0.001$), fasting blood glucose ($p<0.001$), HbA1c ($p<0.001$), total cholesterol ($p<0.001$), LDL-cholesterol ($p=0.003$), triglycerides ($p=0.017$), IL-6 ($p=0.045$) and a significant increase in serum Irisin ($p<0.001$) were observed compared to baseline. After 12 months of treatment no significant differences were found compared to the levels at 6 months. The change in Irisin from baseline (Δ_Irisin) was significantly related to the changes in total cholesterol (Δ_total cholesterol) ($r=-0.293$; $p=0.020$), while the change in IL-6 (Δ_IL-6) was significantly related to the changes in WC (Δ_WC) ($r=0.347$; $p=0.006$). Conclusions: Additive treatment with GLP1-analogues results in an increase in serum circulating Irisin levels and a decrease in IL-6. Although, the change in Irisin was correlated with a decrease in total cholesterol and the changes in IL-6 were correlated with a decrease in WC, the exact mechanisms by which the GLP1-RA increase Irisin and decrease IL-6 levels remain to be clarified.

EP118

Translational analyses: from the patients to the Patients-Derived Organoids (PDOs) through a deep sequencing technology on DNA

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Colorectal cancer (CRC) is considered the third most common and second deadliest malignancy for both sexes combined [1]. Fortunately, during the last years, thanks also to improvements in screening, the incidence of new cases and the mortality is decreased [2,3]. Overall, the CRC bears a strong association with environmental and genetic risk factors. Approximately 5-7% of all CRC cases are related to inherited syndromes [4,5], but 10-15% of unselected patients carry germinal putative pathogenic variants in related genes. In this context, from one side the mutational status in predisposing genes may be a tool to identify at risk subjects, from the other the study of preclinical model such as Patients-Derived Organoids (PDOs) represent a milestone toward a successful precision medicine [6]. We enrolled 94 patients affected by CRC, who underwent surgery and, from 16 of them we established PDOs. So, to better understand the mutation pattern of each patient and of each stabilized PDOs, we carried out molecular analyses: first, using a customized multigene panel (n=58) containing variants/genes already known to be related to CRC, and then using whole genome sequencing (WGS) with Oxford Nanopore Technology (ONT). These analyses were performed on 4 different genomes derived from the same patient (blood, PDOs, tumor-derived tissue and locally paired healthy tissue). Using this strategy, we found in the germinal line 15 relevant mutations in 11 different patients; in accordance with the literature, 11.7% of our patients bear pathogenic mutations in genes related to CRC predisposition. Moreover, we found 51 pathogenic mutations at somatic level in the 16 PDOs, found also in the tumoral tissue of the same patient. Finally, the same strategy was also carried out using WGS, which allowed us to confirm the mutations found with the panel and, to further investigate aspects of epigenetics such as methylation. Thus, with this strategy is possible to obtain a precise correspondence for the "Precision Medicine" assigned to each patient. In conclusion, to the aim of precision medicine, the full spectrum of somatic mutations, as demonstrated by this work, is an essential

tool for the subsequent drug screening leading to target specific therapies.

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EP119

Genetic alterations in a large population of Italian children and adolescents affected by neurodevelopmental disorders.

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Neurodevelopmental disorders are a group of conditions whose onset and clinical expression occur during infancy/childhood or adolescence and characterized by impairments in cognition, communication, behavior and/or motor skills resulting from abnormal brain development. A strong genetic basis and the influence of environmental factors are involved in the etiology of these disorders. The copy number variants (CNVs) represent the genetic aberrations mostly related to neurodevelopmental disorders. The array comparative genomic hybridization (a-CGH) and next-generation sequencing (NGS) analyses allow the identification of CNVs and mutations in specific genes as significant causative factors in neurodevelopmental disorders. In the present study, we describe pathogenetic CNVs and variant of uncertain significance (VOUS) containing in some cases a single gene potentially related to these disorders found in a cohort of 1125 subjects with autism, intellectual disability, language delay, psychomotor developmental delay, ADHD, and other diagnostic suspects using the a-CGH. High resolution a-CGH analysis was performed on genomic DNA from the patient by using oligonucleotide probes that cover the whole genome with an average spatial resolution of 13 Kb and an alteration resolution of 25 Kb. Microarrays were analyzed on an Agilent G2600D scanner and image files were quantified using Cytogenomics software (V4.0.3.12, Agilent). We observed on the basis of the CNVs identified in patients with neurodevelopmental disorders a greater involvement of some chromosomes than others. In particular, using a-CGH and consulting the literature and different databases we focused on the following genes: CNTN4, CNTN5, CNTN6, MACROD2, CHRNA7, GABRG3, ANKS1B, ANKRD11, RBFOX1, CTNNA3, CNTNAP3. These genes are involved in different stages of brain development and their involvement in neurodevelopmental disorders when altered makes them potentially causative genes.We want to highlight the diagnostic efficacy of a-CGH and its importance as a routine genetic test in patients with neurodevelopmental disorders to identify new molecular alterations, containing specific genes, underlying or contributing to the clinical manifestations.

EP120

Liraglutide reduces atherogenic small dense low-density lipoproteins and carotid intima media thickness in patients with type-2 diabetes: a 4-month prospective study.

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Background: Liraglutide demonstrated to have surprising non-glycemic properties in subjects with Type 2 Diabetes Mellitus (T2DM) including beneficial effects on plasma lipids, such as a reduction in Low Density Lipoproteins-Cholesterol (LDL-C) concentrations. However, its effect on atherogenic small dense (sd) LDL is still unknown. Methods: Sixty two patients (31 men and 31 women; mean age: 61±9 years), with T2DM naïve to incretin-based therapies have been treated with liraglutide (1.2 mg/day) for 4 months. Fasting plasma samples were collected for laboratory analyses, including all the 7 distinct LDL subclasses by gel electrophoresis (Lipoprint, Quantimetrix Corporation, USA). Carotid Intima Media Thickness (cIMT) was assessed by color doppler ultrasound. Results: Significant reductions were found in fasting glycemia and glycated hemoglobin (HbA1c) (from 9.1±4.0 to 7.3±2.0 mmol/L and from 8.4±1.5 to 6.9±1.1 %, respectively; p<0.0001 for both), body mass index (from 30±5 to 29±5 kg/m2, p<0.0001) and waist circumference (from 106±13 to 103±12 cm, p<0.0001). Also, lipid parameters reduced significantly after liraglutide therapy: triglycerides (from 1.9±1.1 to 1.6±0.7 mmol/L, p=0.0061), LDL-C (from 2.7±1.1 to 2.3±0.8 mmol/L, p=0.0089) and VLDL (from 23.4±4.1to 20.9±4.3 mmol/L, p<0.0001), while no significant changes were found in high density lipoprotein-cholesterol. Cholesterol (C) content (in mmol/L) in each LDL subclass was calculated and we found an increase in LDL1-C (from 17.4±4.7 to 23.6±5.6, p<0.0001), accompanied by reduction in LDL3-C and LDL4-C (from 9.8±4.7 to 5.3±2.8, p<0.0001 and from 3.1±4.6 to 1.7±2.8, p=0.0233, respectively). C content in any of the other LDL subclasses did not change significantly. In addition, liraglutide significantly ameliorated cIMT (from 1.1±0.3 to 0.9±0.2 mm, p<0.0001). Moreover, among the 7 distinct LDL subclasses a significant positive association was found between cIMT and LDL-3 (r=0.510; p<0.0001) only. Conclusion: In patients with T2DM liraglutide therapy after 4 months significantly reduced atherogenic sdLDL and had a beneficial effect on apo-B containing lipoproteins, beyond glycaemic control. Also, cIMT decreased significantly. If this would translate into an effective CV prevention remains to be established by future studies.

EP121

Molecular characterization by using the a-CGH in a girl with epilepsy and obesity

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Microarray technologies has revolutionized clinical cytogenetics, as it provides a relatively quick method to scan the genome for gains and losses of chromosomal material with significantly higher resolution and greater clinical yield. In particular the Array Comparative Genomic Hybridization (a-CGH) allowed to identify the copy number variations (CNVs) that can contribute to the risk of developing complex diseases. Currently, several studies have highlighted their involvement as susceptibility factors for complex diseases of unknown etiology, mainly neurological disorders and multiple congenital anomalies. In this study, we describe a female child with diagnosis of obesity and epilepsy. High resolution a-CGH analysis on genomic DNA from the patient using 4×180 K SurePrint G3 Human CGH (Agilent Technologies), that was performed following manufacturer's recommendations, with sex-matched reference DNA. The a-CGH analysis allowed the identification of a deletion of 256.4 Kb in the 4p15.2 region that involve Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha (PPARGC1A) gene and a deletion of 54.4 Kb in the 14q23.4 region that contain Gephyrin (GPHN) gene. PPARGC1A encodes for a transcription coactivator that regulates the expression of energy metabolism's genes and interacts with a broad range of transcription factors that are involved in a wide variety of biological responses including adaptive thermogenesis, mitochondrial biogenesis and glucose/fatty acid metabolism. GPHN encodes a neuronal assembly protein that anchors inhibitory neurotransmitter receptors to the postsynaptic cytoskeleton and it is essential for postsynaptic clustering of glycine (GlyRs) and GABA type A receptors. Gene deletions have been identified in patients with epilepsy and neurological disorders such as autism, schizophrenia and seizures that can result in the expression of truncated variants. The involvement of the PPARGC1A gene in the regulation of metabolism highlights a role of this gene in the development of disorders such as obesity; and the presence of the GPHN gene could explain the epilepsy in the patient, being an essential protein involved in the regulation of inhibitory postsynaptic density.

EP122

Role of new laboratory parameters in the management of acquired Thrombotic Thrombocytopenic Purpura patients treated with Caplacizumab and anti-CD20.

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Background: Acquired Thrombotic Thrombocytopenic Purpura (aTTP) is an acute, severe, rare syndrome of difficult diagnosis related to antibodies anti-ADAMTS13. We evaluate, by clinical and laboratory parameters, the response to the treatment of relapsing forms of aTTP treated with Caplacizumab and subsequent administration of anti-CD20 (Rituximab).Methods: A cohort of 4 patients affected by relapsed aTTP was followed over a period of 18 months. The response to treatment with Caplacizumab and anti-CD20 was assessed by platelets count, ADAMTS13 Activity levels, and titers of anti-ADAMTS13 antibodies, measured by both chemiluminescence (CLIA) and immunoenzymatic techniques.The cohort consists of four patients with aTTP diagnosed in previous years, relapsing, undergoing traditional therapies (PEX, Rituximab and corticosteroids) plus Caplacizumab.All four patients received Caplacizumab during PEX and after its suspension, according to recommended indication. Three of them were also treated with anti-CD20 during hospitalization at the end of PEX, while the other two received anti-CD20 in the following months during follow-up for ADAMTS13 Activity below 20%.Results: In two patients who benefited from rituximab treatment only in the months following PEX + Caplacizumab treatment for various reasons, different relapses were observed over time. This may be due to the delay in the introduction of rituximab and probabily the only therapy with PEX + Caplacizumab is not effective in preventing relapse.The course of the disease in the other two patients would confirm the hypothesis that the closest extraordinary administration of anti-CD20 to Caplacizumab promotes complete remission and does not predispose to further repercussions, at least in the short term.Conclusions: ADAMTS13 Activity is the cornerstone of the correct diagnostic framing of aTTP, but it does not seem to be a valid predictive indicator of relapse. For this purpose, the antibody titer, inversely proportional to the ADAMTS13 Activity, may be a more useful tool.The preliminary data described show that the new laboratory parameters are configured as excellent candidates to improve the clinical-diagnostic scores, making them more effective in preventing relapses.

EP123

Il ruolo del laboratorio di biochimica clinica nella scelta di ricorrere ad un trapianto autologo in un paziente con amiloidosi AL

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Il caso clinico è quello di un uomo di 42 anni con amiloidosi AL cardiaca e renale valutato presso il nostro centro per decidere il percorso terapeutico. Gli esami di laboratorio alla diagnosi mostravano una componente monoclonale IgGlambda, proteinuria di Bence Jones lambda, catene leggere libere lambda 225 mg/L (v.r. 8,3-27 mg/L), rapporto kappa/lambda 0,05 (v.r. 0,26-1,65), proteinuria 3,45 g/24h (v.r. >0,15 g/24h), creatinina 1,15 mg/dL (velocità di filtrazione glomerulare stimata [eGFR] 78 mL/min/1,73 m2), frammento amino-terminale del peptide natriuretico di tipo B (NT-proBNP) 5700 (v.r. <227 ng/L), troponina I ad alta sensibilità (cTnI) 93 ng/L (v.r. <47 ng/L come 99°percentile della distribuzione). Nonostante la giovane età del paziente, la presenza di NT-proBNP >5000 ng/L e di cTnI >70 ng/L, rappresentava una controindicazione al trapianto autologo di cellule staminali emopoietiche (ASCT). Pertanto, è stato iniziato un trattamento con ciclofosfamide, bortezomib e desametasone. Dopo 4 cicli è stata osservata una risposta ematologica parziale (PR) senza una significativa risposta d'organo. Il paziente è stato rivalutato per decidere come proseguire la terapia per ottenere una risposta ematologica più profonda. Poiché la PR era stata accompagnata da un miglioramento dei biomarcatori di danno cardiaco (NT-proBNP 4320 ng/L; cTnI 36 ng/L) con funzionalità renale che permaneva nella norma (eGFR 80 mL/min/1,73 m2), il paziente è risultato candidabile all'ASCT. L'eleggibilità all'ASCT è stata in seguito confermata in ambito ematologico con indagini strumentali necessarie. Dopo tre mesi dall'ASCT non erano più visibili le componenti monoclonali sieriche ed urinarie all'immunofissazione, la concentrazione delle catene leggere libere circolanti lambda era 25 mg/L, il rapporto kappa/lambda 0,44 (v.r. 0,26-1,65), la proteinuria 2,35 g/24h, la creatinina 1,16 mg/dL (eGFR 77 mL/min/1,73 m2), il NT-proBNP 832 ng/L, la cTnI 29 ng/L, indicando l'ottenimento di una remissione ematologica completa ed un miglioramento della disfunzione cardiaca. Il laboratorio di biochimica clinica ha un ruolo fondamentale nell'aiutare i clinici nella scelta della terapia nell'amiloidosi AL e in particolar modo nell'identificare i pazienti che possono essere eleggibili all'ASCT.

EP124

Autoimmune profile evaluation on vaccinated subjects divided in first, second and booster dose

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Background: Coronavirus disease 2019, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapidly disseminated worldwide. The clinical research for developing vaccines takes several years but considering the state of emergency, all scientific and technological forces have concentrated towards the formulation of vaccines for the disease containment. In less than one year (December 2020) a first messenger RNA vaccine (Comirnaty, BioNTech/ Pfizer) was authorized. However, the vaccines were administered in phase 4 and the scientific community investigated about eventual side effects on the immune system, discussing the vaccination strategy and possible autoimmune implications. Aim: Since the vaccines development process has undergone an unprecedented acceleration, to evaluate a relationship between SARS-CoV-2 vaccine administration and impact on the autoimmune level, the determination of circulating immune-complexes (CICs) concentrations, the presence of anti-nuclear antibodies (ANA) and second level tests (dsDNA, ENAscreen and ENAprofile), were studied on vaccinated subjects, after first, second and third dose of Pfizer vaccine. Methods: The recruited subjects were divided according to anti-SARS-CoV-2 IgG RBD antibodies concentrations in: Group I <10 BAU/ml (N=114); Group II >1000 BAU/ml (N=112); Group III >2500 BAU/ml (N=78). CICs were determined by commercial ELISA kit; ANA by indirect immune-fluorescence on Hep-2 cells.Results: CICs concentration, did not show significant differences, giving the following median values: Group I =5,44 U/ml; Group II =5,49 U/ml; Group III =4.29 U/ml.Regarding ANA test: 23.7% of samples (27/114) were positive at 1:80 screening dilution and 4.4% (5/114) at 1:160 clinically relevant dilution, in Group I; 15.2% of samples (17/112) were positive at 1:80 screening dilution and 2.7% (3/112) at 1:160 dilution, in Group II; finally 10.2% of samples (8/78) were positive at 1:80 screening dilution and 1.2% (1/78) at 1:160 dilution, in Group III. No specific positivity was found in any group.Conclusions: our study did not show significant results variations in the different vaccinated populations examined, suggesting the exclusion of a correlation between vaccine administration and the onset of autoimmune disorders.

EP125

Identification of a deletion involving the RBFOX1 gene in twins with intellectual disability

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Copy number variants (CNVs) are an important class of genetic variation, and play a clear role in the etiology of many neurodevelopmental disorders, in particular intellectual disability (ID). Recently, the Array Comparative Genomic Hybridization (a-CGH) allowed to identify new alterations involved in the development of cognitive deficits. A strong genetic contribution to the development of intellectual deficit is provided by twin studies as the risk of recurrence is significantly higher in monozygotic twins than in dizygotic twins. In this study we report the case of two twins with a diagnosis of ID. Molecular analysis was conducted on DNA extracted, using the a-CGH platform 4×180 K SurePrint G3 Human CGH Microarray (Agilent Technologies, Santa Clara, CA, USA), with an average spacing of 13 kb, allowing resolution of 25 kb. The microarray was scanned on Agilent G2600D scanner. Image files were quantified, and data were visualized by using Agilent's Cytogenomics software version 4.0.3.12 (Agilent Technologies, Santa Clara, CA, USA). The a-CGH analysis showed the presence of a deletion of approximately 44,041 Kb on chromosome 16 in the p13.3 region that includes the RNA Binding FOX-1 Homolog 1 (RBFOX1) gene. This gene encodes the RNA Binding Protein, Fox-1 Homolog 1, also known as Ataxin-2-binding protein, a splicing factor that plays an important role in the regulation of the alternative splicing of large neuronal gene networks important for brain development. It is expressed mainly in the nervous system, heart, and muscle. In particular, RBFOX1 is a high-level regulatory factor in early brain development, so it is not surprising that a growing number of patients with neurodevelopmental phenotypes have been identified with mutations disrupting RBFOX1. Infact, haploinsufficiency of RBFOX1 gene causes severe neurodevelopmental phenotypes including include syndromes of autism spectrum disorder (ASD), intellectual disability, and epilepsy. Deletion of the RBFOX1 gene found in twins could lead to the production of a non-functional protein potentially causing the cognitive impairment found in patients.

EP126

The application of array-Comparative Genomic Hybridization for the identification of genetic variants potentially causative of autism spectrum disorder

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The Array Comparative Genomic Hybridization (a-CGH) is a fundamental technique used to identify genetic alterations associated or involved in neurodevelopmental disorders, in particular in autism spectrum disorder (ASD). ASD is a heterogeneous group of neurodevelopmental abnormalities characterized by impairment of social interactions, problems in communication, and a restricted range of behaviors and interests with a prevalence of around 1–2% in the general population. Genetic and environmental factors play an important role in etiology of ASD. The wide application of a-CGH allows to identify copy number variations (CNVs) containing genes potentially causative of ASD, that code for proteins involved in brain development. We report a case of a male child with diagnosis of ASD; a-CGH analysis was performed on genomic DNA from the patient by using 4×180 K SurePrint G3 Human CGH Microarray (Agilent Technologies, Santa Clara, CA, USA) containing 180,000 probes with an average spatial resolution of 13 Kb. The CNVs contained in the report were analyzed using Alissa bioinformatic software (Agilent) consulting several databases such as Clinvar, Decipher, DGV and OMIM. By using a-CGH, we detect a deletion of 105.1 Kb on the 9 chromosome at q33.1 region that includes Astrotactin 2 (ASTN2) gene. ASTN2 is an integral membrane protein, expressed in the brain, that plays a central role in neural-glial adhesion during neuronal migration and in modulation of synaptic activity. Several rare CNVs in ASTN2 gene were identified in patients with neurodevelopmental disorders including ASD, attention deficit–hyperactivity disorders and intellectual disability. Infact, according to SFARI databases, which includes all the risk genes for autism, ASTN2 is reported such as strong candidate with score 2, that shows that variants present in this gene could have a functional effect and relate with an increased risk of developing autism. The role of ASTN2 in modulation of synaptic activity during neural development provides a likely explanation for its association with ASD. For this reason, the a-CGH allows the discover of new candidate disease genes associated with ASD, which could contribute to clarify the molecular basis of this disorder.

EP127

Quantitative detection of SARS-CoV-2 antibodies from dried blood spots in a newborn cohort.

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Background: The Severe Acute Respiratory Syndrome Coronavirus 2 related (SARS-CoV-2) has rapidly spread originating into the fifth documented pandemic wave. Dried blood spot (DBS) provides an alternative method to the venous blood samples to determine Ab, presenting several advantages, including the practicability, especially in infants, and the possibility to test a higher number of samples in a limited time. The main goal of this study was to measure seroprevalence of anti-SARS-CoV-2 IgG Ab in newborns, using DBS collected from January 2020 to December 2021.
Methods: Anti-SARS-CoV-2 IgG levels were determined using DBS by Anti-SARS-CoV-2 QuantiVac IgG ELISA assay (Euroimmune, Lubeck, Germany).
Results: Preliminary analyses included 515 DBS from newborns, 54% males and 46% females, collected 2-3 days after birth. Overall, mean IgG levels, although below the positive threshold (≥ 35.2 kBAU/L), were significantly higher in 2021 (Feb/21 and Mar/21), with respect to 2020 (Feb/20: 8.6±3.5, n=99 vs Feb/21: 28.1±42.8, n=40; Mar/20: 11.6±5.1, n=94 - Mar/21: 16.8±11.6, n=39, mean kBAU/L ± DS, $p<0.005$). Moreover, an increased number of positive samples were found in 2021 (4/44 Gen/21; 8/40 Feb/21; 2/39 Mar/21). As expected, a trend of increase around 5% in DBS tested positive for anti-SARS-CoV-2 IgG were found in December 2021, where IgG were above the positive threshold in 41.54% of DBS (450.926±873.291, mean kBAU/L ± DS, n=65).
Conclusions: In these preliminary data, newborn DBS seems to reflect population-wide infection rates during the studied periods. This suggests a potential role for DBS in COVID-19 surveillance, especially in infants and in areas where viral testing is limited.

EP128

Characterization of gut microbiota in patients with dysbiosis: a photograph of Italian population

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ABSTRACT

The microbiota influences physiologic processes in the host. The predominant Phyla of the species in the intestinal microbiota are Bacteroidetes (B) and Firmicutes (F), which represent 60-80%, and Proteobacteria and Actinobacteria [1]. When some of the species of the bacterial Phyla are imbalanced, the dysbiosis status is manifested. A clinical laboratory index is F/B ratio<0.8. An elevated proportion of F and a reduced population of B were observed in diabetes type 2 (T2D) subjects [2]. T2D is characterized by the decreased production of butyrate, which supports the function of pancreatic β-cells, especially after food intake [3]. This study aimed to detail the dysbiosis status in the Italian population, focusing on pathogenic spectrum (T2D).

334 fecal samples were analyzed to perform a gut microbiota genetic test, using MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostic, Switzerland) for the extraction and An Illumina® MiSeq ™ 6000 system platform for sequencing. Taxonomic and bioinformatic analysis were performed using the MicrobAT® software (SmartSeq S.r.l., Italy). 314 samples were analyzed statistically.

The results indicated microbiota biodiversity labeled in the low (mean:306.06, number (n):201) and mild range (mean:472.00; n:70). A trend in over imbalance was observed in the percentage of Proteobacteria (median value: 6.75%; interquartile range (IQR): 3.57-17.29%). A statistically significant association ($\chi 2$ p=0.033) was observed between type of dysbiosis and T2D patients with an Odds Ratio (OR) of 1.86 (95% CI: 1.05-3.29). It was noted that females with cystitis/candidosis are significantly prevalent in T2D females (p<0.01; OR:3.59; 95% CI: 1.43-8.99), characterized by severe dysbiosis. Although, in non-diabetic males, sugar craving is significantly associated with the rate of dysbiosis in non-diabetic males (p<0.05; OR 1.07; 95% CI 1.00-1.16).

The overall data confirmed the state of art in microbiota dysbiosis. In T2D patients, Bacteroidetes/Firmicutes ratio was biased in favour of Proteobacteria, probably due to the diet. The increase of Proteobacteria in T2D females had altered gut permeability, supporting the development of opportunistic pathogens and infections in other internal organs, as the vaginal tract [4].

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EP129

Circulating calprotectin as a marker of inflammation and sepsis

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Background: Sepsis is an infection-induced syndrome of pathological and biochemical abnormalities believed to be a serious public health problem. The Third International Consensus (Sepsis-3) defined sepsis as "a life-threatening organ threat caused by a dysregulated host response to infection". Besides, sepsis patients with concomitant coronavirus disease (COVID-19) have been related to high morbidity and mortality rate. By its identification and characterization, fecal calprotectin (CP) has emerged as a biomarker for intestinal bowel diseases. Recently, its concentration has been documented to increase in serum and plasma samples of patients affected by autoimmune diseases. Since, the biochemical markers used in laboratory diagnosis are often unable to detect the onset of sepsis in a timely manner and given the growing interest in circulating calprotectin (cCP) as a generic marker of inflammation in recent literature, its role has been investigated in emergency room patients requiring hospitalization.Methods: Patients were divided into four groups: healthy subjects (CTRL); patients hospitalized for causes other than sepsis and negative to SARS-CoV-2 infection (GROUP 1 "NO SEPSIS"); septic patients negative to SARS-CoV-2 infection (GROUP 2 “SEPSIS”) and septic patients positive to SARS-CoV-2 infection (GROUP 3 “SEPSI-COV”). The cCP concentration was determined by a fully automated chemiluminescence immunoassay.Results: Circulating calprotectin values detected were significantly higher in groups 1, 2 and 3 (2.08 μg/mL; 4.10 μg/mL; 2.25 μg/mL), compared to the control group (0.50 μg/mL) ($p < 0.0001$), and in group 2 "SEPSIS", compared to group 1 "NO SEPSIS" ($p < 0.0028$). The receiving operating characteristic (ROC) curves showed good AUC (area under curve)(0.888; 0.945; 0.922), sensitivity (80%, 86%; 81%) and specificity (99%) values in group 1, 2 and 3, discriminating healthy subjects from patients. To find a cut-off value able to identify sepsis patients, data from group 2 and 3 were combined and compared to group 1 (AUC=0,598; sensitivity=60%; specificity=63%).Conclusions: Our results confirmed cCP as a marker of inflammation. Furthermore, the increase in cCP levels in patients with sepsis, suggests its importance as a good marker of sepsis.

EP130

A pilot study on COVID-19 T-Cell in vaccinated healthcare workers, using Interferon-gamma Release Assay

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ABSTRACT

As is known T-cells play a central role in the immunological response [1]. Nowadays new assays are being developed for the indirect quantification of T-cell memory activity [2]. The aim of this work was to demonstrate Interferon-gamma Release Assay (IGRA) test could be useful for vaccination monitoring.

23 vaccinated healthcare workers were enrolled in the study after 8 months of the Pfizer BioNTech vaccination. The antibody levels were assessed through Chemiluminescence immunoassay. T cells were indirectly analyzed by an ELISA against INFγ. Lymphocyte subtyping was evaluated. Statistical analyses were processed.

The patients were divided into 3 different groups based on S-RBD and ACE-2 antibody levels: the S-RBD and ACE-2 antibodies were significantly lower in Group 1 than in Group 2 ($p<0.001$). However, T cells revealed no significant difference between Group 1 and Group 2. Group 3 was the negative control.

The results supported the actual role of SARS-CoV-2 T cell, expressed after the vaccine administration and persisting at high concentration over time, despite the antibody levels [3] [4]. Consequently, the new IGRA test was revealed to be an immunological screening that offers information on the protection from SARS-CoV-2 and suggests new strategies for doses administration.

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EP131

Distribuzione delle varianti genetiche del gene HFE in pazienti con sovraccarico di ferro nella provincia di Caserta

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L'emocromatosi ereditaria è un disordine genetico caratterizzato da un'alterazione del metabolismo del ferro che comporta un aumentato assorbimento intestinale ed il suo progressivo accumulo nell'organismo con conseguente danni irreversibili all'organismo.Il sovraccarico marziale che caratterizza l'emocromatosi è causato da un'anomalia dei geni che controllano la quantità di ferro assorbita con l'alimentazione. Il principale gene responsabile della patologia è l'HFE, localizzato sul cromosoma 6 in prossimità della regione che codifica per l'HLA-A. Diverse sono le mutazioni che possono determinare il fenotipo emocromatosico: la C282Y, responsabile della forma più severa; la H63D e la S65C, associate a forme più lievi sia in omozigosi che in eterozigosi composta. Altre mutazioni rare possono presentare un fenotipo più o meno severo in omozigosi o in eterozigosi con C282Y. La mutazione più frequente è la C282Y presente soprattutto nelle popolazioni del Nord Europa, tuttavia questa frequenza sembra essere più bassa nelle popolazioni del Sud Europa. Lo scopo del nostro lavoro è stato quello di valutare la frequenza delle mutazioni dell'HFE in pazienti con sovraccarico di ferro nel territorio casertano. Nel nostro laboratorio sono stati analizzati 314 campioni (229 maschi e 85 femmine) di sangue periferico di pazienti con sovraccarico di ferro nel periodo compreso tra luglio 2007 e marzo 2022. L'analisi genetica è stata eseguita con amplificazione delle sequenze in PCR end-point e rilevazione mediante ibridazione inversa, previa estrazione con sistema automatico.I risultati ottenuti hanno mostrato, nel campione da noi testato, una prevalenza della mutazione H63D. Si può ipotizzare che i risultati ottenuti, possono essere compatibili con l'azione di fattori concomitanti, come ad esempio epatopatie virali, nel determinare il sovraccarico marziale anche in presenza di una mutazione con effetto patogenetico più lieve.

EP132

SARS-CoV-2 specific T Cell: clinical validation of a rapid and easy to use method based on direct real-time PCR amplification

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Introduction: SARS-CoV-2 immune-response is mediated by both humoral and cellular immunity. However, since Ab levels wane faster than SARS-CoV-2 specific T cells immunity, cellular immunity represents an important factor for COVID-19 immune defence. Determining immunoreactivity of SARS-CoV-2 specific T cells is of clinical relevance in transplant recipients or patients treated with immunomodulant therapy. SARS-CoV-2 specific T cells assays are currently based on ELISA, whilst rapid tests are pivotal for real-time patients' evaluation. In this study, a novel direct real-time PCR (dRT-PCR) targeting mRNA of CXCL10 for measuring SARS-CoV-2 specific T cells, was tested and evaluated.
Method: A total of 104 healthcare workers, with two or three doses of homologous (Pfizer/BioNTech, n = 82) or heterologous (Pfizer/BioNTech and Vaxzevria or Moderna, n = 22) vaccinations were asked to collect a blood (Li-He) sample. Blood was stimulated overnight with SARS-CoV-2 spike peptides (S-peptide) or treated with non-stimulating substance. Stimulated/treated samples were diluted in Buffer A, mixed with dqTACT MS then loaded into the cartridge. The analysis was performed using SCV2 T Activation kit, bCube and bApp (Hyris srl, Lodi, Italy), equipped by an automatic result interpretation based on artificial intelligence. For a subgroup of 49 samples, IFN-y releases to SARS-CoV-2 spike peptides were tested by Quant-T-Cell SARS-CoV-2 and ELISA (Euroimmune, Lubeck, Germany).
Results: Seventy-nine (75.9%) and 25 (24.1%) were females and males, respectively. Twenty-nine subjects were previously infected by SARS-CoV-2. Overall mean age (± SD) was 45.9±13.3 years. At qualitative analyses, 97 subjects (93.2%) resulted reactive to S-peptides, 3 (2.8%) were borderline and 4 were negative (3.8%). These negatives had their third vaccinal dose in December/November 2021. Previous infected individuals presented reactivity to S-peptides, with the exception of one subject with resulted reactive also in the untreated sample. Samples tested with both dRT-PCR and ELISA perfectly agreed (100%) with both methods. At quantitative analyses, between-assay correlation was 0.32 ($p<0.001$).
Conclusion: Hyris dRT-PCR appeared accurate for determining presence or absence of immunoreactivity of SARS-CoV-2 specific T cell, especially when rapid analyses are required, such as for organ transplantation.

EP133

Predictive value of Hepcidin level in COVID-19 intensive care unit patients

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Coronavirus disease 2019 (COVID-19) caused by the recently uncovered human coronavirus SARS-CoV-2 [1] presents a clinical spectrum that ranges from an asymptomatic condition to critical illness [2]. Patients with critical illness present respiratory failure, septic shock and/or multi-organ failure induced by the so-defined "cytokines storm" [3]. Hepcidin modulates cellular iron export to plasma and extracellular fluid through ferroportin, which acts as hepcidin receptor and cellular iron exporter in vertebrates. Hepcidin is not routinely measured in COVID-19 patients, but some preliminary studies showed that high levels of hepcidin are associated with the severity of disease [4] as well as low levels of serum iron are correlated with the severity and mortality of disease and severe hypoxemia in intensive care unit (ICU) patients [5]. The aim of this study was to analyze retrospectively the levels of hepcidin in a group of COVID-19 patients admitted to the intensive care unit (ICU) of the Policlinico Tor Vergata of Rome, Italy. Thirty-eight patients from November 2020 to May 2021 because of pneumonia caused by SARS-CoV-2 were enrolled in the study. Based on the clinical outcome, the patients were assigned to two groups: survivors and non-survivors. A series of laboratory parameters were monitored during the stay of the patients in the ICU and their levels correlated to the clinical outcome. The Hepcidin was determined with "Intrinsic Hepcidin IDx™ ELISA kit" (Intrinsic Lifesciences, San Diego, CA, USA) that is a competitive immunoenzymatic assay based on a monoclonal antibody (mAb) with high affinity to the N-terminus of hepcidin-25. Of the parameters measured, significant statistical differences in the level of hepcidin, IL-6, LDH, leucocyte count, LNR, NLR, neutrophils level, CRP, and TNF-α were observed between the survivors and non-survivors groups. Specifically, a higher level of hepcidin, IL-6, LDH, leucocyte count, LNR, NLR, neutrophils, CRP and TNF-α were measured in the non-survivor group compared to the survivor group. In conclusion, Hepcidin can be prognostic of the clinical outcome, moreover, it could be used together with other markers in a predictive algorithm of disease severity.

EP134

Management of an elderly man with eye bleeding associated with pemphigus in the emergency department.

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Alterations in haemostasis tests accompanied or not by haemorrhagic manifestations in individuals without a personal or family history of coagulation disease may be due to the presence of specific anti-factor inhibitors, most frequently directed against FVIII (acquired hemophilia A). Such disorders still have a high lethality rate for bleeding complications (3-15% in the most recent registries) and are not always detected and treated promptly. Antibodies are often associated with autoimmune and neoplastic diseases, in post-partum, following surgery or drug administration and less frequently with dermatologic diseases (1-4%), but in 50% of cases are idiopathic [1-2]. BG (M, 81 years old) entered the Emergency Department of the Misericordia Hospital of Grosseto presenting eye bleeding and muscle haematomas associated with a prolongation of aPTT (2.46), with PT in range and hemoglobin 131 g/L. The patient suffered from Parkinson's disease and pemphigus receiving corticosteroids. The personal and family history was negative for hemorrhagic syndromes. Thus an acquired hemorrhagic syndrome was suspected. The patient was referred for further diagnostics to the University Hospital of Siena and in depth tests were performed at the Coagulation Unit. PTmix was not performed as the PT was within the normal. aPTT mixture test revealed a non-correction both at room temperature and more markedly after incubation at 37°C for 2h, indicating the presence of an intrinsic pathway inhibitor. The Lupus Anticoagulant assays ruled out its presence and the factor assays were performed. Among the factors dosed on ACLTOP 750 with plasmas deficient in FVIII, FXI, FXII, FIX, FX, FV, FII, FXIII (Werfen), only the activity levels of factor VIII were extremely low (0,1%). Von Willebrand Antigen was 224% and Ri:Cof>200% according to the acute phase of acquired hemophilia. The titer of FVIII inhibitor (Bethesda modified Nijmegen) results in 6UB confirming the diagnosis. The patient was treated with corticosteroids for the eradication of the inhibitor. After 21 days of therapy, aPTT returned to normal ranges (0.96) with an increase in FVIII activity (54%) and an increase in hemoglobin (142 g/L). The close cooperation between institutions enabled early detection and management of the case.

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EP135

Importanza delle informazioni pre-test nel percorso diagnostico per le emoglobinopatie e nella valutazione dell'HbA1c: il caso dell'Hb Lepore

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La crescente prevalenza ed eterogeneità delle variazioni genetiche dell'emoglobina nella popolazione italiana impongono che l'approccio diagnostico di laboratorio, oltre all'utilizzo di strumenti appropriati, si accompagni all'acquisizione di informazioni essenziali sul paziente. La mera applicazione di metodiche, una lettura acritica del pattern separativo e la mancanza di dati che contestualizzino la richiesta dell'esame contribuiscono fortemente a compromettere la corretta interpretazione del risultato diagnostico. Un esempio tra tanti è il caso di un uomo di 52 anni a cui è stata richiesta la determinazione di HbA 1c misurata con metodo separativo in elettroforesi capillare
(Capillarys HbA 1c kit - Sebia, Lisses, France) risultando pari a 71 mmol/mol (8,7%) ma con un profilo che veniva segnalato "atipico" e quindi da valutare. La separazione delle componenti Hb con metodo dedicato in CE (Capillarys 3 Tera con kit Capi3 Hemoglobin, Sebia Lisses, France) segnalava in particolare un picco minore in "zona (D)" dell'1,1% e HbF 5,7%. Tale anomalia veniva individuata anche con metodica alternativa HPLC (Variant II Dual Kit Beta-Thal, Bio-Rad, California, USA) coeluendo con picco di HbA 2 pari a 3,4%.
L'emocromo non risultava particolarmente alterato ma con una discreta anemia (Hb=103 g/L). Il medico richiedente veniva quindi contattato e rivelava che il soggetto, periodicamente ma con una certa regolarità, veniva trasfuso per una emoglobinopatia non definita. L'esame molecolare successivo ha consentito di stabilire la presenza di Hb Lepore allo stato omozigote. Questo caso ci consente di esporre alcune considerazioni: a) l'utilizzo di un metodo separativo per l'HbA 1c permette di osservare eventuali situazioni "atipiche" e quindi di poter valutare in modo obiettivo la misura dell'emoglobina glicata e l'eventuale effetto della presenza di varianti Hb; b) la richiesta di misura dell'HbA 1c in un soggetto trasfuso si deve ritenere non appropriata; c) l'assenza di informazioni pre-test in generale o la segnalazione di trasfusioni recenti in particolare, possono innescare conclusioni presuntive non definitive, conclusioni erronee o percorsi di laboratorio inutili e onerosi.

EP136

A new diagnosis of monoclonal B-cell lymphocytosis with cytoplasmic inclusions in a patient with COVID-19

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A 75-year-old man with a history of chronic ischemic heart disease with a previously normal blood count, presented to the emergency room with fever and tachycardia. There was no hepatosplenomegaly or lymphadenopathy. An electrocardiogram showed left bundle branch block. Because of the fever the patient underwent SARS-CoV-2 RNA testing with positive result.
The patient's blood count showed a WBC of 10.46 × 109/L, lymphocytes 4.51 × 109/L, hemoglobin 129 g/L, and platelet count 233 × 109/L. D-Dimer was 659 µg/L (normal range <500) and IL6 was 76.3 pg/ml (normal range <6.4). A computed tomography scan of the chest showed bilateral interstitial infiltrates associated with multiple enlarged mediastinal lymph nodes. Following a rapid and unexpected increase of the WBC to 17.49 × 109/L with lymphocyte count of 8.37 × 109/L, a blood film and immunotyping were performed. The film showed small/medium sized lymphocytes, with a variable N:C ratio and moderately basophilic cytoplasm. Smear cells were present. About 25% of the lymphocytes showed the negative images of one to three rod-shaped crystals (average 2 per cell). Some immature monocytes and neutrophils showed mild toxic granulation or abnormal nuclear shapes, consistent with COVID-19. Flow cytometric immunotyping showed an increased number of circulating B cells (93% of lymphocytes, 7.78 x 109/L) with lambda light chain restriction and expressing CD19, CD5, CD23, weak CD20, CD43, and CD200; CD10, CD79b, CD81, FMC7, and CD38 were negative. At this stage the clinical picture could not be distinguished from chronic lymphocytic leukemia (CLL).
Two months later the WBC and lymphocyte count returned to normal and immunotyping showed only 0.63 × 109/L CD5-positive clonal B cells. Lymphocytes with cytoplasmic crystals were still present. A diagnosis of monoclonal B-cell lymphocytosis (MBCL) was made. Patients with CLL in whom COVID-19 led to a marked but transient increase in the lymphocyte count have been reported. In our case, COVID-19 in a patient with MBCL led to an increase in the lymphocyte count simulating CLL but follow-up indicated the correct diagnosis. We report here the observation of endocellular crystals, attributable to crystallization of immunoglobulin, in MCBL, a phenomenon previously reported in CLL.

EP137

A correlation study between the Westergren gold standard method and Cube 30 Touch and Mini-Cube, for a clinic determination of Erythrocyte Sedimentation Rate

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ABSTRACT

Erythrocyte Sedimentation Rate (ESR) is an indirect measure of blood fibrinogen, and it is altered by all those pathological conditions that modify the aggregation of red blood cells [1]. Therefore, the ESR is a non-specific and non-invasive laboratory test used to help diagnose conditions associated with acute and chronic inflammation, including infections, cancers, and autoimmune diseases [2-3]. Even though the international guidelines by the ICSH (international council for standardization in Hematology) [4] define the Westergren method as the gold standard for ESR, this method is completely operator-dependent, time-consuming, and it can only be worked once, due to the patient's blood consumption. Therefore, the validation of new ESR analyzers is needed. This work focused on the method validation of CUBE 30 TOUCH and MINI-CUBE, two DIESSE automated ESR analyzers.

242 blood EDTA samples were collected at the University Hospital of Tor Vergata, of which 38 were from cancer patients. A comparison between the two automated instruments and the gold standard was performed. Statistical analyses were processed by MedCalc software. The comparison analysis performed on the overall samples, has reported a good agreement for both methods, showing a Spearman rank correlation coefficient of 0,910 ($p<0,001$) and 0,920 ($p<0,001$), respectively for CUBE 30 TOUCH and MINI-CUBE, compared by Westergren test. The statistical analysis on the 38 tumor patients only, proving Spearman rank correlation coefficient of 0,907 ($p<0,001$) and 0.864 ($p<0,001$), respectively for CUBE 30 TOUCH and MINI-CUBE. The Bland-Altman analysis showed the mean bias of 1 (upper limit 27.5; lower limit of -23.1) for CUBE 30 TOUCH and 3,9 (upper limit 27,6; lower limit of -19,7) for MINI-CUBE. Concludingly, results from both automated ESR analyzers asserted a good correlation rate with the gold standard. It was observed a good alignment between CUBE 30 TOUCH and MINI-CUBE; the two performances are comparable. Consistently with previous funding [5-6], samples with low ESR levels correlated better than high levels. A further investigation would be conducted to assess precision and eventual clinical interferences.

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EP138

MALATTIA DI ALZHEIMER: BIOMARKERS LIQUORALI E INQUADRAMENTO CLINICO, LA NOSTRA PROSPETTIVA

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Le principali modificazioni fisiopatologiche della malattia di Alzheimer (AD) sono rappresentate dalla deposizione extracellulare di placche di βAmiloide e dalla presenza di grovigli neurofibrillari intracellulari di proteina tau iperfosforilata (p-tau). Inoltre, la proteina total-Tau (t-tau) aumenta in maniera aspecifica nelle altre patologie neurodegenerative [1]. L'obiettivo del presente studio è la valutazione della concordanza tra la classificazione laboratoristica, basata sulla presenza di varie componenti biochimiche, e l'inquadramento diagnostico clinico basato su parametri radiologici, biochimici e clinici.
Dal 2019 ad oggi abbiamo determinato le concentrazioni di Aβ-42/Aβ-40, T-tau e P-tau (classificazione ATN) su liquor cerebrospinale di 200 pazienti tramite il sistema automatizzato Lumipulse G600 II System (Fujirebio, CLEIA). Ogni marcatore è stato poi convertito in base al proprio valore di cut-off, definito dalla metodica, in 1 (associabile con lo sviluppo di AD) o 0 (associabile ad altre condizioni neurodegenerative), differenziando, così, i soggetti in base ad un punteggio variabile tra 0 e 3 (score-lab).
Le diagnosi cliniche sono state suddivise in due sottogruppi, AD e non-AD e confrontate con lo score-lab dicotomizzato in 0 e 1-3 e, di conseguenza, ne sono state determinate la sensibilità (76%), la specificità (65%), l'accuratezza (68%) e la predittività positiva (52%) e negativa (85%).
I risultati ottenuti suggeriscono l'uso della determinazione dei marker liquorali in termini di supporto diagnostico che sfrutti la buona predittività negativa evidenziata.
Con la recentissima introduzione del loro dosaggio anche su prelievo ematico, attualmente disponibile solo a scopo di ricerca, si può auspicare un'applicazione di tali determinazioni quale metodica di screening nella popolazione a rischio.
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EP139

Evaluation of the automated urinalysis system Atellica® 1500 as a screening tool for diagnosis of urinary tract infection

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Urinary tract infection (UTI) is one of the most common disease occurring in both hospitalized and community subjects. A quantitative urine culture is the key microbiological test for the diagnosis of UTI, but approximately 80 % of urine cultures are negative. In order to reduce the number of unnecessary urine cultures, we performed a study with the aim to evaluate the use of the automated urinalysis system Atellica® 1500 (Siemens Healthineers, Erlangen, Germany) as a screening tool for rule out UTI. A total of 319 urine specimens from outpatients that had simultaneous request for both urinalysis and urine culture during April – December 2021 were evaluated. The Atellica® 1500 leukocyte (WBC) and bacteria (BAC) counts were used in combination to classify a sample as affected by UTI (WBC ≥ 20 cells/µL and BAC ≥ 180 elements/µL) or not UTI. Of the samples enrolled, 60 (18.8%) were positive for urine culture, while 259 (81.2%) were negative. Among positive specimens, Atellica® 1500 detected UTI in 46 cases (diagnostic sensitivity, 77%; negative predictive value, NPV, 92%), 14 were false negatives. Among negative samples, Atellica® 1500 ruled out UTI in 240 cases (diagnostic specificity, 93%; positive predictive value, PPV, 74%), 19 were false positives .Reviewing literature, some authors proposed the use of automated sediment analyzers, such as UriSed, IRIS iQ200, and sediMAX, as screening methods for the detection of UTI combining bacteria and leukocytes counts. Comparing our results with their data, we observed that our NPV was lower, a result that not fully justifies the use of bacteriuria and leukocyturia as a combined parameter to rule-out UTI. Increasing the study population and defining specific thresholds, based for example on age and gender, might improve the NPV and make the urine test potentially useful for excluding UTI without perform an urine culture.

EP140
Rapid monitoring of SARS-CoV-2 variants through high resolution melt analysis

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Since September 2020 the current global pandemic of COVID-19 caused by the SARS-CoV-2 coronavirus is characterized by a succession of waves of infection due to the emergence of new variants of the original virus, presenting various genomic mutations. Many mutations are present in the gene encoding the Spike protein, the main target of the nucleic acid-based vaccines. The Variants of Concern that have been reported since autumn 2020 include Alpha/ B.1.1.7 and sublineages, Beta/B.1.351, Gamma/P.1 and sublineages, Delta/B.1.617.2 and sublineages, Omicron/ B.1.1.529 and sublineages. The rapid and cheap variant monitoring in the population is pivotal for epidemiological studies and for the prompt detection of SARS-CoV-2 variants characterized by high transmissibility or reduced susceptibility to neutralizing antibodies induced by vaccination. Surveillance of genomic variants is currently based on viral whole genome sequencing (WGS) performed on a random fraction of samples positive to molecular tests. WGS involves high costs and extended analysis time compared to a PCR-based diagnostic test, as well as specialized staff and expensive instruments. To rapidly identify the variant in samples positive to SARS-CoV-2, different rapid tests based on real-time PCR and high-resolution melting (HRM) were designed and applied on 88 oropharyngeal swab samples collected from October 2020 to February 2022 (84 positive samples and 4 negative samples). The HRM results were confirmed by PCR product sequencing. Overall, the assays showed 100% specificity and sensitivity compared with commercial PCR assay for COVID-19 testing. Moreover, 83 samples out of 84 (98.8%) were correctly identified as follows: 8 Wuhan (wild type), 12 Alpha, 23 Delta, 37 Omicron BA.1, 1 Omicron BA.1.1, 2 Omicron BA.2. With our lab equipment, about 10 samples can be processed every 3 hours at the cost of 8.5 € per sample, including RNA extraction. The identified variants overlapped with mutation and case prevalence over time in Italy (as reported in outbreak.info, which collects genomic data from the GISAID Initiative), accounting for the feasibility of this approach.

EP141
Valutazione del sistema Microsemi CRP 767G all'interno del PS Pediatrico della ASST di Cremona

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Nella ASST di Cremona sono presenti diversi strumenti POCT (emogas, bilirubinometri, coagulometri) tutti governati dal Laboratorio Centrale che si occupa di affiancare il personale di reparto durante le fasi di istallazione e collaudo del nuovo strumento, della formazione del personale, di monitorare la qualità del buon funzionamento rilevando periodicamente i dati dei controlli di qualità eseguiti. L'addestramento del personale ed un controllo assiduo sono condizioni necessarie per mantenere la qualità di un sistema che viene utilizzato da personale in continuo ricambio. Nell'ottica dell'introduzione del sistema POCT (Microsemi CRP 767G di Horiba) per l'esecuzione di emocromo e PCR presso il PS Pediatrico si è resa necessaria la valutazione/ validazione del dato generato dal POCT. Sono stati confrontati gli esiti generati dal nuovo sistema con il metodo di riferimento collocato in Laboratorio Centrale (Sysmex XN - Dasit). Sono stati processati in doppio 37 emocromi in giornate diverse, eseguite da operatori diversi: il #% e il test di Student sono stati utilizzati per confrontare i risultati. I parametri della serie rossa hanno mostrato una buona correlazione tra i due sistemi: RBC (valore medio #%: 1.2% range -15.3% ; 38.9%) p= 0.1996; Hb (valore medio #%: 3% range -15.3% ; 40.2%) p= 0.031; RDW (valore medio #%: 1.9% range -17.7% ; 13.8%) p= 0.1771; Il parametro MCV ha mostrato invece una costante sovrastima (valore medio #%: 5.1% range -2.3% ; 11.6%) p=3.33e-13. Per quanto concerne la serie bianca, i parametri che hanno mostrato una costante sottostima rispetto al sistema di laboratorio sono stati: WBC (valore medio #%: -6.6% range 9.9 ; -37.2), t-test associato a due code: p=0.0002; PLT (valore medio #%: -8.9% range 19.5% ; -43.4%) p=0.0008, e monociti (valore medio #%: -43.6% range 10% ; -75.7%) p=1.397e-08. L'analisi preliminare pone in evidenza la necessità di ampliare la casistica analizzando nel dettaglio le procedure della fase preanalitica e analitica del nuovo sistema POCT rispetto al sistema di riferimento.

EP142

Comparison between neutralizing antibody titres and anti-RBD SARS-CoV2 IgG

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Objectives: Serological assays for virus-specific antibody response are commonly employed to establish the immunological response to vaccines and correlates of protection. We aimed to establish the usefulness of immunoassays for the quantitation of IgG antibody response to SARS-CoV2 receptor binding domain (anti-RBD) in the prediction of neutralizing antibody response. Methods: An observational study was performed, included 97 subjects with positive anti-RBD SARS-Cov2 IgG from Mauriziano hospital S.C. Laboratorio Analisi between December 2021 and June 2022 divided into two groups: the first group (A, n=37) with positive nasal swab for SARS-CoV2 or presence of IgG direct to Nucleocapside antigens (anti-N > 0.30 AU/mL with SARS-CoV2 IgG method, Abbott Diagnostics) and second group (B, n=60) with no evidence of late infection. All samples was tested for SARS-CoV2 neutralizing antibody titres (NAb, SGM Italia). Anti-RBD SARS-CoV2 IgG were measured with automated chemiluminescent immunoassay, the results were expressed in Binding Antibody Units (BAU/mL); NAb were performed with quantitative latex immunoturbidimetry method and the results were expressed in neutralizing percent (NAb %). Statistical analysis was performed with Analyze-it. The comparison between anti-RBD levels and neutralizing antibodies has been studied using correlation by Spearman method and receiver operating characteristics (ROC) curve analysis. Results: A significant correlation between anti-RBD IgG levels and NAb titres was found in both A and B groups (rs=0.90 and 0.91 respectively, p<0.0001). The 99% probability of hight NAb titer (>56%) was reached at 212 BAU/ml (A) and 238 BAU/mL (B) and the area under the receiver operating characteristic (ROC) curve was 0.99 (CI: 0.98-1.0) and 1 in A and B respectively. Conclusion: Our study confirm that there is a hight degree of concordance between anti-RBD antibodies and NAb. Based on our data, a threshold of 220 BAU/mL is highly predictive of strong neutralizing antibody response. However these results sure need further confirmation on a higher number of samples.

EP143

Impatto della centrifugazione integrata nel sistema Aptio by Impeco sui test PT e aPTT

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Nel luglio 2021 è stato attuato un processo di consolidamento con integrazione della fase preanalitica nella automazione del Laboratorio Centrale. Tale fase risulta estremamente delicata per alcuni parametri: è noto infatti come l'affidabilità dei test di coagulazione risenta della corretta gestione del campione dalla fase del prelievo alla fase di analisi. In particolare allo scopo di una possibile integrazione al sistema automatizzato degli strumenti per la determinazione di PT e aPTT, sono state effettuate prove di correlazione tra campioni gestiti con centrifugazione manuale e campioni centrifugati sul sistema automatizzato. Nello specifico sono stati prelevati in doppio n° 101 campioni di pazienti in terapia con farmaci anti-vitamina K, trasportati rapidamente e centrifugati entro 1 h dal prelievo e processati secondo il seguente protocollo: una provetta (gruppo 1=x) è stata centrifugata a 2000 giri per 15 minuti a T di 20°C, la seconda provetta (gruppo 2=y) è stata caricata sul sistema Aptio by Impeco (Siemens Healthineers) e centrifugata a 2600 giri per 10 minuti a T di 20°C. Entrambe le provette sono poi state caricate sugli analizzatori dedicati (STA-R- Stago), nella stessa seduta analitica. I dati appaiati sono poi stati confrontati utilizzando il test t-Student, coefficiente di correlazione r e regressione lineare. I risultati ottenuti per l'analisi PT sono i seguenti: t-test associato a due code: 1,2557 (p-value 0,2121); correlazione 0,995; retta di regressione y=0,998x -0,006. Per l'analisi aPTT i risultati sono i seguenti: t-test associato a due code: 0,2688 (p-value 0,7886); correlazione 0,988; retta di regressione y=0,953x +0,056. Il confronto tra i due metodi è risultato soddisfacente. Come atteso il test aPTT ha mostrato maggiori variazioni rispetto al test PT.

EP144
Hb J Paris: un'insidia per il monitoraggio del paziente diabetico

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Laboratorio Generale AOU Careggi

La Regione Toscana con D.R. 1371/2018 ha inserito la valutazione dell'assetto emoglobinico nel pannello dei test di screening prenatale. In occasione di tali screening, una paziente di 34 anni giunge alla nostra attenzione sia per la valutazione dell'assetto emoglobinico, che dell'Hb glicata (HbA1c), essendo affetta da diabete di tipo II. Il cromatogramma dell'HbA1c, ottenuto mediante HPLC (Arkray HA-8180V), non mostrava alterazioni qualitative delle frazioni di Hb fornendo un risultato di 35 mmol/mol, invece l'assetto emoglobinico eseguito in HPLC (9210 Premier Resolution) evidenziava la presenza di una variante veloce con percentuale del 25,3%. Come noto la presenza di varianti emoglobiniche può interferire con la quantificazione dell'HbA1c, pertanto abbiamo deciso di confermare i dati mediante un metodo separativo alternativo, l'elettroforesi capillare (EC) (Capillarys 3 Tera, Sebia). L'elettroferogramma di HbA1c (kit Capillarys HbA1c) ha riportato un valore di 46 mmol/mol. In questo caso è evidente la presenza di un picco ben separato sia da HbA1c che da HbA0 ascrivibile ad una variante emoglobinica con percentuale del 27%. Vista la discrepanza dei due valori di HbA1c (>5 mmol/mol) in HPLC e EC, il dato è stato confermato con metodo immunologico (Celltac Chemi CHM-4100k, Nihon Kohden), con un risultato di 43 mmol/mol, in linea con quello della EC. La presenza della variante emoglobinica è stata confermata in EC (kit Capillarys Haemoglobin), evidenziando un picco in zona Z12 con una percentuale pari al 24,5%, comparabile alla percentuale descritta sopra. La variante è stata quindi caratterizzata molecolarmente mediante Next Generation Sequencing (kit Devyser, piattaforma Illumina) ed è risultata essere: HBA2:c.38C>A (Hb J-Paris-I) in eterozigosi. L'Hb J Paris è una variante che in HPLC determina una sottostima della misura dell'HbA1c. Come recentemente discusso nel documento di consenso SiBioc (BC, 2022;vol46;n°2), la refertazione di HbA1c in presenza di varianti emoglobiniche può essere insidiosa ed è fondamentale un'attenta valutazione dei singoli casi per l'appropriato inquadramento del paziente diabetico. La disponibilità di metodiche basate su principi analitici diversi all'interno della stessa realtà diagnostica costituisce un valore aggiunto.

EP145
Abbott Cyclosporine new assay and through levels damage thresholds evaluation

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Introduction: Abbott released a reformulation update at the end of 2020 using a new monoclonal antibody for the Cyclosporine assay. This work aimed to evaluate the new reagent (Ref. 3R30) compared to the old in-use assay (Ref. 1L75) in patients monitored before and after the therapeutic drug administration.Methods: One hundred seventy samples from patients in therapeutic monitoring at Belcolle Hospital in Viterbo (Italy) were compared with Ref 3R30 and REF 1L75 methods on an extended range. Ninety of these patients were supplemented and monitored for peak concentration after two hours of the drug administration.Results: Precision was improved from the previous CV% of 12% to a current 6 %. The comparative analysis between the previous method (1L75) and the reformulated method (3R30) showed a good correlation (r2= 0.97) but with a non-linear recovery profile. While no adaptation in the 20-120 ng/ml range was needed, the Weighted Deming regression over 120 ng/ml up to 1000 ng/ml was represented by equation y=0.68x+2.8. Fold increase after two hours from the time of therapeutic drug administration was evaluated, and a difference of near one-fold between the two methods was found. Nephrology Department applied medical decision thresholds for drug induction associated toxicity were recalculated and adapted as follows: Proteinuria threshold = from 110 ng/ml Damage Through Level (12h) = from 600 to 515 ng/ml; Damage Through level (24h) from 900-1200 to 767-1080 ng/ml. Comments: The recorded underestimation entailed a risk of interpreting the induction efficiency only the first time, comparing the old and new levels reached after drug administration. The response to the therapeutic drug produced a proportional increase in the 2 hours after administration that has never exceeded the higher toxicity thresholds.

EP146

Does PLR reflect inflammation from chronic hyperglycemia?

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BACKGROUND
Recent studies have examined the relationship between stress-induced hyperglycemia, determined by the glycemic gap between admission glucose levels and average glucose levels derived from A1c, and disease severity and/or unfavorable clinical outcomes. Diabetes patients who have chronic hyperglycemia experience identical consequences to those caused by stress-induced hyperglycemia, including increased oxidative stress, inflammation, and the activation of stress-responsive kinases. The Platelet to Lymphocyte Ratio (PLR) has recently been proven as an inflammatory marker during the Covid-19 outbreak. The purpose of this study was to assess how the glycemic gap and PLR, a measure of inflammation in chronic hyperglycemia, correlate with one another.

METHODS
Retrospective data from 696 outpatients with medically declared diabetes from the year 2019 was obtained. For the results of the CBC and HbA1c, all records were complete. Only 638 of the 696 records contained blood glucose data. PLR was computed for each of the 696. For 638 cases, the estimated A1c-Derived Average Glucose (ADAG) level was computed using the formula 28.7 x HbA1c(%) x 46.7 [1], and was then subtracted from the blood glucose level to measure the glycemic gap. According to the percentages of HbA1c (4,5–6,4; 6,5–6,9; 7,0–8,0; >8,0), four groups were formed, and the average PLR and glycemic gap were determined for each group. The data was then evaluated to see how HbA1c and PLR related to glycemic gap.

RESULTS
PLR and glycemic gap were 119,45 and 13,76 in group 1 (HbA1c % 4,5–6,4; n = 223), 112,50 and 16,79 in group 2 (HbA1c % 6,5–6,9; n = 144), 119,27 and 25,23 in group 3 (HbA1c % 7,0–7,9; n=201), and 115,88 and 46,04 in group 4 (HbA1c % > 8; n=114), respectively. By using correlation analysis, we found that the PLR increased proportionally to the glycemic gap but did not rise with rising HbA1c.

CONCLUSIONS The finding of proportionally higher PLR revealed a correlation between a higher glycemic gap, a sign of chronically induced hyperglycemia, and an elevated pro-inflammatory state.

1 Glycemic Gap as a Useful Surrogate Marker for Glucose Variability and Progression of Diabetic Retinopathy. SC Hsing, C Lin , JT Chen , YH Chen, WH Fang. J Pers Med 2021 Aug 16;11(8):799. doi: 10.3390/jpm11080799

EP147

The clinical importance of Hevylite assay in follow-up of MM: a case report

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A 76-year-old Caucasian woman affected by multiple myeloma (typed as a IgA Kappa in beta2 zone) since 2012, she is in follow-up at the Haematology Department of the ASST Monza. During these years she is undergoing chemotherapy treatment. A restaging was performed in March 2022: S-ELF (Capillarys 2 Sebia) didn't show morphological changes in beta2 zone 0.34 g/dL (normal value 0.23-0.47 g/dL); FLC Kappa was 6.29 mg/L (normal value: 3.30-19. 40 mg/L), FLC Lambda was 1.4 mg/L (normal value: 5.71-26.30 mg/L) and K/L ratio 4.49 (total range 0.26-1.65) (Optilite Binding Site); total S-IgA (Cobas Roche) 126 mg/dL (normal value 70-400 mg/dL). Moreover it was performed the quantitative assay of Hevylite (HLC) IgA Kappa 3.12 g/L (95° range 0.58-2.98), HLC IgA Lambda 0.32 g/L (95° range 0.432-2.035) and ratio 9.67 (95° range 0.911-2.416) (Optilite Kit Binding Site); the assay showed a value of HLC IgA Kappa and ratio above the normal limit, indicative of the presence of minimal residual disease (MRD) despite of normal S-ELF. On the basis of the results obtained it was decided to proceed with S-IFE (Hydrasys 2 Sebia), which detects the presence of the monoclonal IgA Kappa component. In April a S-ELF showed a visual morphological alteration in the beta2 zone 0.55 g/dL; FLC Kappa 11.67mg/L, FLC Lambda 0.87 mg/L and K/L ratio 13.41; total S-IgA 321mg/dL; HLC IgA Kappa was 7.10 g/L, HLC IgA Lambda 0.7 g/L and ratio 10.11. In May S-ELF showed a significant morphological alteration of the beta2 zone 1.42 g/dL; FLC Kappa 37.21 mg/L, FLC lambda 0.83 mg/L and K/L ratio 44.83; total S-IgA 1163 mg/dL. The HLC IgA Kappa value was increased at 25.86 g/L, the HLC IgA Lambda was below the sensitivity limit of the method and the ratio could not be determined. Conclusion: in this clinical case the HLC IgA Kappa and IgA Lambda assay with relative ratio, in association with FLC assay, showed a better positive predictive value than the morphological evaluation of S-ELF in the follow up of monoclonal component recurrence.

EP148
A case of cryoglobulinemia in Waldestrom's Syndrome vasculitis

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A 64-year-old woman known to have an indolent Waldestrom's syndrome in follow-up since 2019, came to the emergency department for asthenia, declivous oedemas and persistent fever. She referred a previous diagnosis of pleurisy treated with antibiotic therapy. She was admitted for medical case management in the Medicine Department. In the suspect of her disease progression haematochemical exams were performed showing: Hb 7 g/dL, CRP 15 mg/dL, a monoclonal M peak in the gamma zone, typed as IgM Lambda 2.4 g/L; BJ protein test was positive for monoclonal Lambda free light chain. Moreover serum free light chains (FLC) assay was performed: FLC kappa 23.00 mg/dL (normal value: 3.30-19.40) and FLC Lambda 131.95 mg/dL (normal value: 5.71-26.30), ratio 0.17 (total range: 0.26-1.65). The PET scan showed an increase of the known diffuse adenomegaly; however bone marrow and lymphonode biopsies didn't not show a progression in high grade disease. During the hospitalization was observed a gradual reduction of the declivous oedemas with an increase of the purpuric rash: this finding suggested a vasculitis and further chemical chemistry exams were requested: rheumatoid factor (RF) was negative, complement C4 suppressed, and C3 decreased; the investigation of cryoglobulins was positive with an after 7 days cryocrit of 14%; a new sample was requested in order to proceed with the typing of the cryoprecipitate; on the second sample the cryocrit was increased at 19%; the sample was treated with betamercarptoethanol plus fluidil, obtaining a single type I cryoglobulinemia (according to Brouet's classification) with a double monoclonal component (IgM Lambda). Although this finding was expected, it was useful to clinicians to better understand the etiopathogenesis and the differential diagnosis of the patient’s vasculitis.

EP149
Evaluation of an immunofluorescence screening assay for the detection of 9 drugs of abuse

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Background The demand for rapid toxicology screening is rising, especially for samples coming from the emergency department. Recently, a new rapid toxicology screening test, the Triage TOX Drug Screen 94600 (Quidel, USA), which can detect 9 drugs of abuse simultaneously with an instrument-read cartridge, was developed. In this study, we evaluated the concordance of this new fluorescent immunoassay using 23 routine urine specimens; the results were compared with those obtained using the current screening methods in use.Methods Amphetamines (AMP), methamphetamines (mAMP), barbiturates (BAR), benzodiazepines (BZO), cocaine (COC), methadone (MET), opiates (OPI), tricyclic antidepressants (TCA) and cannabinoids (THC) were screened on all urine samples (N = 23) using both Triage TOX Drug Screen 94600 and the visual-read immunochromatographic test TOX/See Drug Screen (Bio-Rad, USA) following manufacturer instructions. In addition, the AMP (N = 15), BZO (N = 13), COC (N = 22), MET (N = 14), OPI (N = 22) and THC results (N = 19) from both rapid methods were compared to those of the corresponding assays on the AU680 (Beckman Coulter, USA) or iLab Taurus (Werfen, Spain) analyzers. Quantitative clinical chemistry results were defined as positive or negative based on the corresponding cut-offs declared by the manufacturers.Results Total percent agreement (TA%) between rapid methods was 100% (23/23) for AMP/mAMP/BAR/COC/MET and 95.7% (22/23) for BZO/OPI/TCA/THC. Of the four total discordant results, clinical chemistry results were available for OPI (439 ng/mL, Triage TOX true positive) and THC (82 ng/mL, Triage TOX true positive). TA % with chemistry results for the other assays was comparable.Conclusion The results of the study indicate that the performance of the Triage TOX Drug Screen 94600 is comparable to current laboratory practices (TA 95.7-100%, N = 207) and clinical chemistry results (TA = 90.9-100%, N = 105). For OPI and THC, two positive samples were not detected by the visual method, suggesting a higher sensitivity for Triage TOX for these two specific assays. Other advantages of the immunofluorescent method can be found in the ease-of-use and the automated, objective results interpretation.

EP150

The Hevylite test: the contribution to the diagnosis and monitoring of patients with plasma cell dyscrasias

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Monoclonal gammopathies include a broad spectrum of clinical disorders characterized by uncontrolled proliferation of clonal plasma cells in the bone marrow resulting in the production of monoclonal immunoglobulins (M protein). M protein may consist of intact immunoglobulins and/or monoclonal fragments such as free light chains (FLCs), which can be detected in serum and/or urine. Diagnosis requires identification of M protein using serum protein electrophoresis (SPE), serum and urine immunofixation (sIFE/uIFE), and measurement of FLCs [1]. The serum FLC assay has shown an important supporting role in monitoring and prognosis of these pathologies and has been included in IMWG Guidelines for the evaluation of response criteria to therapy. Recently IMWG revised response criteria to include the assessment of minimal residual disease (MRD) using sensitive assays [2]. In particular, the test for the determination of specific immunoglobulins IgG/A/M Kappa/Lambda (Hevylite-HLC) was recognized as an additional method for the management and monitoring of patients with oligosecerning Multiple Myeloma (MM) or with a monoclonal component of IgA type migrating in the βzone. Moreover, HLC assay is technically useful when non-linear dye binding in the case of IgG monoclonal proteins and inaccuracy at low concentrations has been observed determining M-proteins underestimation [3]. The aim of this study was to investigate the usefulness of HLC test for the diagnosis and monitoring of patients with MM, during treatment and in the post-transplant/maintenance phase. The final outcomes were compared to the data obtained in capillary ETFs.Preliminary data show substantial agreement with routine capillary ETFs, with a Cohen's kappa coefficient of 0.68. In addition, 6 patient samples were assayed before and after induction therapy showing a percentage reduction of about 70%. These preliminary data suggest HLC assay can be a useful tool in routine analysis to confirm migrating monoclonal components in the β 2 zone and useful for monitoring patients with plasma cell dyscrasias. Therefore, HLC assay furnishes additional information that might help to ameliorate the clinical decision-making since it complements SPE, sIFE and the serum FLC assay [4].

EP151

RATE OF ADHERENCE TO GUIDELINES FOR ALBUMINURIA LABORATORY TESTING: A PRELIMINARY SURVEY FROM THE LITERATURE

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Albuminuria is a diagnostic and prognostic biomarker for diabetic nephropathy, chronic kidney disease (CKD), and cardiovascular disease (CVD). Multiple consensus statements and international guidelines highlighted the importance of test standardization from the pre- to the post-analytical phase. This study aimed to evaluate the current adherence to the KDIGO 2012 guidelines across published papers from June to September 2021. PubMed database was searched using medical subject headings (MeSH) terms and keywords in the title or abstract fields; we used: "urine albumin [Title/Abstract] OR UACR [Title/Abstract] OR albuminuria [Title/Abstract] ("2021/06/01 [Date – Create]: 2021/09/26 [Date – Create]"). The search resulted in 278 papers; 2 out of these were unavailable. Articles were screened independently for eligibility by two investigators who evaluated the title and abstract; discrepancies were resolved by mutual agreement. Book chapters, guidelines, position papers, systematic reviews, meta-analysis, in vitro studies, and studies on animal models were ruled out. Finally, 264 papers were examined by reading the text, and five were further excluded because of the lack of data on sample type, methods, results, and cut-off levels. Approximately half papers (49.0%) did not report the method of urine sampling; in the remaining papers, the urine sample was collected as follows: random spot (24.5%), first-morning (10.7%), undefined morning (5.0%) and 24-h (3.1%); eight papers utilized two types of samples. 81.1% of papers used the albuminuria-to-creatininuria ratio (UACR); of these, 79.1% expressed results as mg/g, 13.9% as mg/mmol, 3.1% as g/g, 2.3% were papers reporting errors in the unit of measurement, and 1.6% reported no unit of measurement. 9.4% of papers used the albumin excretion rate (AER); of these, 46.7% expressed results as mg/24 h, 6.7% as mg/L, 6.7% as µg/min, 13.3% as 1+, 2+, 3+. Regrettably, 59.1% and 61.0% omitted to report the analytical method for albuminuria and creatininuria, respectively; 3.8% claimed that creatininuria was measured by an ID-MS traceable method. In conclusion, most published papers utilize UACR; the rate of papers omitting the type of urine sample and analytical method is still considerably high.

EP152
Total Laboratory Automation for the clinical chemistry and immunometry section of the AUSL Romagna Reference Laboratory

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Introduction: Total Laboratory Automation (TLA) allows the management of biological samples from the test order to the laboratory report, ensuring traceability and productivity of the process.In 2020 the installation of new diagnostic systems was completed in the Clinical Pathology Laboratory of the AUSL Romagna. The number of biological samples processed at the reference laboratory, coming from 103 blood collection centers and 405 hospital wards, was around 11 milions in 2021. Clinical chemistry and immunometry section was involved in the processing of around 8 milions of samples. Materials and methods: The benchmarks used to evaluate the efficiency of the automation system were: the capacity, intended as the number of biological samples that can be processed simultaneously in order to guarantee a suitable walk away; the productivity, intended as the maximum number of samples that can be processed every hour.Results: TLA covers the pre-analytical phase (Cobas P512, Roche), transport of samples, automated workflow (Cobas 8100, Roche), analytical phase (Cobas C701, Cobas C502 and Cobas e802, Roche) and post-analytical phase (Cobas p501).Pre-analytical systems have a high capacity (750 samples) and allow the simultaneous management of samples of different size and type. Transport modules deliver samples to the clinical chemistry and immunometry section of the laboratory using baskets with a capacity of 150 samples and a productivity of 707 ± 470 samples per hour.The input/ output modules of the automation are used for loading, sorting, analysis and unloading operations and are able to deal with different test tubes, reaching a productivity of 785 ± 151 samples per hour. The analytical modules have a productivity of 4429 ± 58 tests per hour. Samples that have completed all the analytical processes are sent to the storage (capacity of 27000 samples) and automatically disposed after 48 hours.Discussion: TLA guarantees the optimization of human and financial resources and allows to better meet clinicians and patients' needs, ensuring traceability of the process and minimising the exposure to the biological material. The automation allows to standardize all the laboratory process in order to increase the quality and the safety.

EP153
IL CONTROLLO DI QUALITA' ALLARGATO (CQA) PER TROPONINA: STRUMENTO PER OTTIMIZZARE LA GESTIONE DELLA DIAGNOSI DI IMA

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Per stratificare il rischio dei pazienti con sospetto IMA sono necessari valori di troponina il più possibile esenti da errori. L'uso nell'UOC di Patologia Clinica del PO di Andria di CQI, VEQ e controllo di qualità interno allargato CQA consente la gestione della qualità analitica del dato.
Scopo di questo lavoro è evidenziare come partecipare a un CQA migliori le prestazioni analitiche e garantisca valori di troponina più vicini al dato reale del paziente.
BioRad fornisce controlli Cardiac Marker Plus e software Unity Real Time 2.0 (URT). URT gestisce il CQI e consente di confrontare i dati con quelli di un gruppo di consenso omogeneo per metodo e strumentazione o solo per metodo. Il software invia i dati di controllo a centri di calcolo che elaborano statistiche mensili di confronto fornendo report visualizzabili su www.qcnet.it.
Si considerano i controlli processati su Beckman Coulter Access/DxI a Aprile 2022 valutando media, DS e CV, SDI, indice di deviazione standard e CVR, rapporto del coefficiente di variazione. Questi parametri statistici riflettono accuratezza e precisione rispetto al gruppo omogeneo, attribuendo al laboratorio un valore numerico che va da -2/+2 per SDI (valore ottimale prossimo allo 0) e da 0 a 2 per CVR (valore ottimale prossimo all'unità). L'analisi statistica elaborata rispetto al gruppo omogeneo ha evidenziato CVR = 0.8 e SDI = -0.44 per il livello 1 e CVR = 0.4 e SDI di -0.02 per il livello 2. CVR e SDI sono molto vicini ai valori ottimali. Nei report questi due parametri sono interpolati in un grafico dove è possibile distinguere tre zone di accettabilità: in linea con il gruppo di consenso, accettabile, ai margini di accettabilità; al di fuori del grafico le prestazioni sono inaccettabili e necessitano di azioni correttive. Dalle analisi elaborate e ricevute il confronto con il gruppo di consenso omogeneo rileva per i due livelli di controllo valori in linea con il gruppo di consenso.
Ciò consente di affermare che i valori di troponina sono stati affetti da scarso livello di errore totale in quanto caratterizzati da un ridotto bias (indice di errore sistemico della prestazione analitica) e da un ridotto coefficiente di variazione (indice di errore casuale) e hanno contribuito in modo efficace al percorso diagnostico dell'IMA.

EP154

Analisi di impatto sul budget per Afinion™2 nel monitoraggio del paziente diabetico: l'esperienza di ASL3 Sistema Sanitario Regione Liguria

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Introduzione. Il diabete mellito rappresenta una priorità di salute pubblica a livello globale. Valida alternativa al monitoraggio standard del paziente diabetico è rappresentata dalla strategia del Point of Care Testing (PoCT), che consente l'effettuazione dei test presso il punto di cura in un tempo immediatamente prossimo o contemporaneo alla visita medica.
Scopo. Analisi di impatto sul budget volta a considerare le implicazioni economiche derivate dall'adozione di differenti percorsi di gestione del paziente: senza PoCT e con PoCT.
Materiali e Metodi. La diabetologia di ASL3 dispone di 6 dispositivi dislocati sull'area metropolitana di Genova, per una popolazione di circa 22.000 pazienti che accedono regolarmente alle visite programmate. Afinion™2 (Abbott) è un analizzatore multiparametrico compatto, capace di determinazioni quantitative di HbA1c, ACR, PCR e profilo lipidico, tramite un prelievo di sangue capillare.
Risultati. Sono stati creati 2 scenari: senza PoCT e con PoCT e analizzati 3 percorsi differenziati: percorso standard in cui le tappe si verificano in regolare successione, il percorso di pazienti afferenti al centro in assenza degli esami prescritti e il percorso in condizione di emergenza metabolica. I PoCT si sono rilevati utili per la valutazione dei pazienti giunti senza esami (14%) e di quelli in urgenza metabolica (4%). Per queste due categorie di pazienti l'utilizzo del PoCT si è rivelato costo efficace, con un risparmio rispettivamente del 35% e del 29% rispetto alla procedura tradizionale. Il costo più elevato dei test eseguiti con PoCT viene ampiamente ammortizzato dal risparmio sulla seconda visita non più necessaria e dal risparmio sui costi indiretti, ovvero la perdita di giornate lavorative.
Conclusioni. L'impiego dei PoCT nell'ambito di una struttura ambulatoriale operante con diverse sedi dislocate sul territorio rappresenta un interessante strumento di miglioramento della qualità dei servizi erogati. Ulteriore punto di forza è dato dai vantaggi in termini di costi indiretti e di qualità della vita dei pazienti, aspetti che rivestono sempre maggiore importanza nell'ottica di una sanità che si deve far carico anche del miglioramento della capacità produttiva di una popolazione, quale sottoprodotto del miglioramento della salute stessa.

EP155

Digital healthcare for Bacterial Infection diagnosis: the case of urinary tract infection. From screening to Antibiotic prescription in 3 h.

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Current methods for the diagnosis of urinary tract infections with antimicrobial susceptibility testing take 2–3 days and require a clinical laboratory. The lack of a rapid, point-of-care antibiotic susceptibility test (AST) has contributed to the misuse of antibiotics on treating urinary tract infections (UTIs) and consequently to the rise of multi-drug-resistant organisms [1]. The current clinical approach has led to reduced treatment options and increased costs of diagnosis and therapy. To address this issue, novel diagnostics are needed for the timely determination of antimicrobial susceptibility. We present a rapid, point-of-care, phenotypic AST device that can report the antibiotic susceptibility/resistance of a uropathogen to a panel of antibiotics in as few as 3h by utilizing fluorescent-labelling chemistry and a highly sensitive particle-counting instrument [2,3]. We analysed 744 urine samples from outpatients and inpatients of two Italian hospitals. The 130 UTI-positive patient urine samples were measured using a panel of six common UTI antibiotics plus a growth control. By comparing our results to hospital laboratory urine cultures, we obtained an overall sensitivity = 81%, a specificity = 83%, an SPV (sensitivity predicted value) = 95%, and an RPV (resistance predicted value) = 54%. According to our preliminary data, the sensitivity predicted value for a single antibiotic agent was 95%, thus allowing (in the vast majority of cases) an early (within 3 h) recognition of an effective agent for a single patient.The instrument and protocol described in this study represent a prototype point-of-care (POC) device to perform rapid phenotypic UTI AST in a clinical evaluation. Our AST device does not require any preprocessing steps simplifying the necessary instrumentation. Unlike culture-based AST, our device provides objective, quantitative information to the end user, and is not vulnerable to inaccurate or subjective interpretations due to variables, such as user-dependent differences in sample preparations, plating techniques, and zone-diameter measurements.Moreover, such diagnostic tool well fit in the context of digital healthcare [4]. Providing clinical information to physician through a POC platform, allowing him to send therapy prescription to pharmacy, where patient can directly go and get his drug.

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EP156

A LC-MS/MS method to monitor urinary biomarkers of gluten free diet (GFD) adherence

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Objective: The identification and quantification of novel gluten exposure biomarkers is of great importance to help patients and clinicians in evaluating the adherence to gluten-free diet (GFD), the only treatment currently available for Celiac disease (CD)1. The 3,5-dihydroxybenzoic acid (DHBA) and the 3-(3,5-dihydroxyphenyl)-propanoic acid (DHPPA), gluten derived urinary metabolites, were proposed as potential molecular biomarkers for short-term monitoring of GFD2. The aim of this work has been to set-up and validate an analytical method using ultra high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) to identify and quantify these biomarkers in human urine samples.
Methods: Molecules and internal standards were extracted from urine using a liquid-liquid extraction (LLE) procedure. Chromatographic separation was obtained by hydrophilic interaction on a HILIC column with ammonium acetate buffer in water and acetonitrile as mobile phases.
Results: We developed, optimized and validated a new UHPLC-MS/MS analytical method for the identification and quantification of DHBA and DHPPA in human urine. For the validation we used a standard addition method (SAM), a typical approach for the quantification of endogenous metabolites. As a proof-of-concept we applied our method to a pool of urinary samples from healthy volunteers undergoing GFD.
Conclusions: Monitoring DHBA and DHPPA by UHPLC-MS/MS might represent a promising tool to help CD patients to keep their diets under control and physicians to understand the causes of any potentially occurring adverse events. The methodological approach proposed provided good efficiency and wide applicability, although a further clinical evaluation on a larger number of celiac patients is needed to foresee an effective use of this method to clinical practice.

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EP157

1-year monitoring of antibody response after SARS-CoV-2 vaccination in healthcare workers

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The global strategy to control coronavirus disease 2019 (COVID 19) was based on the availability of COVID-19 vaccines [1]. Measurement of post-vaccination neutralizing antibodies (Abs) titer, has been shown to be related to protection from SARS-CoV-2 infection [2]. This work aims to improve vaccination data through the evaluation of neutralizing antibodies in triple-dose individuals. To this end, we have conducted a surveillance program focusing at measuring the concentration of IgG Abs against the Receptor Binding Domain (RBD) and neutralizing Abs (NT) anti-SARS-CoV-2 that block the interaction between RBD and the surface receptor cellular angiotensin converting enzyme (ACE2), in the serum of individuals in the vaccination course. The study was conducted on workers from the University of Rome "Tor Vergata" (nTOT=169) who received the Vaxzevria/AstraZeneca vaccine (n=56) and on healthcare workers of the PTV University Hospital who received the Comirnaty/Pfizer-BioNTech vaccine (n=113). Initially for both vaccines two doses were administered: Vaxzevria (12 weeks apart), Comirnaty (2 weeks apart). After the second dose, for the two vaccines has been registered an increase in Abs values both for RBD and NT Abs. As the second dose of the two vaccines has been given at very different time, Pfizer vaccine resulted to response with a higher Abs values earlier in time than Astrazeneca. Moreover, Abs values recorded for those who received Pfizer vaccine are higher up to an order of magnitude. After 6 months from the first dose, the average value Abs titer was 300 BAU/ml and 200 BAU/ml for Pfizer and Astrazeneca respectively. All patients received the Pfizer vaccine as third dose. This last dose gave rise again to an increase of the Abs levels, the average values obtained were 5300 BAU/ml and 3900 BAU/ml for Pfizer and Astrazeneca respectively. As concern NT Abs, we observed a similar pattern to RBD one. After 5 months from the third dose, almost one year from the first dose, antibodies level was over 1000 BAU/ml. Recent work provided Abs cut-off value of immunity against SARS-CoV-2 infection. Values reported range from 200 to 600 BAU/ml [3,4].From this perspective our data have shown a low risk of infection after 1 one year for subjects with a complete vaccine cycle.

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EP158
DOES CEREBROSPINAL FLUID LACTATE ADD DIAGNOSTIC POWER TO DETECT BACTERIAL INFECTIONS?

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Background and aim.
Diagnosis of cerebrospinal fluid (CSF) infection is based on cell count and differential, biochemical markers, bacterial culture and Multiplex PCR. However, the diagnostic value of lactate compared to other CSF parameters has not yet been elucidated. We aimed to determine if lactate, alone or in combination with other CSF markers, can add diagnostic power to detect infectious pathogens in the CSF of patients with meningitis and/or encephalitis signs.
Methods.
Concurrently ordered 1) CSF clinical chemistry panel (WBC, glucose, total protein (TP), lactate), 2) FILMARRAY™ Meningitis/Encephalitis Panel (Multiplex PCR) and 3) CSF culture have been considered. Out of 2380 CSF clinical chemistry panels, a result for Multiplex or for CSF culture was available in 512 and 908 subjects.
Results.
Multiplex and CSF culture were positive in 26/512 (5.1%; 6 bacterial, 20 viral infections) and 29/908 (3.2%). Patients were subgrouped into: G1) neg CSF culture AND neg Multiplex (n=361); G2) pos Multiplex with viral identification (n=20); G3) pos CSF culture AND/OR pos Multiplex with bacterial identification (n=33). Groups were different for WBC (median 3 vs 24 vs 141 WBC/uL; p<0.001), glucose (67 vs 58 vs 40 mg/dL; p<0.001), TP (45 vs 49 vs 135 mg/dL; p<0.001), lactate (1.86 vs 1.90 vs 3.84 mmol/L; p<0.001). At the univariate logistic regression WBC (p=0.005), glucose (p<0.001), TP (p=0.006) and lactate (p<0.001) were associated with bacterial positivity (G3 vs G1+G2); however, only glucose (p=0.001) and lactate (p<0.001) were found to be independent predictors at the multivariate analysis. Although WBC and the multivariate model displayed same AUCs (0.84), the latter displayed higher sensitivity (34% vs 9.4%) and comparable specificity (98.9% vs 99.7%). 4 out of 6 samples with pos bacterial identification at the Multiplex were correctly classified by the model (only 1 using WBC), whereas none of the samples with pos viral identification at the Multiplex was misclassified.
Conclusions.
Our data suggest that CSF lactate may add diagnostic power to detect CSF infections. High CSF lactate and low CSF glucose are independent predictors of bacterial meningitis. The multivariate model showed a high AUC, with high specificity but low-to-moderate sensitivity.

EP159
EARLY ASSESSMENTS OF ANALYTICAL PERFORMANCE FOR A POSSIBLE TRANSITION FROM AN INTERNAL QUALITY CONTROL (IQC) SYSTEM BASED ON THE TOTAL ERROR MODEL TO A SYSTEM BASED ON MEASUREMENT UNCERTAINTY

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INTRODUCTION. While Total Error (TE) is still predominant in monitoring the analytical quality of results, the Measurement Uncertainty model (MU) has been proposed to implement an IQC system. To assess the feasibility of switching IQC from the TE to the MU model, we compared MU of clinical chemistry methods performed at the CoreLab of the Spedali Civili of Brescia with MAu (Maximum allowable uncertainty of measurement), the analytical performance specifications (APS) based on biological variability (BV) recently proposed by the EFLM for MU (https://biologicalvariation.eu/).
METHODS. IQC data generated between H2 2019 and H2 2021 from 3 Roche Cobas c702 modules with third-party controls were used to calculate the bias and coefficient of variation (CV) of 64 measurands over six months. According to ISO 20914:2019, MU was calculated as 2*(ucal^2 + uRw^2)^1/2, where ucal is the combination of MU of the higher-order reference selected by the IVD manufacturer to implement traceability and the MU derived from the calibrator value assignment process (including any bias correction), while uRw is the CV of the repeated measurements performed on a QC material during a recommended period of 6 months. Finally, MU was compared with MAu.
RESULTS. The lack of ucal and MAu data prevented the analysis of 22 of the 64 measurands made with Cobas instruments. For the remaining 42 measurands, out of a total of 1098 half-year determinations, MU exceeded MAu in 238 cases (21.7%); the measurands with the highest number of mismatched MU were bicarbonates and sodium (100%), albumin (81%), calcium (75%), chloride and magnesium (67%) and total protein (47%). In 91.6% of cases, the deviation between the six-month average and the peer group consensus value was less than the APS for bias.
CONCLUSIONS. Implementing an IQC system within the MU framework requires obtaining metrological traceability data of the calibrators from the manufacturer and selecting MU-specific APS, but these are often missing. Furthermore, another crucial issue is to establish criteria for the acceptance of IQC results in daily practice. For this reason, setting up an IQC system based on measurement uncertainty is a challenging task that needs to be fine-tuned before this model is successful.

EP160

REVISION OF STATE-OF-THE-ART BASED ANALYTICAL QUALITY SPECIFICATIONS FOR HORMONES AND TUMOUR MARKERS INFERRED FROM AN INSTITUTIONAL EQA PROGRAM

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INTRODUCTION. Analytical performance specifications (APS) are an indispensable tool for evaluating the analytical quality of results. Of the three models proposed at the 2014 EFLM Strategic Conference in Milan, the "state-of-the-art" model requires deriving analytical goals from the performance of methods and instruments available on the market, but without providing comprehensive guidance on how to set them. For this reason, the SIBioC WG "Analytical Quality" and the "Centro di Riferimento Regionale per la Qualità dei SmeL" of the Lombardy Region have proposed a procedure for calculating this type of APS for hormones and tumour markers, in which acceptable performance goals are set based on data submitted by participants in a EQA program conducted by the centre (Biochim clin 2018;42:S63). In the present work, the APS were revised using data collected after the initial survey and compared with the APS of the biological variability (BV)-based model.

METHODS. For 23 measurands, 181148 results submitted by 230 laboratories in 45 exercises (years 2017-2020) were used to calculate the percentage difference between each result and the robust consensus mean of their peer group (same instrument/method): for each measurand, the 95th percentile of differences was considered the APS. These APS were then compared with those based on BV (if available) and published on the EFLM website, for both measurement uncertainty (MU) and total error (TE).

RESULTS. Compared to the first survey, 14 out of 21 APS are comparable and 7 show a change of more than 5% from the previous value, with 5 of them worsening. For 6 measurands for which concentration-dependent APS had been identified, the concentration ranges for 4 were redefined, so they are not comparable. In the 18 cases where the measurands collected on the EFLM website have a BV value, 13 state-of-the art APS (72% of cases) correspond to at least one of the three BV-based levels for MU (4 minimum, 5 desirable, 4 optimal) and in 83% of cases to those for TE.

CONCLUSIONS. Data collected in EQA schemes are a source of information on the current technology and can be used both to derive APS based on the current state-of-the-art and to assess the ability of methods and instruments to meet clinical requirements for analytical quality.

EP161

T Cell mediate immune response: Real-Time PCR test for the rapid determination of SARS-CoV-2 specific CD4 and CD8 T Cells

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Introduction

Cellular immunity is pivotal for SARS-CoV-2 protection, but measurements of cellular-specific response are rarely performed due to costs and technical requirements. Hyris developed a direct Real-Time PCR (dRT-PCR) assay that measures CD4 and CD8 T cells immunoreactive to SARS-CoV-2 Spike protein by targeting CXCL10 mRNA (upregulated in response to IFN-γ secreted by antigen-specific T Cell). This test uses a modular T cell activation method based on pool of synthetic peptides to stimulate S-specific T cell, and those able to tolerate amino acid (AA) mutations that characterize new variants. The aim of this study was to evaluate the assay performances.

Methods

Whole blood (Li-He) of 13 SARS-CoV-2 naïve and 116 vaccinated individuals were stimulated with a pool of S-peptides (15 AA long) covering whole S protein (Wuhan) and left unstimulated (SCV2 T Activation kit). Whole blood of 78 healthy vaccinated individuals was stimulated with two sets of peptides covering the constant and mutated region of S protein of Omicron BA1.2 (SCV2 Omicron T Activation kit) and left unstimulated. After overnight incubation T cell activation was detected by bKIT dqTACT MS (CXCL10 and ACTIN mRNA) on the HYRIS bCUBE.

Results

The vaccinated subjects statistically shown level of relative mRNA expression of CXCL10 greater than unvaccinated subjects; 109 vaccinated individuals were detected as reactive to stimulation (mean relative expression 0.043) with SARS-CoV-2 S peptides (Sensitivity 94%), while 10 naïve were detected as not-reactive (mean expression -0.25) (Specificity 77%). Considering the 129 samples the AUC value of is 90% (CI 82%-98%). 78 samples activated with the constant and mutated region of S of Omicron peptides showed different quantities of T cells response. The 62% of sample assessed resulted reactive (with a ratio between the two stimulations above 15%) despite the AA mutations, showing that AA mutations can affect the magnitude of S T cell response.

Conclusions

Combining the modular utilization of synthetic peptide pools as activators and the direct measurement of CXCL10 mRNA in whole blood, dRT-PCR method was proven to be a reliable and efficient assay for the measurement of the individual antigen-specific T cells measurement of SARS-CoV-2 cellular immunity.

EP162

Validation of new serological tests with MAGLUMI 800 for determination of antibody response for HIV, Treponema pallidum, HBV and HCV.

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Human Immunodeficiency Virus (HIV), Treponema pallidum, Hepatitis Virus B (HBV) and Hepatitis Virus C (HCV) are four viruses that cause severe pathologies: AIDS (Acquired Immune Deficiency Syndrome), Syphilis, Hepatitis C and B respectively. The gold standard diagnoses involve the use of molecular biology and biopsy. After viral infection, the immune system produces immunoglobulins: early IgM and later IgG antibodies against the same set of antigens. The antibodies assay is a tool for monitoring the disease trend and for screening of blood donations [1,2]. The purpose of this work was to validate new qualitative and quantitative serological tests with the CLIA (chemiluminescence immunoassay) MAGLUMI 800. A methodological comparison was made with the method used in laboratory routine to evaluate the efficiency of these new diagnostic kits. 256 serum samples were collected at the University Hospital of Tor Vergata, respectively: 88 HIV, 112 HBV, 73HCV, and 83 with Syphilis. The serum samples were analysed on CLIA MAGLUMI 800 with four different methods HIV, HCV, Treponema pallidum antibodies, and quantitative determination of HBV antibodies (SNIBE Co ltd; Shenzhen, China,). Precision was assessed using 3 replicates for 5 days (quality control), Carry-over was performed by dividing samples in 11 "low" aliquots (L) and 10 "high" aliquots (H) and were loaded into the analyser in the following order: L, L, L, H, H, L, H, H, L, L, L, L, H, H, L, H, H, L, H, H, L. The difference between the mean of the low measurements results after a high measurement, and the mean of the low measurements after a low measurement is a measure for carry-over.The percent coefficients variation values confirmed that declared by the manufacturer both for between- and within- run. The agreement for HBsAg and HIV was 100%, while for HCV and Treponema pallidum antibodies has been obtained a discrepancy rate of 1%. The carry-over effect was not observed in all tested methods.The comparison data obtained in this work support the new methods as proper approaches for a first-level rapid screening of infected patients [3–5]. These methods due to shorter response times, low cost, and a good performance represent an excellent and automated diagnostic solution, useful in the lab workflow.

EP163

Validation of new Serum Amyloid A chemiluminescence immunoassay with Maglumi 800.

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Serum Amyloid A (SAA) is an acute-phase protein mainly produced by the liver in response to pro-inflammatory cytokines. SAA is primarily produced by hepatocytes in response to the inflammatory cytokines tumor necrosis factor–α (TNF-α), interleukin (IL)-1β and IL-6. In healthy individuals, plasma SAA level is within 0¸10 mg/L. However, under inflammatory conditions, plasma SAA levels can increase exponentially, even reaching 1000 mg/L or more in some cases. Human SAA expression is upregulated during the acute phase of various viral infections, including cytomegalovirus, herpes simplex virus, measles virus, mumps virus, rubella virus and varicella-zoster virus, and returns to normal during the convalescent phase of infection. Moreover, can be a useful biomarker to predict COVID-19 patient's severity and prognosis.The aim of this study was to evaluate a new chemiluminescence immunoassay for SAA.All serum samples were measured on Maglumi (Snibe platform) and compared with BN ProSpec (Siemens platform), which is used in the routine of the clinical laboratory of the 'Tor Vergata' University Hospital. Analytical precision, correlation coefficient and linearity were assessed. The precision of CLIA system was evaluated by using the commercial normal and high-quality control materials (IQC) recommended by the manufacturer for evaluating precision of SAA. Precision estimation was performed by evaluating triplicate measurements of aliquots of the same samples, performed for a total of five non-consecutive days. Precision data correlated with those declared by the manufacturer. The linearity test was performed using a series of serial dilutions (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 and only diluent) with dilution sample by manufacture. The linearity test showed a correlation coefficient equivalent to 0.997. The results from Snibe platform correlated well with those obtained by Siemens platform with a correlation coefficient of 0.974 ($p<0.001$). This study demonstrated that the new Snibe SAA test has excellent analytical performance and good reliability and can be used in routine analysis.

EP164

AUTOMATED BLOOD COUNTER OR MANUAL PROCEDURE IN BURKER'S CHAMBER FOR CELL COUNTING IN BODY FLUIDS? OUR EXPERIENCE WITH ABBOTT ALINITY HQ

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Introduction: Body fluid cell counting provides important information for diagnosis and treating various medical conditions. Manual microscopic cell counting chambers are considered the gold standard, but such tecnique requires more time and skilled testing personnel (possible high imprecision rate among different operators). For overcoming such drawback, automated blood counters would be very important for counting and differentiation cells in body fluids. Nevertheless this automated count isn't approved by FDA/EMA yet. The aim of our study is to compare cell counting in body fluids with Abbott Alinity Hq hematology analyzer and microscopic count in Burker's chamber. Abbott Alinity Hq hematology counter provides determination Total Nucleated Cell (TNC), Red Blood Cell (RBC), Polymorphonuclear cell (PMN) and Mononuclear cell (MN), expressed as percentage and absolute values. Methods: Samples were collected in AO Ordine Mauriziano, Torino, from January 2022 to June 2022. Cells counts were determinated on a total of 80 body fluids (10 synovial, 3 cerebrospinal, 1 pericardial, 26 pleuric and 40 peritoneal fluids). Statistical analysis were performed using Analyze-it software. Results: Background concentration limits were ≤1000 cells/µl for RBC counts and ≤3 cells/µl for TNC counts. The limit of detection was 1000 RBC/µl and 5 TNC/µl. Imprecision was evalued based on CV: CV% was inversely correlated with blood cell concentrations. Carryover was <0.1% for RBC and 0.5% for TNC. Optimal Spearman's correlation (rs=0.974: CI 95% 0.960 to 0.984) was seen between manual and Abbott Alinity Hq TNC count. A slight positive bias was observed with Passing-Bablok regression (slope 1.077: CI 95% 1.020 to 1.176). Worst correlation (rs=0.698: CI 95% 0.561 to 0.798) was demonstrated for RBC count and a more positive bias (slope 1.286: CI 95% 1.042 to 1.818). A good agreement between two TNC and RBC counting methods was observed with Bland-Altman plot. Conclusions: in agreement with previous study we confirmed comparable performance for body fluid cell counting between Abbott Alinity hq hematology analyzer and manual microscopic examination. Automation offers benefits of standardization, time saving and reduced human error.

EP165

EVALUATION OF ANALYTICAL PERFORMANCE BY PERIODIC RETROSPECTIVE ANALYSIS OF INTERNAL QUALITY CONTROL (IQC) DATA USING THE TOTAL ERROR MODEL

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INTRODUCTION. Estimating the total error (TE) of a measurement procedure (linear sum of imprecision and systematic deviation from a "true" value) is a way to assess the analytical quality of results and monitor the ability of measurement systems to meet analytical performance specifications (APS). For this purpose, we used the retrospective analysis of IQC performed every 6 months for 3 years at the CoreLab of the Central Laboratory of the Spedali Civili of Brescia.
METHODS. 104 measurands determined with 3 Cobas c702, 1 c502, 2 ISE and 2 e801 modules were subjected to IQC from the 2nd semester 2019 to the 2nd semester 2021 using third party controls and Biorad IQC software. Every six months, for each measurand, level and instrument, the mean and CV were extracted from the software and reprocessed using an Excel spreadsheet equipped with macros to calculate the bias % from the reference value (inter-laboratory peer group mean or control-assigned value), the laboratory TE and the Sigma value of each analytical procedure, and to perform a comparison between TE and the APS (represented by acceptable TE, TEa) originally set or revised during the bi-annual surveys.
RESULTS. Over the 3 years, TE was higher than the established TEa in 118 of 2189 (5.4%) determinations (4.8% of cases for Cobas c702/c502 and 7.9% for e801), while the Sigma value (an index of the analytical methods' ability to meet the APS) was < to 3 in 39% of cases, between 3 and 4 in 26%, between 4 and 5 in 18%, between 5 and 6 in 8% and > 6 in 9%. Both metrics improved over time, with the percentage of non-compliant TEs gradually decreasing over the semesters (10.5%, 6.7%, 6.4%, 2.3% and 2.5% respectively) and Sigma improving (for example, Sigma < 3 procedures went from 46% in the first survey to 31% in the last). This improvement was due to real performance gains related to instrument familiarisation and fine-tuning of process control, as well as revisions to TEa (81 changes, including 54% with a less restrictive and 46% with a more restrictive target).
CONCLUSIONS. Periodic "long-term" IQC analysis serves the dual purpose of monitoring and improving the efficiency of the implemented IQC process and verifying the ability of the analytical systems to achieve predefined quality goals.

EP166

Is the interaction of technology useful in laboratory haematology diagnostics?

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Background: Monoclonal B lymphocytosis (MBL) increases with age and individuals with high count MBL progress to chronic lymphocytic leukaemia requiring therapy at a rate of ~1%-5% per year. These cases usually have atypical lymphocytes at the microscope, abnormal representation in the scattergram, and positivity of flags. Using XN9000 (Sysmex), we noticed cases of MBL without this correlation. We studied customized gates for discovering MBL cases of our interest. Methods: We considered 212 peripheral blood samples with known phenotypes: 76.7% negative and 23.3% positive for B, T, or NK lymphocytes clones. We created gates studying the XN9000 FCS files in Diva software to identify new areas for better delimiting subpopulations of our interest and calculating sensitivity and specificity. Results: We found significant differences between negative and positive groups for Q-flag “Blasts/Abn Lympho?” (B/AL) and LY-X ($p < 0.05$) with lymphocyte counts below 5×10^9/L. A new gate P1 normalized by P2 (P1n) differentiated between phenotypes much better than Q-flag B/AL with lymphocyte counts ≤ 5 x10^9/L. Moreover, cases with MBL CD5 positive had higher medians ($p < 0.05$).Conclusion: We propose a gate P1n as a new Q-flag for lymphocytes count ≤ 5 x10^9/L, in order to hypothesize the presence of MBL CD5 positives.

EP167

Il laboratorio in un caso di grave anemia

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Caso clinico: donna di 91 anni, accede al Pronto Soccorso per profonda astenia. All'esame emocromocitometrico, eseguito con lo strumento Sysmex XN, si evidenzia un quadro di grave anemia macrocitica (Hb 44 g/L, HCT 14,0%, MCV 121 fL, MCH 37,9 pg), lieve leucopenia (WBC 3,68 x 10^9/L) e piastrinopenia (59 x10^9/L), in presenza di alterata funzionalità renale (creatinina 2.36 mg/dL), bilirubina e LDH nei limiti di riferimento. L'analisi dei citogrammi mostra una distribuzione anomala della popolazione leucocitaria, che pone il sospetto della presenza di plasmacellule. All'esame microscopico dello striscio di sangue periferico si conferma la presenza di plasmacellule (4%), per cui vengono richiesti esami di approfondimento. L'elettroforesi capillare su siero evidenza un doppio picco monoclonale in zona gamma e ipogammaglobulinemia, associati ad un'over espressione di catene leggere libere lambda (rapporto kappa/ lambda <0,01) e aumento di beta 2 microglobulina (17,5 mg/L). All'immunofissazione si evidenzia una banda intensa costituita da sole catene leggere libere lambda. Viene quindi eseguita una immunofissazione di approfondimento con gli antisieri anti-IgD, anti-IgE e anti-lambda free, da cui si evidenzia la presenza di una componente monoclonale costituita da IgD-lambda e una componente monoclonale costituita da sole catene leggere libere lambda. Viene quindi effettuato l'esame del midollo e posta la diagnosi di mieloma multiplo IgD-lambda. Il mieloma multiplo IgD è una malattia rara, rappresenta meno del 2% di tutti i mielomi, e ancor più rara se associata ad una eccessiva produzione di catene leggere libere. Il decorso clinico è severo, spesso accompagnato da insufficienza renale e prognosi sfavorevole. In questo caso il riscontro di plasmacellule ha indirizzato il successivo iter diagnostico, consentendo la corretta diagnosi e la terapia appropriata. Il mieloma IgD, associato a una produzione eccessiva di catene leggere libere, può essere erroneamente diagnosticato come mieloma a catene leggere se non viene eseguito un approfondimento con antisieri IgD specifici. La conoscenza di questo raro sottotipo di mieloma e delle sue caratteristiche epidemiologiche e cliniche è fondamentale ai fini diagnostici e per migliorare il decorso clinico della malattia.

EP168

Confronto di metodi tra strumentazione decentrata POCT e analizzatori centralizzati di laboratorio per i principali ioni ematici dosati in urgenza.

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Introduzione.
I principali ioni ematici richiesti dal medico in regime di urgenza (Sodio, Potassio, Cloro e Calcio) sono parametri essenziali per la gestione clinica dei pazienti di area critica. Per cui l'esecuzione dei test attraverso strumentazioni POCT (Point-Of-Care) vicino o al punto di cura del paziente, in situazioni critiche ed emergenziali, permette la disponibilità dei risultati in tempo molto breve per una diagnosi e/o decisione terapeutica [1]. Lo scopo di questo lavoro è quello di effettuare un confronto di metodi tra la strumentazione POCT decentrata e quella centralizzata del laboratorio.
Materiali e Metodi. Sono stati eseguiti 100 campioni ematici per eseguire l'allineamento tra il Cobas 8000 (Roche) e lo strumento Fuji (DRI-CHEM 4000). Per il confronto tra metodi, i risultati sono stati analizzati con i test statistici di regressione Passing-Bablok e grafico Bland-Altman.
Risultati. Per la determinazione di sodio i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,82, intercetta= 26,9 ed r=0,888; all'analisi Bland-Altman il Bias= -0,66. Per la determinazione di potassio i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,98, intercetta= 0,1 ed r= 0,98; all'analisi Bland-Altman il Bias= 0,01. Per la determinazione di cloro, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,76, intercetta= 25,2 ed r= 0,746; all'analisi Bland-Altman il Bias= -0,21. Per la determinazione del calcio, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 1,18, intercetta= -0,9 ed r= 0,872; all'analisi Bland-Altman il Bias= -0,341.
Conclusioni. I risultati del confronto hanno mostrato un'eccellente correlazione per la determinazione degli ioni inclusi nello studio. La distribuzione dei valori ha una distribuzione omogenea per sodio, potassio e cloro, mentre per il calcio abbiamo una costante sovrastima che tuttavia non è significativa per le decisioni cliniche.
Bibliografia. [1] F. di Serio, T. Trenti, P. Carraro, Gruppo di studio SIBioC "Point-of-Care testing" Raccomandazioni per l'implementazione e la gestione del POCT. Biochimica Clinica 2011; 35(3) 242.

EP169

UN CASO DI BATTERIEMIA RILEVATO ALL'ESAME MICROSCOPICO DI SANGUE PERIFERICO

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Presentiamo il caso di una paziente di 78 anni, ricoverata presso un reparto di medicina. L'esame emocromocitometrico, effettuato con l'analizzatore Sysmex XN, evidenzia la presenza di anemia macrocitica (Hb=7,7 g/dL, MCV 102 fL, MCH 30.7 pg), modesta piastrinopenia (piastrine=110x10^9/L), leucociti=7,01x10^9/L, neutrofili=5.58x10^9/L, presenza di granulociti immaturi (9,6%) ed eritroblasti (5.1%), con variazione rispetto ai valori dell'emocromo eseguito nei giorni precedenti. Gli esami ematochimici rilevano un aumento degli indici di flogosi (proteina C reattiva=368.5 mg/dL, fibrinogeno=954 mg/dL) e alterata funzionalità renale (creatinina=2.26 mg/dL). L'analisi dei citogrammi strumentali mostra uno scattergram, relativo alla differenziazione leucocitaria, anomalo con un cluster granulocitario con distribuzione alterata. E' stato quindi eseguito l'approfondimento morfologico dello striscio di sangue periferico, che ha evidenziato anomalie morfologiche dei granulociti neutrofili e la presenza di formazioni omogenee intra ed extracellulari suggestive di granulociti fagocitanti batteri e di batteri extracellulari in ammassi. Nel sospetto di setticemia batterica è stato contattato tempestivamente il reparto richiedente per comunicare il riscontro microscopico, ma la paziente, affetta da subocclusione intestinale in carcinosi peritoneale in stadio terminale, era deceduta. La setticemia è una grave sindrome clinica caratterizzata da segni sistemici di infezione, shock e insufficienza d'organo sistemica, che richiede una corretta e precoce diagnosi ai fini terapeutici e prognostici. La diagnosi di sepsi batterica viene eseguita mediante emocoltura, che conferma la presenza di microrganismi nel sangue e ne consente l'identificazione, ma è un'indagine che richiede tempo. In corso di sepsi, è possibile osservare all'esame microscopico dello striscio di sangue periferico la presenza di corpi batterici extracellulari o fagocitati dai granulociti. Questo riscontro, sebbene di bassa sensibilità, può essere di notevole aiuto per fornire una diagnosi preliminare rapida e costituisce un'emergenza, che richiede una tempestiva comunicazione al medico curante, consentendo di intervenire immediatamente prima degli esiti colturali.

EP170

Confronto di metodi tra strumentazione decentrata POCT e analizzatori centralizzati di laboratorio per parametri di chimica clinica

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Introduzione.
I test ematici (Glucosio, Creatinina, Urea, Amilasi, GOT e GPT) sono parametri essenziali per la gestione clinica dei pazienti. L'esecuzione dei test attraverso strumentazioni POCT (Point-Of-Care) vicino o al punto di cura del paziente, in situazioni critiche ed emergenziali, permette la disponibilità dei risultati in tempo molto breve per una diagnosi e/o decisione terapeutica [1]. Lo scopo di questo lavoro è quello di effettuare un confronto di metodi tra la strumentazione POCT decentrata e quella centralizzata del laboratorio.
Materiali e Metodi.
Sono stati eseguiti 100 campioni ematici per eseguire l'allineamento tra il Cobas 8000 (Roche) e lo strumento Fuji (DRI-CHEM 4000). Per il confronto tra metodi, i risultati sono stati analizzati con i test statistici di regressione Passing-Bablok e grafico Bland-Altman.
Risultati.
Per la determinazione glucosio, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 1, intercetta= 2,3 ed r= 0,99; all'analisi Bland-Altman il Bias= -0,99. Per la determinazione creatinina, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 1, intercetta= -0,1 ed r= 0,98; all'analisi Bland-Altman il Bias= 0,025. Per la determinazione di urea, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 1,07, intercetta= 0,0 ed r= 0,99; all'analisi Bland-Altman il Bias= -1,62. Per la determinazione dell'amilasi, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 1,01, intercetta= 2,8 ed r= 0,99; all'analisi Bland-Altman il Bias= -1,8. Per la determinazione di GOT, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,97, intercetta= 2,7 ed r= 0,99; all'analisi Bland-Altman il Bias= -0,885. Per la GPT, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 1,04, intercetta= 0,8 ed r= 0,99; all'analisi Bland-Altman il Bias= -0,83.
Conclusioni.
I risultati hanno mostrato un'eccellente correlazione per tutti i parametri in studio. Le differenze riscontrate sono minime e non sono significative ai fini clinici.
Bibliografia.
[1] F. di Serio, T. Trenti, P. Carraro, Gruppo di studio SIBioC "Point-of-Care testing" Raccomandazioni per l'implementazione e la gestione del POCT. Biochimica Clinica 2011; 35(3) 242.

EP171

Confronto di metodi tra strumentazione decentrata POCT e analizzatori centralizzati di laboratorio per parametri ematologici

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Introduzione
I test ematologici (WBC, RBC, emoglobina, ematocrito e PLT) sono parametri essenziali per la gestione clinica dei pazienti di area critica. L'esecuzione dei test attraverso strumentazioni POCT (Point-Of-Care) vicino o al punto di cura del paziente, in situazioni critiche ed emergenziali, permette la disponibilità dei risultati in tempo molto breve per una diagnosi e/o decisione terapeutica [1]. Lo scopo di questo lavoro è quello di effettuare un confronto di metodi tra la strumentazione POCT decentrata presso il Pronto Soccorso e quella centralizzata del laboratorio per la determinazione di parametri ematologici.
Materiali e Metodi
Sono stati eseguiti 100 campioni ematici per eseguire l'allineamento tra il contaglobuli XN (Sysmex) e lo strumento Swelab α. Per il confronto tra metodi, i risultati sono stati analizzati con i test statistici di regressione Passing-Bablok e grafico Bland-Altman.
Risultati
Per la determinazione WBC, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 1,02, intercetta= -0,1 ed r=0,99; all'analisi Bland-Altman il Bias= -0,03. Per la determinazione RBC, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,97, intercetta= 0,1 ed r= 0,98; all'analisi Bland-Altman il Bias= 0,02. Per la determinazione di emoglobina, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,95, intercetta= 0,6 ed r= 0,986; all'analisi Bland-Altman il Bias= -0,02. Per la determinazione dell'ematocrito, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,99, intercetta= -0,6 ed r= 0,97; all'analisi Bland-Altman il Bias= 0,44. Per la determinazione delle piastrine, i risultati della regressione Passing-Bablok hanno mostrato una pendenza= 0,96, intercetta= -0,5 ed r= 0,97; all'analisi Bland-Altman il Bias= 4,4.
Conclusioni
I risultati hanno mostrato un'eccellente correlazione per la determinazione di WBC, RBC, emoglobina, ematocrito e piastrine. Le variazioni non sono significative per le decisioni cliniche.
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EP172

Caso clinico di un paziente in DOAC: l'importanza del ruolo del laboratorio nel management dei DOAC.

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I DOAC (Direct Oral AntiCoagulant) hanno rivoluzionato la terapia anticoagulante orale perché presentano molte caratteristiche in termini di efficacia e sicurezza sovrapponibili agli anticoagulanti anti vitamina k ed inoltre grazie alla loro farmacocinetica non necessitano di monitoraggio routinario di laboratorio infatti vengono somministrati a dosaggio fisso e hanno minor rischio di interazioni con altri farmaci e con il cibo.Ciò nonostante si possono presentare diverse circostanze in cui risulta determinante il loro dosaggio per una adeguata gestione del paziente.Il caso clinico presentato riguarda un paziente di 80 anni diabetico pervenuto al PS per dolori e malessere generale. Vengono eseguiti gli esami ematici in ingresso: emocromo, coagulazione, procalcitonina e PCR . Si conferma paziente diabetico, GLU 381 mg/ dL, INR 1,36, APTT ratio 1.27, PCR 3,54 mg/dL, HB 12,5 g/dL, bianchi totali 10.450/mmc, granulociti 82%, i restanti parametri nella norma.Il paziente viene ricoverato in medicina, il giorno successivo vengono ripetuti gli esami.I globuli bianchi sono in aumento 18.540/mmc, nelle urine si riscontrano leucociti e batteri. Refertata IVU da enterobacilli. Si evidenzia un valore di INR stranamente molto alterato 6,31 mg/dL. Si comunica il dato al reparto.Il giorno successivo vengono ripetuti gli esami della coagulazione: INR 9,30 e APTT ratio 4.67. Il pomeriggio INR 9.66 e APTT ratio 5.04. Il reparto viene contattato nuovamente e vengono chieste informazioni sulla terapia seguita dal paziente. Dall'anamnesi emerge che il paziente era in terapia con Pradaxa e che il giorno precedente aveva iniziato il trattamento con il konakion. Essendo questo un DOAC è stato ricordato che non essendo un antagonista della vitamina K chiaramente la somministrazione del konakion non avrebbe migliorato i valori della coagulazione. Inoltre è stato fatto presente che i DOAC vanno ad interferire sul risultato dei test PT e APTT, e che forse sarebbe stato opportuno dosare la concentrazione di farmaco presente nel paziente.Dal dosaggio del Dabigatran è risultato un valore di 1029 ng/mL, un sovradosaggio tale da giustificare la graduale riduzione dell'hb fino a 10.8 g/dL, per probabile emorragia gastrointestinale con una normale funzionalità epatica e renale. Dal confronto con il reparto è emerso che per una incomprensione era stata somministrata una quantità di farmaco superiore alla dose indicata per il Dabigatran . E' stato quindi attivato un percorso di emergenza per la possibile intossicazione da farmaco. Il paziente è stato attentamente monitorato nelle ore successive e nei giorni seguenti. I valori degli esami si sono normalizzati: INR 1.09, APTT ratio 1.09, HGB 13.6 e quindi dieci giorni dopo il ricovero il paziente è stato dimesso. Come considerazione finale si evidenzia ancora una volta come per la corretta interpretazione degli esami della coagulazione sia necessario conoscere i farmaci assunti dal paziente e quanto sia importante la comunicazione tra i clinici e il laboratorio.

EP173

Case report: Fetal hydrops in subject with Bart's hemoglobin

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Introduction

The hemoglobin (Hb) Bart's disease (hydrops fetalis syndrome) is one of the common fetal development abnormalities and the most severe form of α-thalassemia. This severe α-thalassemia is characterized by the absence of all four a-globin gene loci (–/–). In this paper we describe the case of a pediatric patient with Bart's hemoglobin disease [1,2].

Case presentation

An 8-month-old pediatric patient was admitted to the pediatric ward of the San Donato Hospital (Arezzo) for symptoms that are not well defined. CBC (Complete blood count) showed the following results: white blood cell 6.7 x 10^9/L, red blood cell 0.90 x 10^12/L, haemoglobin 30 g/L, haematocrit 0.125 L/L, mean corpuscular volume 138.9 fL, mean corpuscular haemoglobin 33.3 pg, mean corpuscular haemoglobin concentration 240 g/L, red blood cell distribution width 19.2%, platelet 263 x 10 ^ 9/ L and erythroblasts 1.5%. The presence of anisochromia and erythrocyte anisopoikilocytosis was shown in the peripheral blood smear. The glucose 6 phosphate dehydrogenase was 49 U/gHb and the screening of fractionated hemoglobins showed: Hb A1 83.3%, Hb F 14.5%, Hb A2 2.2%, other Hb 16.2%.

Conclusion

The rapid identification of pathological hemoglobins allows the clinician to frame the patient clinically by planning the diagnostic investigations in specialized centers avoiding the inappropriate request for useless laboratory tests. The clinical pathologist with the screening methods is required to identify the pathological hemoglobins, highlighting their method-dependent biochemical characteristics, easily available in international archives.

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EP174

Diagnostic and Prognostic value of Presepsin in the management of sepsis at the Emergency Department

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Background: Sepsis is a life threatening condition due to a dysregulated response of the organism to infection, both viral and bacterial. Clinicians need to diagnose the infection rapidly in order to undertake an adequate and effective therapeutic treatment before septic shock occurs [1]. Therefore, clinical laboratories could help accelerate the diagnosis using specific biomarkers. Along with clinical signs, a range of biomarkers can be very helpful in the early diagnosis of sepsis. Presepsin (P-SEP) is one of such biomarkers, not only important for sepsis diagnosis but also useful for prognosis [2]. The aim of the present study is to evaluate the role of P-SEP in diagnosis and risk stratification of septic patients in emergency department using AIA-360 (Tosoh Corporation) platform to diagnose sepsis early, quickly, affordable and at low cost. Methods: This study was conducted at Tor Vergata University Hospital of Rome. A total of 96 K-EDTA blood samples were collected from patients at the Emergency Department: 15 samples were obtained from patients with confirmed sepsis diagnosis and 44 samples with suspected sepsis but not confirmed. As control group, 37 samples from healthy blood donors were collected. For statistical analysis, Shapiro Wilk test for distribution analysis and the non parametric Kruskal Wallis test were used to evaluate significative differences among the groups ($p<0.05$). ROC curve analysis to evaluate sensitivity and specificity of P-SEP biomarker was also performed.Results: The control group has a median of 473 pg/ml, while non-sepsis group and sepsis group has a median of 866 pg/ml and 1467 pg/ml, respectively. Therefore, there is a statistically significative difference among all groups. The ROC curves highlight a good sensitivity (93%) and specificity (76%) for P-SEP values and suggest a best cut-off of 890 pg/ml.Conclusions: The P-SEP test with AIA-360 can improve the diagnosis of sepsis upon the admission to the Emergency Department. Furthermore, the measurement of P-SEP levels, through this fast assay, reveals valuable prognostic capacity to predict sepsis in respect to other biomarkers.

EP175

Non-nephrotic proteinuria as suspected initial amyloidosis: clinical case

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Background: Amyloidosis (AL) is a disease caused by abnormal extracellular deposition of insoluble fibrillary protein in various organs and tissues resulting in severe organ dysfunction. At the time of diagnosis ~69% of patients already have more than one organ involved, so it becomes vital not only to diagnose AL early, but also to control plasma cell dyscrasias and thus to halt escalating organ damage. Patient and Methods: A 70-year-old woman was admitted to Department of Nephrology and Dialysis (S. Eugenio Hospital, Rome) for diagnostic investigations of non-nephrotic proteinuria detected in routinary blood tests. During hospitalization, haematochemical tests performed at UOC of Clinical Pathology (S. Eugenio Hospital, Rome) confirmed renal normofunction (creatinine 0.59 mg/dl), good hematopoiesis (Hb 14.5 g/dl), dyslipidemia (total cholesterol 241 mg/dl, LDL 151 mg/dl) and hypoalbuminemia. Serum protein electrophoresis and immunofixation were negative. Urinalysis showed proteinuria (1.1 g/l) and in a 24-h urine collection were found free monoclonal lambda light chains that cannot be measured by densitometry. Thoracic X-ray was negative and abdominal ultrasound showed multiple bilateral renal parapyelic cysts. Subsequently, the patient underwent to haematological and cardiological examinations and renal biopsy. Haematological examination showed an altered kappa/lambda ratio with a monoclonal lambda component of 177 mg/dL, for cardiological examinations troponin and BNP were 5.6 pg/ml and 33 pg/ml respectively. The renal biopsy showed amyloid deposits, while immunohistochemical examination suggested a stretch for lambda light chains with deposits at the interstitial peritubular, mesangial and vascular wall levels. According to the clinical data collected, the diagnosis was early onset renal light chain amyloidosis (lambda). Conclusions: AL presentation could be rather deceitful due to its mimicry of various conditions. The issue of this disease is early diagnosis, the finding of a proteinuria at a routinary blood tests can provide clinicians the suspicion of AL and prevent organ dysfunction. Thus, a close collaboration between laboratory doctors, hematologists, nephrologists, cardiologists is essential to implement the best patient management strategy.

EP176

Utilizzo del biomarcatore Krebs von den Lungen-6 (KL6) in pazienti con Covid-19 che sviluppano compromissione polmonare severa

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Krebs von den Lungen-6 (KL-6), una glicoproteina ad alto peso molecolare (200 kDa) con una catena di acido sialico riconoscibile da specifici anticorpi, è prodotta principalmente dai pneumociti alveolari Tipo II danneggiati o in via di rigenerazione e risulta aumentata in malattie interstiziali polmonari (ILD) quali fibrosi polmonare idiopatica e polmonite da ipersensibilità. Scopo dello studio: valutare le informazioni cliniche fornite dalla determinazione di KL-6 in pazienti con Covid-19 che hanno sviluppato una compromissione respiratoria di grado severo con esiti sfavorevoli in alcuni pazienti. Sono stati valutati 215 pazienti (144 maschi, 67%; 71 femmine, 33%); essendo lo studio ancora in corso, verranno presentati i risultati relativi a 72 pazienti (55 maschi, 76,4%; 17 femmine, 23,6%) che hanno sviluppato una grave forma di malattia e nei quali KL-6 è stato determinato durante il ricovero in terapia sub-intensiva respiratoria; ad oggi 18 dei 72 pazienti (25%) sono stati sottoposti a monitoraggio ambulatoriale. Le concentrazioni seriche di KL-6 sono state determinate con metodo immunoenzimatico in fluorescenza (FEIA) su analizzatore AIA 360 (TOSOH Biosciences). I risultati hanno evidenziato: 1) Ad elevati valori di KL-6 si associa un peggiore decorso ospedaliero in unità sub-intensiva (OR 23,33, p<0,004) con trasferimento in terapia intensiva od esito infausto, indipendentemente da età e sesso. 2) Gli stessi elevati valori di KL-6 presi singolarmente non si associano a “morte” (OR 4,62, p<0,113) se non in concomitanza a comorbidità di tipo metabolico (diabete, obesità) (OR 8,76, p<0,047). 3) All'analisi di regressione lineare non si evidenzia correlazione tra valori di KL-6 osservati durante il ricovero e durata totale della degenza (mediana: 22 gg, range: 8-98; media: 28,46 gg, DS ±19,81; p = 0,417) né con quella del ricovero in terapia sub-intensiva respiratoria (mediana: 9 gg, range: 1-61; media 11,18 gg, DS ± 9,78; p = 0,272). I risultati preliminari descritti, suggeriscono l’utilità di KL-6 nella predizione di decorso ospedaliero complesso in pazienti Covid-19; i risultati del monitoraggio ambulatoriale, potranno confermare l’utilità nel predire il decorso di malattia post-ricovero per il possibile sviluppo di fibrosi polmonare di cui il marcatore è specifico indicatore biochimico.

EP177

Health Technology Assessment of Sebia Capillarys Flex piercing and Tosoh HLC-723G11 to evaluate glycated haemoglobin and haemoglobin variants

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La determinazione dell'emoglobina glicata (HBA1c) è richiesta sia per la diagnosi di diabete mellito sia per la valutazione retrospettiva del controllo glicemico (ogni 3-4 mesi) negli affetti. La determinazione dell'assetto emoglobinico è, invece, richiesta in caso di sospetto di emoglobinopatie ed è parte dei protocolli di screening neonatale. Nel Servizio di Medicina di Laboratorio dell'IRCCS Ospedale San Raffaele vengono eseguite circa 200 analisi di HBA1c al giorno per sei giorni a settimana e circa 50 assetti emoglobinici due giorni a settimana. Degno di nota è che per ciò che riguarda l'HBA1c si prevede un trend in crescita considerando che la prevalenza del diabete mellito, soprattutto di tipo 2, è in continuo aumento. I dati della IDF (International Diabetes Federation) indicano che attualmente in Europa 61 milioni di adulti convivono con il diabete mellito (con una previsione di 67 milioni nel 2030) e che nel 2021 sono stati spesi 189 bilioni di dollari. Pertanto, sarà importante avere analizzatori affidabili clinicamente e sicuri, ma anche efficienti dal punto di vista organizzativo ed economico. Ad oggi è possibile usare per queste analisi sia l'elettroforesi capillare zonale, ma anche la cromatografia liquida ad alta prestazione. È stato condotto un HTA confrontando il Sebia Capillarys Flex piercing e il Tosoh HLC-723G11, e come modello di riferimento è stato scelto l'EUnetHTA Core Model® versione 3.0, uno strumento operativo in grado di garantire la diffusione e la condivisione delle informazioni in modo affidabile e trasparente. In particolare, è emerso che entrambi gli analizzatori sono di facile e immediato utilizzo; il Tosoh HLC-723G11 ha un turnaround time molto vantaggioso: impiega 1 minuto per determinare l'HBA1c e circa 5 per l'assetto emoglobinico, mentre il Sebia Capillarys Flex piercing è risultato di poco più lento; il software collegato a quest'ultimo analizzatore è risultato, però, più user-friendly rispetto a quello del Tosoh HLC-723G1.L'HTA ha permesso di evidenziare le caratteristiche che differenziano gli analizzatori tra loro diventando uno strumento per creare un legame diretto tra innovazione scientifico-tecnologica e gestione della salute.

EP178

EQA PROGRAM FOR THE DETERMINATION OF GLUCOSE: ANALYTICAL VARIABILITY OF THREE GLUCOSE "POCT" DEVICES USED IN A HOSPITAL SETTING

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The use of "point-of-care-testing" (POCT) for the determination of glucose in a hospital setting may be necessary in situations where therapeutic decisions need to be made in times that are not compatible with the turnaround time of the laboratory.It is therefore fundamental to monitor the glucometers located in the various departments by an External Quality Assessment Program (EQAP), in addition to the internal quality control. Since 2016 the Center of Biomedical Research has been managing a specific EQAP, including 4 surveys of two samples each per year. In 2022 cycle, 28 laboratories and 272 departments participate in the EQA Program.
Aim: To describe the analytical variability and performance and the degree of harmonization of three POCT devices: Nova Biomedical Nova Pro (NP), Roche Accu-Check Inform (ACI), Abbott Freestyle Optium (FO).
Methods: Data relating to 44 liquid control samples, prepared from human serum, distributed from 2017 to June 2022, were analyzed with the following concentration ranges: NP 62-226 mg/dL; ACI 66-274 mg/dL; FO 65-264 mg/dL. The mean CV% of each of 3 POCT devices was evaluated on the basis of the quality specifications of the glucose imprecision (<2.8% desirable and <4.2% minimum).Moreover, we calculated the different levels of performances: optimum (O), desirable (D), acceptable (A), unacceptable (UN), on the basis of the following Acceptability Limits (AL): for values <100 mg/dL, AL = 12 mg/dL, for values >100 mg/dL, AL = 12.5%.
Results: Number and (range) of sample results: NP = 17 (11-38); ACI = 97 (58-170), FO = 48 (24-71). Mean CV % and (range): NP = 2.9 (0.4-6.7), ACI = 1.9 (0.0-3.2), FO = 3.7 (0.4-7.6).Performances: NP = 68.3% O, 19.9% D, 8.7% A, 3.1% UN; ACI = 92.7% O, 6.6% D, 0.7% A, 0.1% UN; FO = 71.9% O, 21.4 D, 4.9% A, 1.8% UN.Median differences among POCT devices: NP vs ACI = -22.3 ± 12.9 mg/dL (-15.0 ±3.7%); FO vs ACI = -3.9 ± 11.0 mg/dL (-4.3 ±8.3%); NP vs FO = -18.4 ± 18.8 mg/dL (-10.6 ±7.8%).
Discussion and Conclusions: The results describe the analytical variability and the state-of-the-art of performances concerning three POCT devices for glucose determination. The EQAP allows to identify possible improvement needs. In order to guarantee the reliability of POCT results the centralized management is needed.

EP179

Therapeutic drug monitoring services in Italy: a national survey

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In the era of personalized medicine, therapeutic drug monitoring (TDM) may play a pivotal role in laboratory medicine enabling physicians to individualize drug dose regimens in every patient, hence exploiting the maximum therapeutic benefit while reducing the risk of toxic effects. Accordingly, the patients' access to TDM protocols and services is mandatory. However, the diffusion of TDM services across the Italian regions has not completely evaluated yet.
Therefore, the aim of the present study will be to perform a nation-wide survey concerning the geographical distribution of TDM services.
The study will be based on a questionnaire with open and multiple-choice questions. Questions were elaborated in order to harvest information about 4 main domains: a) the responder and the location of his/her institution, b) the number of drugs for which a TDM protocol is available, c) the instrumental platforms with the turn-around-time and d) the participation to an external quality evaluation (VEQ). In particular, the questionnaire includes the main drug classes subjected to therapeutic monitoring, as well as immunosuppressants, antiepileptics, antimicrobials, cardiovascular and respiratory agents, and drugs acting on central nervous system. For completeness, biologics have been also included. For some of those agents, specific questions about the instrumental platforms (i.e., chromatographic or immunometric methods) and corresponding turn-around-time are present.
The questionnaire has been submitted firstly to 6 laboratories (at least 3 respondents/lab) asking whether answering and filling the questionnaire were easy or not. Then, according to received feedbacks the questionnaire was slightly modified and resubmitted to further 6 laboratories.In its final version, the questionnaire will be sent to all SIBioC members. We expect an interim analysis by September 2022 and the final evaluation of collected data by the first quarter of 2023.

EP180

Valutazione della determinazione della proteina C-reattiva su sistema POCT

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Il test point-of-care (POCT) della proteina C-reattiva (CRP) può essere uno strumento prezioso per il processo decisionale nelle cure primarie e nelle strutture territoriali quali Ospedali di Comunità e RSA. CRP POCT può essere utilizzato per rilevare l'infiammazione e può contribuire all'inquadramento del paziente direttamente sul territorio riducendo così gli spostamenti dei pazienti verso gli Ospedali se non strettamente necessari. Scopo del lavoro è stato valutare la concordanza dei risultati di PCR ottenuti con strumentazione POCT rispetto ai risultati ottenuti su strumentazione automatizzata di laboratorio. Sono stati selezionati 104 campioni di plasma pervenuti in laboratorio con richiesta di PCR in un range di concentrazione tra 0,7 e 592 mg/L. Gli stessi campioni, dopo essere stati analizzati sul sistema di laboratorio AU 5800 Beckman coulter con metodo al alta sensibilità, sono stati processati su strumentazione POCT LumiraDx. La PCR viene ritenuta patologica per valori maggiori di 5 mg/L. Il sistema Lumira DX ha come limite di rilevazione <5 mg/L e >250 mg/L, pertanto è in grado di quantificare la PCR per i soli campioni patologici (>5mg/L). I risultati ottenuti sui due strumenti hanno dimostrato una buona correlazione ($y = 0.9647x - 2.6075$ $R^2 = 0.9305$, p value inferiore a 0.05). La concordanza clinica è risultata pari al 92,3% ossia solo il 7.7% dei campioni è risultato discordante da un punto di vista clinico (positivo/negativo). Questi 8 campioni classificati come negativi (< 5) dal POCT avevano un valore su automazione compreso fra 5,1 e 7.3 mg/L. I risultati ottenuti su POCT hanno evidenziato un bias medio di circa -10% rispetto a quelli ottenuti su strumentazione automatizzata. Il sistema POCT valutato dimostra essere un buon sistema per identificare soggetti con uno stato di infiammazione clinicamente significativo, solo 8 pazienti sono stati classificati come negativi dal POCT e positivi dall'automazione ma con valori appena sopra al valore di normalità. Il POCT risulta adeguato per contribuire ad una gestione decentralizzata del paziente fornendo indicazioni utili sul suo stato di infiammazione/infezione. Per un uso più specifico della PCR, con necessità di precisione sui valori bassi di PCR, è indicato l'utilizzo dei sistemi di laboratorio.

EP181

HBOC genes panel testing to improve the molecular diagnosis of hereditary breast and ovarian cancers

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Hereditary breast and ovarian cancer (HBOC) syndrome is a cancer predisposition syndrome that confers an increased lifelong risk of developing cancers, particularly breast and ovarian cancers. Germline, loss-of-function mutations in the BRCA1 and BRCA2 tumor-suppressor genes are the main HBOC risk factor. Nevertheless, BRCA genes mutations have been identified only in about 15% of all cases, suggesting that other genetic risk factors may be involved. In particular, other genes involved in DNA repair mechanisms are emerging as low and moderate penetrance HBOC genes. According to this line of evidences, we have improved the HBOC patients' diagnostic flowchart to include, for the BRCA negative patients, a second level test for the simultaneous analysis of ATM, BARD1, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C, RAD51D, STK11, and TP53 genes. In particular, between November 2021 and June 2022, 80 patients have been totally screened for the HBOC panel. A peripheral blood sample in EDTA was collected from each patient and used for DNA extraction following standard procedures. Then, an enriched library for the analysis of the genes panel was obtained for each patient using the Devyser HBOC NGS kit and sequenced on the Illumina MiSeq system. Sequences were analyzed using the Amplicon Suite software. Totally, 9 patients (11%) carried a pathogenic or likely pathogenic variant. These variants were identified in ATM, PALB2, NBN, RAD51C and BARD1 genes, with a slight prevalence of ATM (N=4) and PALB2 (N=2) mutations. Moreover, several variants of unknown significance were found in 22 patients. These variants were found mainly in ATM, CHEK2, BRIP1 and STK11 genes, rarely in PTEN, CDH1 and NBN genes. The identification of germline cancer-predisposing DNA variants is important to improve early diagnosis, support preventive and therapeutic choices, and counselling the at-risk family members. Based on the data presented herein, we are encouraged in supporting the screening for HBOC panel. Indeed, we were able to identify 9 additional mutations carriers, improving the diagnostic sensitivity of the molecular screening, beyond the BRCA testing. This, in turn, will enhance our understanding of HBOC molecular bases providing novel clues on the role of the additionally tested genes.

EP182

Age-Associated Changes in Circulatory Fatty Acids: New Insights on Adults and Long-lived individuals

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Long-lived individuals (LLIs) are considered a promising model to study healthy human aging. Despite decreased physical functioning, LLIs have a high ability to adapt to age-associated challenges, and the majority of them endure or escape diseases that cause death at younger ages. Blood fatty acid (FA) profile of a cohort of LLIs (90-111 years old, n=49) from Sicily was compared to adults (18-64 years old, n=69) and older adults (65-89 years old, n=53) from the same area. Single nucleotide polymorphisms (SNP) in key enzymes related to FA biosynthesis and metabolism were also genotyped to investigate a potential genetic predisposition in determining the FA profile. Gas chromatography was used to determine the FA profile, and genotyping was performed using high-resolution melt (HRM) analysis. Blood levels of total polyunsaturated fatty acids (PUFA) and total trans FA decreased with age, while the levels of saturated fatty acids (SFA) were similar among the age groups. Interestingly, distinctively higher blood levels of monounsaturated fatty acids (MUFA) in LLIs compared to adults and older adults were observed. In addition, among LLIs rs174537 in the FA desaturase 1/2 (FADS1/2) gene was associated with linoleic acid (LA, 18:2n-6) and docosatetraenoic acid (DTA, 22:4n-6) levels. Likewise, the rs953413 in the elongase of very long fatty acids 2 (ELOVL2) was associated with docosatetraenoic acid (DTA, 22:4n-6) levels. We further found that rs174579 and rs174626 genotypes in FADS1/2 significantly affect delta-6 desaturase (D6D) activity. In conclusion, our results suggest that the LLIs have a different FA profile characterized by high MUFA content, which implies decreased peroxidation while maintaining membrane fluidity.

EP183

BRCA genes copy number variants evaluation trough next generation sequencing data

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In the last 10 years, next generation sequencing (NGS)-based approaches have shown their reliability in the detection of DNA single nucleotide substitutions and small insertions/deletions. Accordingly, NGS is now considered a standard approach in diagnostic settings allowing for diagnostic sensitivity increase, workflow optimization and costs saving. Nevertheless, a main limitation of these techniques is their inability to detect also copy number variants (CNVs). As a consequence, to obtain a complete molecular analysis of genes, like BRCA1 and 2, it is necessary to combine the NGS analysis with another method for CNV's evaluation, like multiplex ligation probes amplification (MLPA). To overcome this issue, several bioinformatic tools have been developed to use the NGS sequences' coverage to estimate the presence of CNVs. The aim of this study was to evaluate the concordance rate between NGS data and MLPA in the detection of BRCA1/2 CNVs. From January 2019 to June 2022, 1296 patients attended to CEINGE Biotecnologie Avanzate diagnostic laboratories for BRCA genes molecular analysis. Genomic DNA was extracted from a blood EDTA sample/patient using the Maxwell 16 instrument. Libraries were prepared using the Devyser BRCA NGS kit and sequenced on the Illumina MiSeq system. Sequence data were analysed using the Amplicon Suite software for both single nucleotide substitutions and CNVs. Moreover, the same samples were analysed with the MRC Holland BRCA probemixes and the Coffalyser software. The comparison between the MLPA results and the bionformatic prediction showed a perfect concordance between the 2 procedures. Nine CNVs have been totally identified in the 2 analyzed genes by MLPA and correctly detected by the bioinformatic analysis. Moreover, false positive MLPA results were identified by NGS as confirmed using a second probemix set. All the remaining patients were negative at MLPA and no false positive calls were highlighted by the NGS software. Our results suggest that the tested bioinformatic pipeline is reliable in CNVs detection supporting the use of NGS-based procedures to carry out a complete molecular screening of specific genes of interest.

EP184

Case report of Candida parapsilosis sepsis detected in peripheral blood smear

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Introduction
During long periods of hospitalization, debilitated and immunosuppressed patients are prone to contracting nosocomial fungal infections, such as Candida parapsilosis, which can cause sepsis. Candida parapsilosis, indeed, is able to form firm and persistent biofilms in central venous catheters (CVC) in addition to other medical devices, thus threatening patients undergoing invasive medical procedures [1]. We report a case of Candida parapsilosis sepsis detected in the peripheral blood smear and by the change of the cytograms of the hematology analyzer before to blood culture positivization.
Case presentation
An 89-year-old woman, positive for the Sars-Cov2 virus, was admitted at the San Donato hospital (Arezzo) for 40 days for Covid symptoms. Laboratory tests show an increase in C reactive protein (10.6 mg/dL), gamma GT (76 U/L), total bilirubin (2.31 mg/dL) and direct (1.46 mg/dL), creatinine (1.00 mg/dL) and reduction of glomerular filtrate (50.4 mL/min /1.73 mq). In addition, at the CBC anemia is detected with hemoglobin of 102 (g/L) and thrombocytopenia (32 x 10^9/L); in the peripheral blood smear the typical morphology of the septate hyphae is detected, attributable to candidiasis with changes in the cytograms of the hematological analyzer. Blood culture confirms Candida parapsilosis infection with a positivization time of 38 hours and 36 minutes.
Conclusion
The recognition in the peripheral blood smear of the morphology attributable to candidiasis, in the absence of a positive blood culture, points towards a localization of the focus on CVC or in other venous/arterial accesses. In cases where there is confirmation of blood culture, the microscopic finding confirms the septic infection. In any case, microscopic recognition represents a high infectious risk where prompt intervention with prophylaxis or therapy is required.
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EP185

Research of new faecal and salivary biomarkers for the non-invasive diagnosis of colorectal carcinoma

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Background. Colorectal (CRC) cancer, among the deadliest cancers worldwide, has late diagnosis and is characterized by several risk factors like family history, obesity, smoking, alcohol and chronic inflammation. Early biomarkers might improve early detection allowing curative surgery thus improving survival. In previous studies, the presence of potentially diagnostic peptides in CRC patients' saliva were identified by MALDI-TOF/MS. In fecal extracts of Crohn's disease, known to increase CRC risk, several salivary derived peptides were identified by Orbitrap analysis, including Basic Salivary Proline-Rich Protein 1 (PRB) 1495.66 m/z (GQ-15) and 1578.75 m/z (GG-17) peptides.

Aim. The aim of the project was to verify whether these two peptides have any biological activity on CRC cell lines in vitro.

Materials and methods. Human CRC cell lines HT-29 and HCT-116 were used for proliferation (24h, 48h, 72h) and signaling experiments. Both CRC cells lines were cultured in presence or absence of EGF (100 ng/mL), GQ-15 (100 nM) and GG-17 (100 nM) peptides. Immunoblot analysis was used to evaluate proliferation (Akt, Erk 1/2, p38), inflammation (NF-κB p65 and p105, Stat-3, IκB-α) and apoptosis (Cleaved caspase-3, Cleaved caspase-8) signaling pathways.

Results. HT-29 proliferation was significantly stimulated by GG-17 peptide after 48h ($F = 5.144$, $p = 0.0005$ for treatment; $F = 25.75$, $p < 0.0001$ for time, $F = 1.659$, $p = 0.0972$ for interaction between GG-17 and EGF). HCT-116 proliferation was not significantly stimulated by GQ-15 and GG-17 peptides. In HT-29 and HCT-116, both peptides increased phosphorylation of the proliferation-related targets p-Akt, p-Erk 1/2 and the inflammation-related target p-NF-κB p65 and p105 more than EGF. p-IκB-α and the apoptosis-related caspase-3 and caspase-8 were undetectable. In HT-29, not in HCT-116, GG-17 peptide increased phosphorylation of the proliferation-related target p-p38 more than GQ-15 peptide and EGF. In HCT-116, not in HT-29, both peptides caused a reduced phosphorylation of p-Stat3.

Conclusions. PRB derived peptides, in particular GG-17, exert an important effect on tumor cell proliferation, acting also on the driver signaling pathways in cancer Akt, MAPK, NF-κB and apoptosis.

EP186

Valutazione dei livelli di citochine pro- ed anti-infiammatorie nell'ambito della malattia COVID19

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INTRODUZIONE. I pazienti affetti da forme gravi di COVID19 mostrano elevati livelli circolanti di citochine infiammatorie, derivati da un'intensa e anomala risposta immunitaria e in grado di influenzare il decorso della malattia.
OBIETTIVO. Valutare in pazienti COVID19 la relazione esistente tra IL6 e TNFα, citochine proinfiammatorie, e IL10, downregulator dell'immunità adattativa e del processo infiammatorio.
METODI. I sieri di 112 pazienti ricoverati presso i reparti COVID dell'AOU di Parma sono stati analizzati presso la SSUO Diagnostica Autoimmune per il dosaggio ELISA dei livelli circolanti di IL6, TNFα e IL10.
RISULTATI. Le percentuali di positività per IL6, TNFα e IL10 sono risultate rispettivamente 80%(≥25 pg/mL), 70%(≥12.4 pg/mL) e 51%(≥3.3 pg/mL). Per valutare la relazione esistente tra citochine pro- e anti-infiammatorie, abbiamo esaminato i valori medio e mediano di TNFα e IL10 sia per i pazienti IL6 positivi(90/112) che per i negativi(22/112). Nei pazienti IL6 positivi, i valori medio(16.6) e mediano(15.2) di TNFα erano elevati così come la percentuale di positività(74%), mentre tra i negativi per IL6, i valori medio(8.5) e mediano(8.4) di TNFα si mantenevano nel range, con una percentuale di positività del 32%. Analoghi dati di positività rispetto a IL6 sono stati ottenuti per IL10. Nei pazienti IL6 positivi, i valori medio(17.0) e mediano(9.3) di IL10 erano elevati, con una significativa percentuale di positività(58%), mentre nei pazienti IL6 negativi, i valori medio(2.6) e mediano(0.1) erano bassi, con una bassa percentuale di positività(14%).
DISCUSSIONE. Dalla nostra analisi, l'andamento dei valori di IL10 appare analogo a quello delle principali citochine proinfiammatorie. Probabilmente, nelle fasi più avanzate, il Sistema Immunitario secerne più IL10 per indurre l'apoptosi delle cellule implicate nella risposta preservando i tessuti normali. Tuttavia, la disregolazione linfocitaria che si verifica in COVID19 fa sì che in questi pazienti la risposta immunitaria si configuri come uno strumento potenzialmente pericoloso. Se all'inizio è finalizzata a combattere l'infezione, il reclutamento continuo di cellule immunitarie per potenziarne gli effetti scatena il cosiddetto storm citochinico, causa di una possibile e grave compromissione multiorgano.

EP187

Evaluation of Interleukin-6 Vs C-reactive protein and Procalcitonin as severe infection biomarkers in course of fever episode in adult hematological patients. Real-life single center prospective observational study

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Background: Sepsis is one of the most common causes of ICU hospitalization with high-cost treatments and elevated mortality rates. Hematological patients represent a population to opportunistic infections, mainly due to the disease itself and chemotherapy-induced neutropenia. So early diagnosis of severe infection and sepsis may improve outcome of neutropenic hematologic patients [Chaftari, 2015 Plos one]. Methods: Hematological patients with at least one fever episode during hospitalization are included in this prospective study, approved by the ethics committee of our institute. Patients are divided into two groups- Group 1: no focus of infection, and Group 2: clinically/microbiologically document infection (patients with at least one peak body temperature >38°C and/or two or more daily fever episodes and/or positive blood culture). Results: A total of 155 episodes of febrile neutropenia in 34 hematological patients were analyzed for serum PCT, IL-6 and PCR levels. In total, 73% were categorized as group 2 and 26% as group 1. In those patients in group 2 with infection, bacteremia was seen in six(E.coli resulted in 33%, S. haemolyticus in 16%, C.species SPP in 16%, S,epidermidis in 33%,and P aeruginosa in 16%). Urine cultures was positive in 50% (K pneumoniae was found in 23%, E. coli in 29%, P aeruginosa 5.8%, S. haemolyticus in 23%, C. albicans and glabrata in 5,8%, and E fecalis in 5,8%). Stool culture was positive in 50% (K pneumoniae was found in 29%, E. coli in 29%, S. haemolyticus in 35%, C albicans and glabrata in 5,8%, E fecalis in 5,8%, P aeruginosa 5.8% and P. mirabilis in 5,8%). The mean values of PCT (4.80ng/ml), IL-6 (146,40) and PCR (11.56) in group 2 were higher than PCT (1.15) IL6 (59.87) and PCR (8.72) in group 1. We noted a statically significant difference ($p<0.04$) for IL6 in group 2 Vs group1. The combined study of sensitivity and specificity by ROC analysis gave a cut-off for PCT of ≥0.1≤, for IL-6 was≥ 130≤ and ≥1.4 ≤was the cut-off for PCR according to Youden's index. The area under curve for PCT, IL-6 and PCR were 0.46,0.56, and 0.54.Conclusions: Our preliminary findings demonstrated that IL-6 may improve outcome of neutropenic hematologic patients with sepsis however these data need to be confirmed in a larger population.

EP188

Mass Spectrometry approach to quantifying orotic acid and its metabolites in urine and plasma

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Background

The orotic acid (2,4-dioxo-1H-pyrimidine-6-carboxylic acid; Vitamin B13) is an intermediate metabolite of pyrimidine nucleotides biosynthesis and represents a minor diet constituent. The precursors of orotic acid in human metabolism are the cytosolic carbamoyl phosphate and aspartate via dihydrorotate: this biosynthesis is catalyzed by CAD gene encoding multifunctional enzyme. The multimeric protein called uridine 5'-monophosphate synthase is constitued by two domains that catalyze uridine monophosphate synthesis: orotatephosphoribosyltransferase (OPRTase; EC 2.4.2.10) and orotidine 5'-phosphate decarboxylase (OMPdecase; EC:4.1.1.23). The complete pathways of orotic acid biosynthesis is reported in Fig. 1. The step (5) represented in Fig.1 is directly involved in metabolism of 5-Fluorouracil (5-FU), because this anticancer drug is competitive substrate of OPRTase. In particular, the transferase activity of OPRTasemulticomplex enzyme is inhibited by 5-FU at 59% level of control. The other end OPRTase is involved in metabolic disorders as congenital orotic aciduria and consequently the urinary orotic acid is quantified in clinical routine analysis for differential diagnosis of hereditary metabolic disease. Therefore,we aimed to develop a high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS)assay that allows the simultaneous and sensitive detection of an orotic acid and orotidine 5'-monophosphate as metabolic and toxicological biomarkers in plasma and urine.

Methods

The implementation and validation of chromatography and spectrometric method are developed in accordance with UNI EN ISO/IEC 17025:2018 and Eurachem Guide Lines (Method validation in clinical chemistry follows theestablished standards and procedures accepted by alldisciplines of chemical metrology). The clinical aspects are tested by analyzing diagnostic proficiency testing (external quality assessment - EQA) and other samples from patients with metabolic and malignant disorders.

Results

The analytes, orotic acid and orotidine-5'-monophosphate are identified and quantified with high performance parameters of repeatability, reproducibility, robustness, precision and accuracy. The quantification method is

based on internal standard approach for signal and matrix effect suppression. Whatever analytes is identified and by two MRM transition with S/N>50 in LOD range. The analytical method clearly distinguish between urine and plasma specimens from the normal and pathological patients at 97.5% of level of confidence.

Conclusions

The HPLC-MS/MS method to be suitable for differential diagnosis of hereditary metabolic disease and metabolic monitoring of toxicity induced by anticancer drug. The analytical protocol is rapid and ideal to be used in routine analysis of clinical chemistry laboratory.

EP189

The relevance of capillary electrophoresis for Hemoglobin analysis

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Hemoglobinopathies, inherited disorders of hemoglobin, are the most common monogenic diseases worldwide. The clinical manifestations are highly variable, ranging from asymptomatic to severe hematological diseases, with multiple end-organ damages. The diagnosis of haemoglobinopathies mainly relies on Complete Blood Count (CBC) and on the analysis of hemoglobin fractions, according to guidelines. Sometimes the diagnosis is casual during routine laboratory analysis. Case report: We report a case of a 46-year-old Caucasian man admitted to the Endocrinology Unit for glycemic decompensation. Thus, he underwent further laboratory investigation, including HbA1C measurement. In our laboratory, routine HbA1C measurement is performed by HPLC on VARIANT II Hemoglobin Testing System (Bio-Rad). The HPLC analysis of the sample showed an undeterminable HbA1C because it coeluted with the Hb variant that was not detectable by HPLC. In order to further investigate such a finding, we measured HbA1C by capillary electrophoresis on Capillarys 3 OPTA (Sebia Labordiagnostische Systeme GmbH, Fulda, Germany). The graph showed decreased HbA2, HbA1C=11.1% (98mmol/mol), and other HbA=3%. Then, we further characterized HbA2 as recommended by guidelines. The assessment of HbA2 by HbA2 SEBIA on Capillarys 3 OPTA showed HbA2=2%, HbA=98%, and the absence of HbF. Thus, since the patient had a normal CBC and did not show anemia, we can hypothesize the presence of delta chain variants that do not allow the detection of HbA1C by HPLC. This case-report emphasizes the high performance of capillary electrophoresis making it a solution of choice for identifying haemoglobinopathies with a high degree of confidence.

EP190

Osservazione e valutazione delle frazioni minori dell'emoglobina durante la diagnosi di Emoglobinopatia: il caso dell'Hb A2-Babinga

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L'HbA2 è l'indicatore principale ed essenziale nella diagnosi di β-talassemia. Una diagnosi che nel soggetto eterozigote può essere definita solo dal laboratorio e nella maggior parte dei casi non ha bisogno di ulteriori approfondimenti o conferme. Il laboratorio ha a disposizione diversi metodi e tecnologie separative per la valutazione di tale componente minore dell'emoglobina (Hb), dell'Hb A ed in alcuni casi dell'Hb F. Tali metodi consentono inoltre, di evidenziare altre frazioni che possono derivare dalla presenza di gran parte delle variazioni genetiche dell'emoglobina o dalla loro trasformazione. L'eventuale presenza di picchi anomali, anche in percentuali apparentemente non significative, deve rappresentare elemento di valutazione che contribuisce a produrre la corretta conclusione diagnostica del laboratorio. Nel corso di esami per le emoglobinopatie abbiamo osservato un uomo di 49 anni originario del Ghana, con emocromo nella norma, che evidenziava in elettroforesi capillare (Capillarys 3 Tera con kit Capi3 Hemoglobin, Sebia Lisses, France) la presenza di una variante pari al 39,6%, confermata come Hb S con test specifico, Hb A2 1,3%, Hb F 5,3% e una ulteriore frazione, non risolta rispetto all'Hb S, parzialmente in "zona (E)". Alcuni sistemi separativi in cromatografia liquida ad alta risoluzione (HPLC) non evidenziavano quest'ultima frazione anomala. Tuttavia, considerando il valore molto basso dell'Hb A2 si è ipotizzato che la frazione minore non quantificabile potesse trattarsi di una variante dell'Hb A2, ipotesi successivamente confermata mediante sequenziamento dei geni δ con l'osservazione dell'Hb A2-Babinga, una variante presente in diverse popolazioni africane. L'Hb F era dovuto invece alla presenza di un polimorfismo allo stato omozigote [-158(C>T)], altrettanto presente in Africa e frequentemente associato all'HbS. Viene ribadita quindi la necessità di una attenta revisione dei tracciati prodotti dai sistemi separativi e successiva conferma molecolare: a) quando si sospettano varianti δ, per poter valutare nel modo adeguato il valore dell'Hb A2-totale, contribuendo alla corretta diagnosi della β-talassemia; b) in presenza di frazioni minori quando si sospettano prodotti di degradazione che, in campioni non invecchiati e ben conservati, si possono associare a varianti "instabili" che possono anche essere "silenti" ai sistemi separativi.

EP191

DEFINITION AND APPLICATION OF STATE-OF-THE-ART ANALYTICAL PERFORMANCE SPECIFICATIONS DERIVED FROM EXTERNAL QUALITY ASSESSMENT (EQA) SCHEMES

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INTRODUCTION. Since 2018, the Regional Reference Centre for Laboratory Quality of the Lombardy Region, in collaboration with SIBioC WG "Analytical Quality", has developed a procedure to determine the limits of acceptability of its EQA schemes. This procedure makes it possible to calculate "state-of-the-art" analytical performance specifications (APS) from the results submitted by the participants. In this paper, we present the APS calculated for the measurands of "Whole Blood Ethanol" and "Thyroid Autoimmunity" programmes, and for the missing measurands of the earlier work done for the "Hormones and Tumour Markers" scheme.

METHODS. 284 results from 14 exercises (programme "Whole blood ethanol"), 3365 from 18 ("Thyroid autoimmunity") and 31921 from 45 ("Hormones and tumour markers B") were processed as follows: subdivision by exercise and by peer groups; calculation of the % deviation of each result from the expected value (robust mean of the peer group, after removal of outliers according to the Hampel approach); calculation of the 95th percentile of deviations for each exercise (as a target based on the state-of-the-art); correlation of the targets identified in each exercise with the corresponding concentration of the samples; identification of a unique or a concentration-specific target.

RESULTS. A single APS was found for Ethanol (18.82%), Anti-thyroglobulin antibodies (21.95%), Anti-thyroperoxidase antibodies (21.18%), 1.25-OH Vitamin D (30.01%), 17-α-OH Progesterone (39.25%), DHEA-S (17.25%), HGH (14.80%), SHBG (14.69%), while concentration-dependent targets were found for 25-OH Vitamin D (22.69% at < 22 and 18.68% at ≥ 22 µg/L), Aldosterone (42.37% at < 60 and 22.66% at ≥ 60 ng/L), Delta-4 Androstenedione (36.25% at < 1.70 and 23.32% at ≥ 1.70 µg/L); IGF-1 (59.13% at < 25 and 28.85% at ≥ 25 µg/L), PTH (71.52 at < 25 and 41.72% at ≥ 25 ng/L), Thyroglobulin (20.88% at < 10 and 14.37% at ≥ 10 µg/L).

CONCLUSIONS. "State of the art" is one of the three models proposed at the 1st EFLM Strategic Conference in Milan in 2014 for setting the APS. Criteria for assigning measurands within this model were mentioned, but there is little guidance on how to set these targets in practise.

Using data from EQA schemes could therefore be an easy way to accomplish this task.

EP192

Long follow-up of BNT162b2 mRNA vaccine in healthcare workers (2020-2022): kinetic of antibody response and impact of booster vaccination

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The management of future booster COVID-19 vaccination requires more and detailed data about the longevity of passive, humoral or cellular, immunity.
We investigated the humoral immunogenicity of BNT162b2 mRNA vaccine in healthcare workers (HCW) up to 12 months. This study was designed to evaluate the kinetic of antibody response measuring sequential anti-S IgG levels. Participants were voluntary and SARS-CoV-2 naïve HCWs of IRCCS Policlinico San Martino Hospital (Genoa, Italy) that they were immunized with two doses of vaccine at December 2020 and a booster dose 9 months later. Blood was sampled prior to vaccine (T0), at 21 days (T1) and 28 days (T2) after the first dose, at 1 (T3), 3 (T4), 6 (T5) and 9 months (T6) after full vaccination, at 1 (T7) and 3 months (T8) after a booster dose. Serological assays were performed at Laboratory Medicine of our hospital using SARS-CoV-2 IgG panel (Bio-Rad, Marnes-la-Coquette, France). It is a multiplex panel of immunoassays intended for the semiquantitative detection of four different IgG antibodies against the receptor-binding domain (RBD), spike 1 (S1), spike 2 (S2) and nucleocapsid (N) structural proteins of SARS-CoV-2. 51 subjects were enrolled among all HCWs and overall, they showed a seroprevalence of 96% (49/51) for RBD and S1 at T1 and 100% (51/51) from T2 to T6. Median values of RBD [100 (51-188) vs 2945 (1693-5364) U/mL] and S1[79 (30.7-131) vs 1574 (833-3256) U/mL] increased remarkable from T1 to T2. These parameters reduced gradually from T3 to T6 reaching a fold decrease of -20 times (CI 95%: 18-23) and -19 (CI 95%: 17-22) for RBD and S1, respectively. At T7, it was observed an increase of antibody level in comparison to T2 (RBD 4 times, CI 95%: 2,5-6; S1 3 times, CI 95%: 1,5-5). All subjects were negative for anti-N IgG from T0 until to T8. HCWs experienced SARS-CoV-2 infection documented by a molecular or antigen assay for 39,2% (20/51) after a median time of 165 (69-184) days.Naïve and healthy people show a protective humoral response with BNT162b2 that it endures up to 12 months with a booster dose at 9th month. Based on the rapid spread of Omicron variants, humoral decrease and booster breakthrough after less of 6 months, an update of vaccine sera booster may be planned for HCWs and patients with failty.

EP193

Oncolytic adenovirus-mediated expression of an anti-PD-L1 scFv induces recruitment and immune evasion inhibition

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Oncolytic virotherapy is an emerging therapeutic approach that could offer an attractive combination of tumor-specific cell lysis together with immune stimulation, therefore acting as potential in situ cancer vaccines. Furthermore, arming an oncolytic virus with immunomodulatory transgenes such as immune checkpoint inhibitors (ICIs) or other molecules can increase the virus ability to target the tumor microenvironment (TME) and, therefore, enhance immune-virotherapy. Adenoviruses (Ads), especially, oncolytic adenoviruses (Onc.Ads), can kill cancer cells in different ways by inducing immunogenic cell death.To increase their anticancer activity, we armed the previously published Ad5D24 to add the ability to block tumor immune evasion. The main pathway involved in immune evasion implicates the interaction between Programmed death ligand 1 (PD-L1) and its receptor PD-1. The PD-1/PD-L1 interaction inhibits CD8+ T cell proliferation turning off the antitumoral T cell-mediated response. We previously isolated a single-chain variable fragment antibody (scFv) that binds to both human and murine PD-L1 with high affinity and developed an Onc.AdΔ24 (Onc.AdΔ24-scFv-anti-PD-L1) that expresses the scFv joining the blockage of PD-1/PD-L1 interaction with the antitumoral activity of Onc.Ads. Firstly, we evaluated onc.AdΔ24-scFv-anti-PD-L1 on B16.OVA melanoma cells, demonstrating the reduction of cell survival in vitro and slow down tumor growth in vivo in a melanoma mouse model. In addition, we observed an increase in the tumor-infiltrating lymphocytes (CD8+) in tumors from treated mice, indicating a favorable immune profile. Subsequently, we evaluated the effect of Onc.AdΔ24-scFv-anti-PD-L1 on TOM-1-GFP-Luc, a murine lung cancer cell line. At this aim, we developed an in vitro tumor model generating 3D spheroid to better mimic tumor structure; preliminary data reveal a significant reduction of spheroids diameter after Onc.AdΔ24-scFv-anti-PD-L1 infection. Altogether, these data suggest that this innovative approach could be effective in the treatment of different tumors supporting and potentiating the available current therapy and, in addition, the combination of immune cells and 3D spheroids can get us closer to obtaining an in vitro model that mimics the complexity of '' in vivo model''.

EP194

Clinical performance of Fujirebio Lumipulse G SARS-CoV-2 rapid antigen test

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Introduction: SARS-CoV-2 infection has plagued the world for the past two years, during which time it has become necessary to use screening tests to prevent the spread of the virus, especially among high-risk patients. The gold standard for the detection of viral RNA is real-time PCR (RT-PCR). This technique requires special care when handling samples, as well as being time-consuming and costly. To meet the need for mass screening, antigenic tests were developed and continuously improved over the years. In our study, we examined the performance of Lumipulse G SARS-CoV-2 Ag (Fujirebio, Japan), a quantitative and automated chemiluminescence enzyme-immunoassay-based antigen test. Materials and Methods: Our study includes 160 subjects (median age 38 years, interquartile range (IQR) 24-58 years; 43% females) screened for SARS-COV-2 at the Service of Laboratory Medicine of Pederzoli Hospital (Peschiera del Garda, Verona, Italy), between August 16 and September 15, 2021. A nasopharyngeal swab was collected for each subject and analyzed at the same time by antigen test Fujirebio Lumipulse G SARS-CoV-2 Ag and by molecular test performed by Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit (Altona Diagnostics GmbH, Hamburg, Germany). Results: A significant Spearman's correlation was found between values of Fujirebio Lumipulse G SARS-CoV-2 Ag and measurable Ct values of SARS-CoV-2 of both the S ($r= -0.94$; $p<0.001$) and E genes ($r= -0.95$; $p<0.001$). The sensitivity and specificity at 1.0 pg/mL (cut-off already used by other authors to discriminate samples positive for SARS-CoV-2) were 0.71 and 1.00. We also used a locally calculated threshold of 0.60 pg/mL (sensitivity 0.88; specificity 0.75). The exclusion of samples tested positive at molecular testing with Ct values comprised between 25-37 enabled the attainment of better diagnostic performance on the 103 residual samples using the 0.60 pg/mL cut-off. Conclusion: the results of our study confirm the good performance of Fujirebio Lumipulse G SARS-CoV-2 Ag (considering cut-off 1.0 pg/mL), especially in samples with high viral load (i.e., Ct value <25), which has proved even better using our locally-calculated cut-off (i.e., 0.60 pg/mL).

EP195

Haemoglobin Variants detected accidentally during HbA1c analysis by Capillary Electrophoresis

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Background: Haemoglobin A1c (HbA1c) is a test routinely used to monitor long-term glycemic control in diabetic patients as well as for the diagnosis of diabetes. Capillary electrophoresis (CE) is a precise and valid method to separate and quantify HbA1c and to detect HbF, HbA2 and haemoglobin variants. In case of detection of Hb variants or elevated HbF during HbA1c testing, a dedicated reagent kit should be employed to perform haemoglobin fractions analysis. The purpose of this study is to report three cases of Hb variants accidentally observed during HbA1c testing but not highlighted by the specific reagent kit for Hb variants. Methods: During a 12-month period (July 2021–June 2022), about 200 000 HbA1c tests had been carried out using Capillarys 3 TERA “HbA1c kit” (Sebia, France) in the Hub Laboratory of AUSL Romagna. To screen for Hb variants interfering with HbA1c determination, Capillarys 3 TERA “Haemoglobin kit” (Sebia, France) was used. Globin gene sequencing was performed by the reference laboratory of the Gaslini Institute (Genoa, Italy).Results: During routine HbA1c analysis, a few subjects carrying HbS, HbC or HbE variants were identified. These variants did not interfere with the HbA1c measurement. In the rare cases in which unknown variants were highlighted, the specific method for Hb variants was employed to obtain more accurate information. In three cases we observed Hb variants interfering with HbA1c assay, but not detectable by the specific “Haemoglobin kit” assay: a 30 year old woman with a variant typed as Hb La Desirade (β129(H7) Ala-->Val), a 50 year old man with a variant typed as Hb Bleuland (α108(G15) Thr-->Asn) and a 56 year old woman with genetic assay in progress.Conclusion: The two identified Hb variants (La Desirade and Bleuland) are clinically asymptomatic; however, when they combine with other Hb variants or thalassemia, they might present with clinical symptoms. HbA1c testing performed by CE is an essential part of routine management of diabetes and in addition has proven to be an effective and powerful method to detect rare or silent Hb variants. We suggest laboratory personnel to be aware of the limitations of their method with respect to the identification of Hb variants.

EP196

IgA kinetics after administration of BNT162B2 COVID-19 mRNA vaccine booster dose in healthcare workers

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Introduction: In a historical period like the one we are experiencing now, the perspective that we will have to coexist with SARS-CoV-2 for a long time is effective. Hence predicting and monitoring the effectiveness of the vaccine response is a task that has become essential for laboratory medicine. Being secreted by the mucous membranes, de facto IgA constitutes the first barrier against Coronavirus. In this study, we focus on describing the trend of IgA neutralizing antibodies in a court of hospital employees who received a booster dose of vaccine, after completing the primary vaccination cycle. Materials and Methods: The study population consisted of 73 healthcare workers (median age 44 years, 54.8% women) of the Pederzoli Hospital (Peschiera del Garda, Verona, Italy), receiving a booster dose of Pfizer COVID-19 vaccine BNT162b2 (Comirnaty). Serum samples were collected before receiving the booster dose (baseline), one month (T1) and three months (T2) afterward. We then measured anti-spike S1 subunit IgA (ELISA, Euroimmun). Results were presented as the median and interquartile range (IQR) or as a ratio with baseline anti-SARS-CoV-2 antibodies value. Results: The median anti-spike S1 subunit IgA titer of our population at baseline was a 1,28 ratio (IQR 0.73-2.21). One month after the booster dose a 4.5-fold increase from baseline occurred (median 5.86 ratio; IQR 4.82-6.22; p<0.001). Three months after vaccination we observed a 16.7% decline of antibody titer from T1 (median 4.88 ratio; IQR 3.45-5.23; p<0.001). 28 subjects, seronegative at baseline had an 8.5-fold increase at 1 month, compared to 45 seropositive subjects at baseline who had only 2.93-fold increase. The decrease at T2 was significantly higher in seronegative group than in seropositive group, respectively 30% and 16,8%. Moreover, only two baseline seronegative subjects out of the entire study population resulted seronegative again at T2. Conclusion: Our study shows that there is a substantial increase in antibody titer after booster dose in the whole study population. Considering that 97.3% of them remained seropositive at T2, our results strengthen the importance of adhering to a vaccination campaign in order to maintain an adequate antibody titer and limit the spread of SARS-CoV-2.

EP197

Anti-SARS-CoV-2 spike trimeric IgG measured 1 month and 6 months after single booster dose Pfizer-BioNTech COVID-19 in healthcare workers

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Introduction: In March 2020, WHO, after assessing the global spread of SARS-CoV-2 infection, declared the outbreak of COVID-19 a pandemic. Several vaccines have been developed during these years, but some studies have shown that their efficacy decreases significantly over time, so a booster dose was required. In November 2021, the administration of the third dose (booster) of the SARS-CoV-2 vaccine was made mandatory. With our study, we want to describe the antibody trend up to 6 months after the booster dose. Materials and Methods: The study population consisted of 255 healthcare workers (median age 47 years, 66,3% women) of the Pederzoli Hospital (Peschiera del Garda, Verona, Italy), receiving a booster dose of Pfizer COVID-19 vaccine BNT162b2 (Comirnaty). Serum samples were collected before the booster dose (baseline), one month (T1), and six months (T2) afterwards. The serum levels of anti-SARS-CoV-2 spike trimeric IgG were assayed with DiaSorin Trimeric spike IgG (DiaSorin, Saluggia, Italy). Results were presented as the median and interquartile range (IQR) or as a ratio with baseline anti-SARS-CoV-2 antibodies value. Results: In our population at T1 (median 7730 BAU/mL; IQR 4462,5-12350) we observed an increase (32,6-folds) of anti-SARS-CoV-2 spike trimeric IgG compared to baseline (median 237 BAU/mL; IQR 129,5-416). At T2 (median 3720 BAU/mL; IQR 1502,5-9495) there was a 51,9% decrease from T1. During the observation period (after the booster dose), 111 subjects resulted positive for a nasopharyngeal swab. This subgroup showed a 16.6% increase in antibody titer between T1 and T2, while the non-infected subjects had a significant decrease (73.2%). Furthermore, in subjects aged 50 years or older, the antibody titer dropped by 60.8% from T1 to T2 compared with the titer of subjects <50 years which decreased by 42.4%.Conclusion: the booster dose of Pfizer COVID-19 vaccine BNT162b2 triggers an excellent immune response in all subjects included in this study. However, the serum levels of anti-SARS-CoV-2 antibodies gradually lower six months after administration, but they still remain discreetly high. Since the decrease is more pronounced in elderly subjects, the assumption to carry out a second booster dose, at least in the most fragile subjects, becomes more concrete.

EP198

Dysregulation of IL-6 cis- and trans-signaling and high frequency of IL6R rs2228145(C>A) in the management of Deep Vein Thrombosis

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Deep vein thrombosis (DVT) usually occurs in the leg and can later develop severe consequences. To date, although the Virchow's triad (stasis of blood flow, endothelial injury, and hypercoagulability state) is thought to contribute to DVT, the mechanisms involved in its onset are still unknown. Among them, the cytokine-driven inflammatory process has been linked to the DVT occurrence. Recently, high plasma levels of the proinflammatory cytokine interleukin-6 (IL-6) have been found in DVT patients, but the involvement of its signaling pathway, e.g. the role of both membrane-bound and soluble receptors (IL6R and IL6ST) as well as IL6R rs2228145(C>A), in DVT is unclear.

To explore the relationship between inflammatory events associated with IL6 signaling and DVT, plasma and PMBC samples from DVT patients (n=19) and healthy controls (n=22) were retrospectively analysed, delving into the activation of IL6 cis- and trans- signaling pathways. Our results demonstrated the over expression of both transmembrane (TM) isoforms of IL6R and IL6ST in leucocytes, as a clear sign of IL6 cis-signaling stimulation. A strong association was observed between the occurrence of DVT and the expression of IL6R TM (OR, 17.07; CI, 3.48-83.75; p<0.001) or IL6ST TM (OR, 10.50; CI, 2.33-47.22; P<0.003). ROC curve analysis revealed a good diagnostic performance for both IL6R TM/IL6R ratio (sensitivity, 78.95%; specificity, 95%) and IL6ST TM/IL6ST ratio (sensitivity, 72%; specificity, 90%). The trans-signaling activation was only supported by the high IL-6 levels detected in patients with DVT (OR,5.7; CI, 1.22–26.3; p<0.05). It is noteworthy that the frequency of rs2228145 SNPs (AC and CC), a major determinant of circulating levels of soluble IL-6R, was strongly correlated with high plasma levels of sIL-6R in all subjects. Specifically, a strong association with DVT was observed when combining the IL6R rs2228145(C) allele with both IL6R TM/IL6R ratio (OR, 15.45; CI, 2.73-87.32; P<0.001) and IL-6 levels (>3.46 pg/mL) (OR, 21.32; CI, 1.10–412.20; p<0.01), highlighting the Asp358Ala variant as possible link between IL-6 overactivation and DVT outcome.

Overall, this study represents a proof of concept for the prediction of DVT development and the targeting of IL-6 signaling as a new therapeutic strategy for the DVT.

EP199

Possible Role of Nano-miRNAs in Diagnostics and Therapeutics

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Background MicroRNAs (miRNA) are short noncoding single-stranded endogenous oligoribonucleotides, key regulators of gene expression.miRNAs play an important role in different biological processes such as cellular development, differentiation, proliferation, metabolism, and apoptosis. The relationships between miRNAs expression and the onset and progression of different diseases, such as tumours, cardiovascular and rheumatic diseases, and neurological disorders, are well known. MiRNAs represent a novel tool for disease detection and treatment. Nanotechnologies can have a strong impact on the diagnosis and treatment of miRNA-related diseases such as cancer.Both approaches (detection and treatment) using nanotechnology-based strategies are analysed, to point out the current trends in this promising field of bio-medical applications. Rationale behind Article SelectionAn electronic search was performed using Medline databases (PubMed interface) for articles published from 2011 to 14 February 2022 (10-year interval). After the exclusion of duplicates, irrelevant articles, reviews, papers not written in English, or papers with missing data, 116 articles were included for review. Discussion Conventionally, miRNA detection is based on real-time polymerase chain reaction (qRT-PCR), microarrays, Northern blotting, and Next-generation sequencing (NGS). These measurement methods just reflect the average gene expression level and cannot provide the heterogeneity and transient spatiotemporal variations of miRNAs in living cells. The main difference between nanotechnology-based and conventional methods lies in the transduction mechanism. Nanoparticles and nanomaterials can have great potential in miRNA detection and biosensing thanks to excellent optical properties, making them ideally suited for the development of sensing strategies. Biosensors represent innovative analytical tools for clinical diagnosis as well as for a better understanding of the molecular mechanisms involved in pathophysiology, and to detect new biomarkers, such as miRNAs. Biosensors can help in the early diagnosis and monitoring of pathological conditions, particularly for oncological diseases, and are useful in prognosis, surveilling the evolution of the disease. In this report are described the progress made in recent years in different biosensing techniques, in particular plasmonic sensor platforms, for the detection of several cancer biomarkers in liquid biopsy samples. Analytical performance of different type of biosensors and their significant advantages are described in terms of high sensitivity and specificity, as well as accuracy and simplicity of workflow, for their future application in clinical practice. Another interesting aspect of nanotechnology is the possibility of using nanomaterials as nanocarriers to deliver miRNAs in cancer therapy. In fact, miRNAs exert a critical role in cancer mechanisms. They are regulators in cancer onset and progression, and in the development of metastatic processes. The inhibition of over-expressed miRNAs with an oncogenic role or the replacement of downregulated miRNAs that protect from carcinogenesis could be an interesting strategy to enhance anticancer therapies. Nanomaterials could overcome the problems related to the delivery of miRNAs to the target cells. Nanocarriers can improve drug stability, permitting and increasing their circulation time and their selective accumulation in cancer sites thanks to the enhanced permeability and retention effect and the presence of fenestrated blood vessels in tumours. most commonly nanosystems used to vehiculate miRNAs to tumour sites, are reported. Conclusions Developing novel methodologies aimed at clinical practice represents a big challenge for the early diagnosis and delivery of miRNAs in specific diseases. Nanomedicine, derived from nanotechnology, can exploit the unique properties of nanometer-sized particles for diagnostic and therapeutic purposes. miRNAs in association with different nanocarriers can be used in target cancer therapy. Through nanomedicine, specific treatment to counteract only cancer-cell proliferation will be improved, while leaving healthy cells intact. Within this context, nanotechnology represents a wide emerging area at the forefront of research over the last two decades, whose potential has yet to be fully attained.

EP200

Pediatric reference intervals veritication for several common chemistry and immunochemistry assays on the Beckman AU5800 and DxI analyzers in Modena, Northern Italy

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IntroductionThe CALIPER study established robust age- and sex-specific reference intervals (RIs) for many biomarkers on several analytical systems in a cohort of children and adolescents. We verified published CALIPER RIs for 32 chemistry assays on the Beckman AU5800 and DxI analyzers in a population of pediatric patients in ModenaMethodsBetween October 2021 and March 2022, leftover samples from 151 pediatric patients (0-18 years of age) collected from outpatient clinics were analyzed for a panel of 18 chemistry and 14 immunchemistry assays on Beckman AU5800 and DxI analyzers. Samples were included if no personal history of acute or chronic disease was found in our LIS (as suggested by clinical data associated with test orders and/or previous laboratory test results). The following assays were measured: albumin, ALP, ALT, amylase, apo-B, AST, total bilirubin, calcium, total cholesterol, creatinine, GGT, iron, LDH, magnesium, phosphate, total protein, transferrin, triglycerides; alfa-fetoprotein, cortisol, estradiol, ferritin, folate, fT3, fT4, TSH, FSH, LH, progesterone, prolactin, vitamin D, vitamin B12The number of laboratory test results that fell within the pertinent CALIPER sex- and age-spefic RI for AU5800 and DxI, accounting for 90% confidence intervals of the reference limits, was calculated for each parameter. When ≥90% of results fell within these limits, the RI was considered verified in our populationResultsOut of the 32 tests assessed, 11 chemistry and 11 imunochemistry assays met the criteria for verification. RIs for amylase, apo-B, total bilirubin, total cholesterol, GGT, LDH, phosphate, folate, vitamina B12 and cortisol did not meet criteria for verication. Of these, the percentage of our cohort falling within reference limits went from 89,8% for total cholesterol to only 15,1% for folate. These discrepancies could be justified by limitations in our patients selection method and/or due to biological differences between our cohort and the CALIPER reference individuals (e.g. Canada mandated folate fortification of food)ConclusionsWith a methodology derived from CLSI-EP28-A3c, we verified CALIPER RIs for several chemistry and immunochemistry assays, allowing the adoption of pertinent reference limits for use in our pediatric population

EP201

Gestione delle variabilità analitiche sul prelievo capillare per gli emogas analizzatori in area critica.

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Il POCT è un modello organizzativo del laboratorio clinico finalizzato al miglioramento della qualità della cura. L'attività diagnostica in POCT nei reparti di emergenza e terapia intensiva riduce il TAT e, di conseguenza, i tempi di gestione dei pazienti. Tale sistema permette inoltre la gestione a distanza di altre attività legate al miglioramento della qualità diagnostica e gestionale tra cui la formazione continua e i programmi di qualità di controllo sul lavoro svolto.

La frequenza degli errori che possono inficiare l'attendibilità dei risultati emogasanalitici in POCT si concentra soprattutto nella fase preanalitica, ed è condizionata da vari fattori, tra cui le modalità di raccolta, il trasporto e la conservazione del campione. A seguito dell'analisi degli errori preanalitici e del confronto con i dati in letteratura, è stato dimostrato che la modalità di prelievo mediante sangue capillare sembra essere la più problematica a causa della formazione frequente di coaguli. La Terapia Intensiva Neonatale, nella nostra struttura, utilizza largamente questo tipo di prelievo per l'esecuzione dei test emogasanalitici e l'influenza delle condizioni preanalitiche è un elemento determinante sulla produttività, a causa dei frequenti fermi strumentali causati dai coaguli.

L'obiettivo di questo studio è di valutare l'effetto della formazione specifica del personale addetto sulla riduzione della quantità di test falliti o con errori, presso l'unità di terapia intensiva neonatale del Policlinico Universitario A. Gemelli IRCCS di Roma.

Lo studio si focalizza sul prelievo capillare che viene utilizzato in emogasanalisi, ideale per i test POCT (1) in neonatologia, poiché la procedura capillare è semplice, poco invasiva e a basso rischio, mentre tra gli svantaggi va considerato il piccolo volume prelevato (10-250 uL), che potrebbe influire sull'accuratezza del test. Nel reparto di terapia intensiva neonatale sono in dotazione analizzatori NOVA Stat Profile Prime Plus che permettono la determinazione di emogas, elettroliti, metaboliti e CO-ossimetria.

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EP202

Dosaggio del recettore solubile dell'attivatore del plasminogeno di tipo urochinasi (suPAR) nella routine clinica: valutazione ad un anno dall'attivazione nel Corelab Alta Automazione del Policlinico Gemelli

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Numerose pubblicazioni hanno evidenziato il ruolo chiave del suPAR nei processi che coinvolgono infiammazione, immunità e coagulazione e recentemente, è stato riconosciuto come marcatore precoce di progressione nei pazienti con COVID19, suscitando l'interesse degli infettivologi.Pertanto, il dosaggio del suPAR è stato attivato nel marzo del 2021 nel Corelab Alta Automazione del nostro Policlinico (primi in Italia ad eseguirlo nella routine clinica ed in urgenza), proprio su richiesta degli infettivologi per individuare i pazienti COVID19 da trattare con Anakinra™. Avendo registrato un progressivo incremento dei dosaggi di suPAR, nonostante la riduzione dei ricoveri per COVID19, abbiamo voluto calcolare ad un anno circa dall'attivazione, il numero di richieste differenziate per tipologia di reparto e patologie, con lo scopo di valutare l'impatto dell'introduzione del nuovo test sui clinici. Dal 13.04.2021 al 13.05.2022 sono stati eseguiti 2532 dosaggi di suPAR presso il Corelab Alta Automazione, usando il dosaggio immunoturbidimetrico (suPARnostic® TurbiLatex, ViroGates A/S, Denmark), implementato su Advia Chemistry XPT (Siemens Healthcare Diagnostics, USA). Negli 8 mesi del 2021 sono stati effettuati 1296 dosaggi, mentre nei primi 5 mesi del 2022 è stato evidenziato un aumento del 40 % (1236). Nel 2021 le richieste da reparti COVID sono state 66.5 %, dal DEA 16.5% e da reparti vari (Medicine, Chirurgie, Rianimazione) il 17%; nel 2022 le richieste da reparti COVID hanno confermato i livelli del 2021 (67.6%), mentre sono diminuite al 7.4% dal DEA ed aumentate al 25% quelle da reparti vari. Abbiamo quindi analizzato le diagnosi dei pazienti, tramite IS intraospedaliero. Se nel 2021 le richieste erano quasi tutte di pazienti COVID, comprese quelle dal DEA, nel 2022 si è registrata una varietà dei quadri clinici: pazienti cardiologici, ematologici, nefrologici, critici con sospetta sepsi o SCA, per la stratificazione del rischio in pazienti acuti extra COVID e non esclusivamente infettivi. In conclusione, il dosaggio del suPAR attivato durante la pandemia da Sars Cov 2, sembra essere diventato uno strumento utile ai clinici per la valutazione prognostica dei pazienti in ambiti diversificati, oltre che per guidare le decisioni terapeutiche.

EP203

COVIDIAGNOSTIX: health technology assessment of serological tests for SARS-CoV-2 infection

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Background

In vitro diagnostic tests for SARS-COV2 have rapidly spread. However mostly single-center technical and diagnostic performance's evaluations have been carried out without an intralaboratory validation process and a HTA systematic approach. Therefore, the HTA for evaluating antibody tests for SARS-COV-2 was applied and the following objectives have been pursued:
-Comparison of tests through the antibody titer in the SARS-CoV-2 vaccination campaign.
-Assessment of the strategy implemented in the SARS-CoV-2 vaccination campaign.

Materials & Methods

A multidisciplinary team conducted the HTA following the DoHTA method. The three methodological and analytical steps used in the HTA were: the selection of the tests to be evaluated; the research and collection of information to support the adoption and appropriateness of the technology; and the drafting of the final report which allows its dissemination and communication.
In addition, alternative vaccination strategies were evaluated through the application of an algorithm that takes into account the ability to reduce the number of deaths and general containment of the pandemic.

Results

Serological tests allow to evaluate antibody response at different time points. In particular, disaggregating the data by sex and by age shows that the antibody response is high in women <48 years and is lower in men >60 years.
The algorithm showed a better performance of the TEST&VACCINE strategy compared to the VACCINE strategy (94.34%vs 83.87%).

Conclusions

Thanks to the availability of real world data relating to the serological analysis it was possible to investigate the evolution of the antibody response to vaccination against SARS-CoV-2 in a large cohort of subjects, and the possibility of a harmonization process of the serological laboratory data, through the use of a conversion factor.
Finally, the possibility of defining differential temporal priorities in the target population constitute an important

element in optimizing the vaccination campaign, due to the ability to reduce the number of deaths and general containment of the pandemic.

EP204

Case report: undetectable HbA1c plasma level in a homozygous Lepore-Boston thalassemia patient

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Background: Glycated hemoglobin (HbA1c) derives from the non-enzymatic bond between the N-terminal end of the hemoglobin β-chains and glucose. Quantification of plasma HbA1c levels is a worldwide standardized, accurate, reproducible, and non-fasting test that reflects glucose levels within the previous three months.Case presentation: We herein report a case of a 49 years-old woman with a hypochromic microcytic moderate anemia (HB=8,70%; MCV=74,30 fL; MCH=22,70 pg), without martial deficiency (SI=95 μg/dL). Using a cation-exchange High-Performance Liquid Chromatography (HPLC) method, the patient's HbA1c level was undetectable. In addition, the chromatogram showed high levels of fetal hemoglobin (HbF=75.0%) and hemoglobin A2 (HbA2=10.3%), and absence of adult hemoglobin (A0). In order to quantify the HbA1c level using the capillary electrophoresis (CE) technology, the same plasma sample was evaluated by another laboratory, confirming HbA1c non-detectable result.Discussion: The reported high values of HbF and HbA2, in combination with the absence of HbA0 and the hematological parameters, were suggestive of a form of hemoglobinopathy. Molecular analysis revealed the presence of a rare homozygous state for Hb Lepore-Boston, a hemoglobin variant caused by a δβ-globin genes in-frame fusion which coelutes with HbA2 during HPLC analysis, justifying its increased value. In the case of homozygosity for this mutation, no HbA2 is produced, therefore, the abnormal peak can be totally ascribed to the Hb Lepore-Boston variant. At the same time, the elevated quantity of HbF could be explained as a compensation for the complete absence of HbA0. Conclusion: In the patient analyzed, the quantification of plasma HbA1c levels was undetectable owing to the lack of HbA0. The HbA1c measurement is limited by the presence of hemoglobin variants affecting the accuracy of this technique, making it unusable for the diabetes diagnosis and follow-up. Alternative laboratory tests, such as fructosamine and glycated albumin assessments, have to be used for medium-term glucose levels evaluation. This work highlights the importance of a correct interpretation of HPLC and CE methods allowing the discover of rare forms of haemoglobinopathy and proposing a better test choice for diabetes monitoring.

EP205

Comparison between 24-hour collection and single spot urines for the quantification of biogenic amines by HPLC/MS-MS in the diagnosis of pheochromocytomas, paragangliomas and neuroblastoma

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Pheochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumors that originate from adrenal medulla or extra-adrenal chromaffin cells, respectively. They produce an excess of one or more catecholamines (dopamine, epinephrine and norepinephrine) and their inactive metabolites (free and total metanephrines, free and total normetanephrines and 3-methoxytyramine). Abnormal levels of these biogenic amines have been also founded in paediatric patients with neural crest tumors, particularly neuroblastomas (NB). Due to the diurnal variation in their renal excretion, the standard practice recommends the quantitative determination of the biogenic amines in urine samples derived from 24-hours collections. However, the collection of 24-hour urine samples cannot be performed easily, especially for children: it is time consuming, it is sometimes difficult to obtain a complete collection and it rarely requires catheterization; moreover, to prevent the degradation of the molecules, the acidification of the samples with hydrocloric acid or citric acid to pH 4 is necessary. In our study, we enrolled 50 patients with clinical symptoms related to PPGL or NB and we determined the concentration values of catecholamines and their inactive metabolites both for spot and acidified 24-hour urine samples using High Liquid Performance Chromatography tandem mass spectrometry (HPLC-MS/MS). To eliminate the variability of the metabolite renal excretion, we expressed all the obtained results as ratio to urinary creatinine concentration, previously determined for each patient and for each type of sample. A correlation study between the levels of each biogenic amine in spot and in 24-hour urine samples was performed using a linear regression analysis model together with the Pearson correlation coefficient. The obtained results provided valuable information for clinical practice and for a safe clinical management of PPGL and NB. In particular, we demonstrated the interchangeability of the two samples using the gold standard laboratory technique such as HPLC-MS/MS. For all the biogenic amines, except epinephrine, there was a strong correlation between the two measures and the Pearson coefficients were always greater than 0.7.

EP206

SARS-CoV-2 surveillance: Nanopore sequencing as potential rapid tool for novel pathogen variants characterization

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Genomic epidemiology is a crucial weapon in the public health fight against infectious diseases.Whole-Genome Sequencing (WGS) of SARS-CoV-2 provides critical insight into viral transmission and evolution.Oxford Nanopore Technology (ONT) works by monitoring changes to an electrical current as a strand of nucleic acid passes through a protein nanopore and the resulting signal from multiple nucleotides is decoded to provide the specific sequence.Illumina sequencing technology, sequencing by synthesis (SBS), detects base-by-base as they are incorporated into growing DNA strands enabling accurate data. In the present study a comparison between ONT performance on MinION and Illumina ISeq was evaluated.Library preparation was performed on 25 SARS-CoV-2-positive specimens collected at Hygiene Laboratory, San Martino Hospital (Genoa, Italy) by COVID panel CE-IVD kit developed at 4Bases and CleanPlex SARS-CoV-2 NGS Panel by Paragon Genomics, respectively to be sequenced on ONT MinION MK1B and Illumina iSeq100 platforms.Data analysis was carried out using the 4eVAR (4Bases) and Sophia DDM Software (Sophia Genetics) platforms and consensus sequence was analyzed using Nextclade webservice. Each variant was classified according to World Health Organization (WHO), Clade and Lineage.Both technologies correctly identified 7 Delta and 18 Omicron variants. Mean coverage on MinION was 763x, after 3 hours of run, covering 98% of the genome. There was perfect match for the WHO, Clade and the Lineage classification except for one difference along the sub-lineage classification of one omicron variant (BA.2 in MinION, BA.2.3 in Paragon).Single mutations analysis showed 96% overlap on average in mutation classification considering all mutations, 98% considering mutations on Spike protein.The two different technologies show a comparable analytical performance, with small differences concerning precise mutation classification. MinION enables real-time analysis, therefore is faster, cheaper and more flexible than standard sequencing techniques. By this analysis it is confirmed to be a promising platform, with the potential to represent a convenient portable and rapid tool for the SARS-COV2 surveillance.

EP207

Evaluation of humoral and T-cell mediated immune responses after the third dose of BNT162b2 m-RNA based vaccine in immunosuppressed patients treated with anti-CD20 monoclonal antibodies

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The benefits of m-RNA vaccines in immunosuppressed patients receiving anti-CD20 monoclonal antibodies such as patients with B-line haematological malignancies or multiple sclerosis (MS) are poor investigated. Several studies demonstrated that anti-CD20 therapies were associated with a reduction/absence of the humoral response but only few data are available on T-cell immunity. In our study, we evaluated the antibodies levels and the T-cellular response of 70 immunosuppressed patients receiving anti-CD20 monoclonal antibodies (45 haematological and 25 MS patients), after the administration of the third dose of BNT162b2 vaccine. We also enrolled 10 healthy individuals, as controls. Anti-CD20 therapies significantly reduced the vaccine-induced antibodies targeting the spike protein (anti-S antibodies) in most patients (both haematological and MS patients). When they were stratified based on time elapsed between therapy infusions and vaccination, the median of anti-S antibody levels showed significant differences: patients vaccinated during the treatment were seronegative; patients who began the therapy after one, two or three doses of vaccine generated increasing antibody titers (109 BAU/mL; 484 BAU/mL; 2532 BAU/mL, respectively); patients who started vaccination 6 months or more after the suspension of the therapy presented good antibody levels (9173 BAU/mL), slightly lower than those of controls (11914 BAU/mL). The magnitude of the T-cell response after vaccination was determined by an interferon (IFN)-γ enzyme-linked immune absorbent spot (ELISPOT) analysis, stimulating peripheral blood mononuclear cells (PBMC) of patients and controls with overlapping peptide pools of the spike protein. The vaccination induced T cell immunity was partially preserved in patients receiving anti-CD20 monoclonal antibodies, even in those without detectable anti-S antibodies. There were important differences between haematological and MS patients: 97% of MS patients developed a good T-cell response after vaccination (with a median value of 96 spots forming units (SFU) per million of PBMC). Conversely, only 59% of haematological patients, treated in association with other cytostatic drugs, produced a protective T-cell response (with a median value of 40 SFU per million of PBMC).

EP208

A cross-sectional correlation study between Lupus anticoagulant tests (dRVVT and SCT) and CLIA anti-phospholipid antibodies (anti-β2glycoprotein I and anticardiolipin) tests.

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease characterized by thrombosis and/ or pregnancy morbidity with the persistent presence of antiphospholipid antibodies (aPL). The main laboratory criteria for APS diagnosis are lupus anticoagulant (LAC), anti-cardiolipin (aCL) and anti-β2glycoprotein I (aβ2GPI) antibodies IgG or IgM. Positivity for all three of these antibodies is associated with a high risk of thrombosis. The aim of this study is to evaluate aCL and aβ2GPI antibody interference in LAC coagulation tests. Methods: aCL and aβ2GPI antibodies were detected using a chemiluminescent immunoassay (CLIA) test (Werfen, Italy); LAC was detected using dRVVT and SCT coagulation tests (Werfen, Italy). A cross-sectional correlation study was performed on 1020 patients enrolled at the "Tor Vergata" University Hospital, Rome, Italy. Inclusion criteria: patient request for LAC, aCL and aβ2GPI antibodies. Exclusion criteria: therapy with oral anticoagulants (DOAC, AVK, UFH). Our data were collected from entries in the hospital database from 01/02/2021 to 31/12/2021.Results: We observed an overall concordance between negative results for both antiphospholipid antibodies and LAC that was higher than 91%. In addition, we found a concordance of over 80% between positive LAC coagulation tests and the absence of aCL and aβ2GPI antibodies.Conclusions: In this study, we confirmed the antiphospholipid antibody family heterogeneity. In addition, we demonstrated that aCL and aβ2GPI antibodies do not interfere in LAC determination. Therefore, LAC is independent from aCL and aβ2GPI antibody positivity. LAC remains one of the main laboratory criteria for the diagnosis of APS, even if it is an indirect method. The factor determining positive LAC coagulation test results remains to be identified.

EP209

An artificial neural network-based system to implement bacteria identification on clinical slides

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Early identification of the bacterial morphology and staining properties in clinical smears still represents, despite the advent of molecular biology, a key approach in diagnostic flowchart as a first and fast step towards adequate antimicrobial treatment choice.A commercial and already available CE IVDR-certified hardware/ software device, able to discriminate bacteria from mycobacteria by colour identification in Ziehl-Neelsen and fluorescence-stained slides, was upgraded with an artificial neural networks (ANNs) based approach, with the aim to identify mycobacteria and discriminate, at morphological level, tubercular Acid Resistant Bacilli (BAAR) from atypical bacilli. Single slide reading is completed in less than 3 minutes.The hardware is a fully automated system composed by a microscope (Olympus BX43), a slide holder (maximum load 60 slides), a chamber with 1600x1100 resolution, an immersion oil dispensing system (100x magnification) and a focusing system. The automation of the whole equipment is managed by a robotic system.The software captures the digital images, divide them into 300 fields and store them. Specifically, trained supervised ANNs are applied to classify and interpret these images.This system was tested on 1000 samples, manually labelled by experts, obtaining recall 89.2 %, specificity 93.3 % and precision 93.0 %.On the basis of the encouraging results obtained, a new project has been started to apply an analogous approach to GRAM-stained reading. Using a set of repository images, the feasibility to further extend the software capability to provide information on bacterial morphology (strepto/staphylococci, bacilli, etc.) and cellular morphology (squamous cells, lymphocyte etc.) is being evaluated.The use of ANNs may support the clinical decision, particularly in emergency setting (sepsis, meningitis etc) when a fully trained microbiologist may not be promptly available on site. The digitalization of the slide, already classified in its key elements may represent a useful tool for remote validation of the diagnosis. The operator can confirm the software proposed identification or modify it by a manual approach on individual points of interest. These manual changes can be used for the continuous upgrade of the learning system.

EP210

Verifica delle prestazioni analitiche dei metodi immunochimici nello screening tossicologico di Ossicodone e Fentanyl

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Introduzione: L'Ossicodone ed il Fentanyl sono analgesici narcotici semi-sintetici e sintetici, diffusi recentemente come sostanze d'abuso. Il laboratorio territoriale ed ospedaliero in supporto agli ambulatori per le dipendenze deve essere in grado di disporre di metodiche affidabili per la discriminazione di tali molecole in campioni urinari. Lo scopo di questo lavoro è la verifica del metodo di dosaggio semi-quantitativo Oxycodone Urine Enzyme Immunoassay Kit (IMMUNALYSIS) e qualitativo ARK Fentanyl Assay Kit (ARK Diagnostics) ottimizzate sull'analizzatore da banco Furuno CA270™.

Materiali e Metodi: La verifica dei metodi, secondo la linea guida "ISTISAN 16/39", ha previsto per il metodo semi-quantitativo la determinazione del limite di quantificazione (LOQ), precisione (ripetibilità intra-sessione - n.10 repliche - e riproducibilità inter-sessione - 15 cicli analitici - in termini di coefficiente di variazione, CV%), accuratezza (errore percentuale %E con modello 4x3x5) e per il metodo qualitativo (verifica del LOQ e la curva di risposta costruita in base alle percentuali dei risultati positivi e delle concentrazioni dell'analita, n.10 repliche per 4 livelli). I risultati sono stati confrontati con le performance dichiarate dal produttore e i limiti di accettabilità adottati dal laboratorio (ripetibilità e riproducibilità: CV<15% per metodi quantitativi e <5% di falsi negativi /positivi intorno al valore cutoff per metodi qualitativi).

Risultati: Per il metodo di dosaggio dell'ossicodone i risultati prodotti mostrano valori di %E medio (8%) e su singoli livelli (L1=4%, L2=11%, L3=14%, L4=5%) nei limiti di accettabilità così come i valori di ripetibilità (CV=3,2%) e riproducibilità (low control CV= 7.7%, high control CV= 4.1%); il valore di LOQ è risultato congruente con quelli dichiarati dal produttore.

Il metodo di dosaggio del fentanyl è risultato pari allo 0% di falsi positivi/negativi in relazione al valore cut-off (1 ng/ mL).

Conclusioni: La proposta dei metodi commerciali verificati in termini di performance in questo lavoro può rispondere alle esigenze pratiche di un laboratorio ospedaliero e territoriale di media/alta produttività per lo screening dell'ossicodone e del fentanyl in campioni di urina.

EP211

A UHPLC-MS/MS method for the simultaneous quantification of the anti-COVID-19 drugs remdesivir, molnupiravir and their active metabolites in human plasma.

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COVID-19 emergency required the development of specific therapeutic choices, especially for patient at higher risk to have a worst prognosis. Among the first and most widely used antivirals are remdesivir (RDV) and molnupiravir (MPV): these are both nucleoside analogs prodrugs, capable to inhibit viral RNA-dependent RNA polymerase by strand termination and a “mutational catastrophe”, respectively. While RDV is only available for intravenous use in hospital, MPV is an oral drug, allowing domestic use. The circulating active metabolites of these drugs, GS-441524 and H-hydroxycitidine (NHC), respectively, are considered for the description of their pharmacokinetics (PK) and are related to their antiviral effect. Nevertheless, PK characteristics of RDV, MPV and their metabolites in the real-life use are still poorly explored, particularly due to the lack of validated methods for their quantification in human matrices. Therefore, in this work, we aimed at validating a fast, reliable and rugged ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) method for the simultaneous quantification of these prodrugs and their active metabolites in human plasma, following FDA and EMA guidelines. Sample preparation consisted in a protein precipitation protocol: 50 µL of plasma are added with 50 µL of a solution of isotope-labeled internal standards (IS) in water:methanol (50:50 v:v) and, then, with 400 µL of a mixture of acetonitrile:methanol (50:50 v:v), vortex mixed and centrifuged. After drying at 40 °C in vacuum centrifuge (1 h), the extracts are reconstituted with water 0.2% formic acid.Then, 5 µL of the extracts undergo UHPLC separation with a gradient run of water (Phase A) and acetonitrile:methanol 50:50 (v:v, Phase B), both with 0.2% of formic acid at 40°C in a core-shell reverse-phase column (Kinetex polar C18, 2.1x100 mm, 2.6 µm). The total runtime is 5 minutes and drug detection is performed by MRM, with 2 specific transitions for each compound and IS. The method fulfilled the requirements from FDA and EMA guidelines in terms of accuracy, precision, recovery, matrix effect, stability and it was applied to samples from a small cohort of patients treated with these drugs, confirming its eligibility for research and clinical use.

EP212

MONITORAGGIO DEI TAT PRE- E POST-INSTALLAZIONE DELL’AUTOMAZIONE NEL CORELAB DELLO SMEL DELL’ASST DI LODI

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Da gennaio a marzo 2021, lo SMEL di Lodi ha riorganizzato il Corelab, in collaborazione con Beckman, Diasorin, Biomedical Services con la nuova automazione che segue la filosofia Lean mirroring: la provetta, in automazione, attraversa l’area di Immunometria, incontra 2 DxI 800 che prelevano un’aliquota di siero liberando immediatamente il campione, per poi andare nell’area di chimica dove sono operativi l’AU5800 (doppio modulo analitico indipendente) e il DxC700AU in grado di gestire sia i test urgenti (prioritari) che di routine.E’ stato valutato quanto l’automazione abbia influito positivamente sulla gestione delle urgenze andando a monitorare i TAT(turn around time) degli esami eseguiti in urgenza prima e dopo l’ installazione della catena. I tempi di validazione clinica a partire dal check-in dei campioni sono stati estrapolati in minuti da Dnlab nelle diverse fasce orarie (00-08, 08-14, 14-20 e 20-00) per i seguenti analiti: K, troponina, Hb (emocromi) e PT e raggruppati per singolo mese da marzo 2020 a dicembre 2020 e da marzo 2021 a dicembre 2021. Per ogni mese è stata fatta la media dei TAT medi di ogni singola fascia oraria. Le medie così ottenute per il 2020 e il 2021 sono state confrontate per determinare la significatività statistica con il Ttest a 2 code e varianza 2. Sono stati esclusi i mesi di gennaio e febbraio 2021 dall’analisi perché coinvolti nell’installazione dell’automazione.L’analisi statistica per singolo mese ha evidenziato una riduzione dei tempi di validazione di 7 minuti per il K ($p<0.005$), di 5 minuti per la troponina ($p<0.005$) e di 6 minuti per PT ($p<0.005$). Non si sono registrate differenze statisticamente significative nei TAT degli emocromi. Analizzando invece i dati per fasce orarie, è emerso che la riduzione dei tempi di refertazione maggiore è stata registrata nel gruppo 00-08 e 08-14: rispettivamente 10.4 e 9.7 minuti per il K ($p<0.005$, $p<0.05$), 5 e 7.6 minuti per la troponina ($p<0.005$) , 9 e 7.4 minuti per il PT ($p<0.005$).I dati dimostrano che la nuova automazione ci permette di affrontare carichi di lavoro più elevati e di rilasciare i test in urgenza in tempi più brevi, soprattutto durante l’orario di routine

EP213

Age-associated modifications in red blood cell counts in an outpatient population of northern Italy: a big data analysis

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Aim: Blood counts are extensively requested in routine laboratory diagnostic investigation as markers of disease conditions. The older subjects may present a decline in red blood cell count, hematocrit and hemoglobin levels as a manifestation of overt disease condition (anemia) or a physiological associated aging change. The objective was to investigate the possible association between changes of cell blood count parameters and aging.

Methods: We examined samples collected by outside hospital patients in the province of Modena, Italy, in the period between January 2010 and March 2020 including hemoglobin, red blood cells, white blood cells, platelets and mean corpuscular value. Data were stored in a Vertica Sequel Server and analyzed by means of the platform Anaconda 3, Python 3.7 and related statistical and graphical packages.

Results: Modena's resident database at the end of February 2020 was composed by 4,007,945 different analyses of the investigated parameters suitable to be evaluated in the study. The distribution of both hemoglobin and red cell count shows that older persons (age 75 and over), both males and females, are largely under the normal value lower limit (respectively, 41.6% and 29.6%). By contrast in the case of white blood cells, platelets and mean corpuscular value not any changes age related were observed. To minimize selection biases, we divided the data by year, and took the average value per single person per year; the trend was similar (32.8% of men and 22.2% of women below the normal boundaries). In order to exclude patients with organ or chronic disease, we limited our observation to subjects with measured normal values of serum glucose, creatinine and transaminase, again considering a single value per year per patient. In this set, composed by 920,012 analyses, 19% of older male subjects were still below normal values.

Conclusions: In the studied outpatients population a relevant proportion of older subjects, especially of male gender, showed hemoglobin levels below the lower limit of the normal range. These findings suggest extreme caution in the interpretation of blood counts in elder population, and might ultimately support a re-definition of the normal laboratory values for eldest people and, in general, for the whole age related population.

EP214

Piano di convalida Nuova Automazione Corelab ASST Lodi

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Da gennaio a marzo 2021, lo SMEL di Lodi ha riorganizzato il Corelab, in collaborazione con Beckman, Diasorin, Biomedical Services con la nuova automazione che segue la filosofia Lean mirroring: la provetta, in automazione, attraversa l'area di Immunometria, incontra 2 DxI 800 che prelevano un'aliquota di siero liberando immediatamente il campione, per poi andare nell'area di chimica dove sono operativi l'AU5800 (doppio modulo analitico indipendente) e il DxC700AU in grado di gestire sia i test urgenti (prioritari) che di routine. La sierologia, eseguita sui LIAISON XL, segue un percorso dedicato.Il piano di convalida dei nuovi strumenti si è basato sulle linee guida SIBioC. Sono stati quantificati 10 analiti da testare sugli strumenti in dismissione e sui nuovi (creatinina, glucosio, LDH, γGT, Lipasi, colesterolo, ALT, proteine totali, bilirubina totale e PCR), per n=30±10 campioni giunti in laboratorio, in sedute analitiche differenti. L'analisi statistica dei risultati ha mostrato un indice di correlazione lineare prossimo a 1 per tutti gli analiti analizzati.Per l'allineamento con lo Spoke di Codogno sono stati quantificati gli stessi 10 analiti sull'AU480 1 e 2, per n=16±1 campioni pervenuti al laboratorio in sedute analitiche differenti. L'analisi dei dati ha mostrato un indice di correlazione lineare prossimo a 1 per tutte le variabili, ad eccezione dell'LDH (indice di correlazione=0.76). Si è proceduto alla calibrazione degli strumenti dello Spoke e sono stati ripetuti i test su entrambi gli analizzatori per n=16 campioni; l'indice di correlazione è salito a 0.95 per l' AU480-1 e a 0.92 per il 2.Abbiamo eseguito un'analisi sui CQI registrati in UnityRealTime valutando 2 livelli di concentrazione (±25%) e calcolando l'imprecisione intra- e inter-laboratorio. Inoltre abbiamo valutato l'indicatore SDI utilizzando la funzione "tabella analisi dati" del programma Unity.Questa nuova soluzione ha permesso allo Spoke di Codogno di passare da una tecnologia a chimica secca (Ortho) a una liquida (Beckman) garantendo uniformità tecnologica ed analitica con il Laboratorio Centrale, grazie a identicità e interscambiabilità dei reagenti, alla medesima interfaccia utente degli analizzatori di chimica e immunometria e al Middleware, condiviso da entrambi i laboratori.

EP215

The appropriateness of laboratory test requests in the USL Umbria 2 in the diagnosis of celiac disease, in light of the most recent guidelines.

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The ESPHAGAN 2020 and ACG guidelines dictate a new prescribing model in the diagnostic pathway for celiac disease.
The first-line test is the serological assay of total tTG-IgA and IgA. The total IgA dosage is essential for the exclusion of the partial or total selective IgA deficiency, linked to the age of the subject and therefore to the maturative state of the immune system or to a congenital defect of the B cell system unable to produce class immunoglobulins. IgA. In this case the recommended serological tests are tTG-IgG and ab anti gliadin IgG. In the case of a positive tTG-IgA, both <10xULN and> 10xULN, the HLA DQ2 / DQ8 test should never be performed as the gastroduodenal biopsy will be decisive.
By virtue of these, the most recent guidelines in laboratory diagnostics for celiac disease, were analyzed of data of the prescriptions in the USL Umbria 2 to verify the state of adherence to the indications of these documents. With reference to the prescription of total IgA associated with tTG-IgA, in order to exclude any selective deficits (dsIgA), the strong discrepancy between the number of requests for tTG-IgA 5636 and 2010 total IgA is evident.
In light of the prevalence of dsIgA ranging from 1: 100 to 1: 1000, the inadequacy of the requests for ab anti Gliadin IgG and tTG-IgG is evident; in 2021 there were 2756 requests for ab anti Gliadin IgG and 792 tTG-IgG out of a total of 5636 requests for suspected CD.
Furthermore, there are numerous requests at first access for tTG-IgA associated with ab anti Endomisio IgA, as many as 3016. The ab anti Endomysium IgA, characterized by a very high specificity (> 99%) are useful for diagnosis only in cases of positive tTG test -IgA, and is therefore to be used as a confirmatory test.
Another evidence of this study is the inappropriateness of the request for DQ2 / DQ8 genetic tests performed at the first access in association with serological tests, in 2021 as many as 603.
At the end of this analysis, the need for a review of the prescribing paths of General Practitioners and Pediatricians is evident and, in addition to this, the introduction of reflex tests in the regional nomenclator is necessary, which allow the request for an initial test which will be followed by cascade other tests based on the initial outcome

EP216

An incidental finding in Clinical Biochemistry: a case of macrotroponin

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Introduction. Cardiac troponin I and T (cTnI/T) are the most sensitive and specific biomarkers of cardiomyocyte injury. In recent decades, tests for cTnI/T have undergone enormous improvements. High-sensitivity assays are recommended over sensitive ones, as they provide improved diagnostic performances at identical cost1.However, it should be noted that the presence of factors interfering with the measurement and false-positives could be encountered making necessary to identify these cases to prevent inappropriate treatment of patients.Patient and methods. To identify the references values of high-sensitivity cardiac troponin I (hs-cTnI) in newborns, a healthy few months old baby was tested. Hs-cTnI plasma concentrations were measured by chemiluminescent microparticle immunoassay on the ARCHITECT i2000SR system (Abbott Laboratories, Wiesbaden, Germany). Hs-TnT were measured by Elecsys ® Troponin-T high sensitive (Roche, Basel, Switzerland). Heterophile antibodies, alkaline phosphatase and rheumatoid factor were evaluated with specific tests. Polyethylene glycol (PEG) 6000 precipitation was used to identify troponin macrocomplexes.Results. The patient showed hs-cTnI levels of 4313 pg/ml. The presence of cardiac diseases was excluded by instrumental methods. Hs-TnT, as a comparative test, showed levels of 0.041 ng/mL, higher than the 99th percentile <0.014 ng/mL. Rheumatoid factor levels were < 10.1 U/mL (reference value <20 U/mL) and heterophile antibodies were negative. Serum of patient treated with PEG determines the precipitation of about 99% of the Hs-cTnI values, allowing to identify the presence of troponin macrocomplexes as the cause of increased cTnI levels.Conclusion. The identification of possible interferences in the assay of hs-cTnI is a crucial point of laboratory medicine, if not recognized they can cause misdiagnosis with harmful consequences for the patient. It is estimated that 5% of false-positives are caused by the presence of troponin macrocomplexes.

EP217

Technological transition from HPLC-ECD to LC-MS/MS for workflow optimization in the analysis of biogenic amines

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Introduction: Biogenic amines (catecolamines, their methylated metabolites metanefrines) are important for the diagnosis of several diseases such as phaeochromocytoma or other tumours of the nervous system. These compounds, when present in high concentrations, cause various toxicological effects and their monitoring and their accurate determination in urine samples is a matter of great importance for unbiased diagnosis of the disease.The most widespread methods for measurement of biogenic amines were based on high-pressure liquid chromatography coupled to electrochemical detection (ECD) at specific voltages. These methods require sample clean-up, commonly performed using solid-phase extraction (SPE) columns, to remove potentially interfering substances prior to chromatographic separation. These methods are time consuming and often suffer from interferences. In recent years several LC-MS/MS methods are developed and manufacturing companies have introduced commercial kits. The aim of this study is to compare the performances of a commercially available LC-MS/MS kit for biogenic amines analysis in terms of analytical quality and turnaround time.
Methods: For this study, 100 samples from the routine were analyzed with the HPLC-ECD method used in the laboratory and with the LC-MSMS method.Results and discussionSamples analyzed with HPLC-ECD and with LC-MSMS gave similar results with mean percent biases lower than 10%.The LC-MS/MS method shows superior specificity, overcoming the chromatographic interference from drugs and other unknown substances detected in the HPLC-ECD method. Furthermore, the use of labeled internal standard and a seven point calibration curve ensure better accuracy and robustness.HPLC-ECD method provide three mobile phases and three different column for the chromatographic separation of the different analyte groups while LC-MS/MS method uses the same mobile phase and the same column for all analytes. Total run time is 18 minutes for the HPLC-ECD method while ranges from 2 to 4 minutes for the LC-MS/MS method. The technological transition to LC-MS/MS has a great impact on laboratory routine considering reduced time of analysis and, most relevant, providing a better accuracy in determination of these analytes.

EP218

A rapid method for determination of underivatized arginine-related metabolites in human plasma using LC-MS/MS

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Introduction. The arginine metabolism is involved in many processes of physiological relevance such as sepsis, endothelial dysfunction, reproduction, immune function and tissue integrity. In literature are present many methods for arginine and its metabolites determination by LC-MS/MS that commonly provide long pre-analytical procedure as solid phase extraction and derivatization.

The purpose of this study was to develop a rapid LC-MS/MS method for the simultaneous determination of underivatized arginine, citrulline, ornithine, symmetric and asymmetric dimethylarginine (SDMA and ADMA) and monomethylarginine (MMA) in human plasma. Materials and Methods. The analytical procedure requires a deproteinization step, performed by adding 300 µL of methanol, containing isotopic internal standards for all analytes except for MMA, to 50 µL of plasma sample. After vigorous agitation, the sample was centrifuged at 14 000Xg for 7 minutes at room temperature, and then 2 of supernatant were injected into UPLC system. Standard stock solutions in water were used to obtain a seven points calibration curve and quality controls by spiking pooled plasma sample. The chromatographic separation was performed with an UPLC Acquity I-Class (Waters Corporation, Milford, Massachusetts, USA), using a Luna HILIC, 3 µm, 200 Å, 100 x 2.0 mm (Phenomenex, Torrance, California, USA), with a gradient of mobile phase A (containing 0.1% formic acid and 20 mM ammonium formate) and mobile phase B (90% Acetonitrile containing 0.1% formic acid and 20 mM ammonium formate). The flow rate was 0.45 mL/min and total run time was 5.5 minutes.Analytes detection was performed with a triple quadrupole Xevo-TQs Micro (Waters Corporation, Milford, Massachusetts, USA) equipped with electrospray ion source operating in positive ion mode.Results and conclusion. The described method allows to determinate the panel of metabolites with an accuracy ranged from 92.2 to 113.8 %, a precision ranged from 1.5 to 5.5 % and a repeatability ranged from 0.9 and 3.3 %. In conclusion, the method reflects our aim to obtain a rapid and easy method for arginine and its metabolites determination.

EP219

Calprotectin as prognostic marker in COVID-19 patients

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Introduction: Calprotectin plays an important role in the inflammatory response and its levels were significantly increased in patients with various inflammatory and autoimmune conditions. Calprotectin is located in the cytosol of neutrophils and is released after their activation. The aim of the study was to evaluate the prognostic role of serum circulating calprotectin in COVID-19 patients. Patients and Methods: We retrospectively analyzed data about 195 COVID-19 adult patients, 10 of which resulting in a fatal outcome. Circulating calprotectin and C-reactive protein (CRP) levels were measured with the ACHITECT i2000R System (Abbott Laboratories, Wiesbaden, Germany). Complete blood count was obtained by a standard method on ADVIA 2120 Hematology System (Siemens Healthcare). Results: Calprotectin levels and neutrophil counts were higher in patients with a fatal outcome respect to surviving patients: 4.26 (2.01-9.84) vs 1.28 (0.54-3.60) with p=0.006 and 20.26 (8.29-30.26) vs 6.57 (4.74-9.38) with p=0.002.Calprotectin levels correlated with levels CRP (Spearman coefficient 0.354, p<0.001) and neutrophil count (Spearman coefficient 0.350, p<0.001). Both high calprotectin levels and high neutrophil counts were associated with death at multivariate analysis.Multivariate analysis revealed the association with death of both calprotectin levels and neutrophil count, with an odds ratio (OR) higher for calprotectin levels 1.843 (1.292-2.630)p=0.001. ROC curve analysis of calprotectin levels revealed a good discriminating power toward survival with an AUC=0.759 (p=0.0004). The best cut-off value for calprotectin was 1.66 mg/L with a sensitivity of 90% and specificity of 58.9%. To test the prognostic value of this cut-off of calprotectin levels toward the exitus, a Kaplan–Meier analysis was performed. Log-rank test revealed a statistical difference in terms of survival between the two groups (p< 0.0005). Conclusion: Calprotectin levels higher than 1.66 mg/L are a predictor of mortality and can be used as a prognostic marker in patients with COVID-19. Calprotectin levels were more efficient in the outcome evaluation than other inflammatory markers, probably because it represents a marker of neutrophil activation.

EP220

Performance evaluation of POCT Statstrip® NOVA Biomedicals: the Careggi experience

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Objective: In agreement with CLSI EP09-A2 guidelines, the study aimed to evaluate the analytical performance, in terms of intra and interday precision, of the POCT Statstrip instrumentation (NOVA Biomedical Inc, Waltham, MA, USA) to Accu-Chek Inform II (Roche Diagnostic, Basel, Switzerland) compared to standard the laboratory method (Cobas 8000 Roche instrumentation) measuring blood glucose levels. Comparability between different matrices was investigated evaluating the estimation of the expanded measurement uncertainty (u). Materials and methods: A total of 38 heparinized whole blood samples were analyzed within 30 to 60 minutes of collection on the 2 glucose meters and then immediately centrifuged for measurement on the laboratory instrument COBAS 8000. These samples were spiked with various volumes of glucose concentrate to extend the glucose range.Finger-prick capillary glucose (N=10) measured on the glucose meters was compared with plasma glucose measured in the laboratory analyzers. We estimated measurement uncertainty of the concentration of glucose according to Brugnoni et Al. (2015), considering precision and bias components of the variability. Results: No systematic difference is observed between 2 glucose meters, while the slope (1.085) and estimated 95% CI (1.016, 1.17) values show the presence of a slight proportional deviation. The evaluation of the Bland-Altman results showed a bias value equal to 13% between 2 meters, and equal to 4% compared to reference method. The intraday and interday precisions were assessed using 3 levels of quality control and 3 levels of spiked whole blood samples. All of the glucose meters achieved precision of less than 5% at all levels. The expanded uncertainty of glucose showed a u value of 17 (k = 2) for the STATStrip meter and 11 (k = 2) for COBAS 8000. Conclusions: No significant differences resulted in intra-, interday variability and in the correlation between the Nova StatStrip and the COBAS 8000 analyzers, and the expanded uncertainty of the two instruments could allow the laboratory to evaluate any pre-analytical or systematic random errors relating decentralized diagnostic analysis perfomed by Point of care testing for glucose determination.

EP221

Comparison of glycated albumin and glycated hemoglobin assay in the healthy and diabetic population of the Salento area in Italy.

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ABSTRACT:
BACKGROUND:
According to the American Diabetes Association (ADA) guidelines (2021), diabetes may be diagnosed basing on plasma glucose criteria or Glycated Hemoglobin criteria. HbA1c represents an average of blood sugar levels over the last 12 weeks. Glycated Albumin (GA) reflects the last 20 days. GA is useful in all those conditions that require short-term monitoring of changes in glycemia. GA can be measured in patients with anemia, hemoglobinopathies, or with renal insufficiency. In this study we verify the accuracy of the GA test compared to HbA1c.
METHODS:
We measured AG (Werfen) on the serum of 290 patients from the Puglia Region in Italy. This subdivision considered the results of HbA1c (Arkray) based on the reference values applied in our laboratory. Two large groups were formed, healthy subjects including those with impaired fasting glycemia (136) and diabetic subjects including those at risk (154). We used Werfen's GA cutoff 14,5%.
STATISTICS:
Pearson's correlation was used where Pearson's correlation index (r) always assumes values between -1 and 1; Correlation is strong when it exceeds 0.70, intermediate values between 0.20-0.70 indicate moderate correlation.
RESULTS:
Comparative studies show a strong correlation (r 0.76) between GA and HbA1c both in diabetic subjects and in the group that includes diabetic and at risk subjects. In these same groups there is a moderate correlation when we compare GA (r 0.46) and HbA1c (r 0.60) with glycemia. In healthy subjects there is a moderate correlation (r 0.21) when comparing GA to both HbA1c and glycemia. Sensitivity (88%) and specificity (86 %) were measured by excluding the subjects who presented renal and hepatic function defects. The analysis to redefine the ideal threshold value based on the subjects studied, performed using the ROC curve, showed a cutoff for GA of 14.8%. At this cutoff, the sensitivity was 76% and the specificity was 74% with 0.840 AUC and P <0.001.
CONCLUSIONS:
Based on our result, we consider the GA test a valid aid to support the diagnosis of diabetes as long as it is used to supplement and not replace the more consolidated HbA1c test, advising every laboratory to redefine and validate their own reference values taking into account the target population and the methods used.

EP222

Controllo statistico del processo per il monitoraggio della raccolta e conservazione del plasma da aferesi per uso clinico.

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Introduzione: L'applicazione del controllo statistico di processo (SPC), ormai obbligatoria dal 2005 per i centri di lavorazione del sangue Europei (direttiva 2004/33/CE), è uno strumento fondamentale nella gestione del Sistema Qualità perché fornisce le evidenze sul monitoraggio del processo e le eventuali derive. Scopo di questo lavoro è stato quello di applicare il SPC per valutare i parametri sottoposti a CQ del plasma da aferesi per uso clinico prima e dopo congelamento.Materiali e metodi: Le specifiche per i controlli di qualità a cui ci siamo attenuti sono le seguenti: volume definito sulla base del sistema utilizzato ±10%; FVIII dopo scongelamento: qualità richiesta ≥70 UI/dL; Cellule residue (Emazie (RBC) e piastrine (PLT): esame emocromocitometrico, leucociti residui (r-WBC): metodica in fluorescenza ADAM by Macopharma), RBC <6×109/L, Plts <50×109, r-WBC<1×106. Per l'applicazione del SPC abbiamo applicato la carta di controllo per le variabili della media e deviazione standard.Risultati: Nell'anno 2021 sono state prodotte 525 unità di plasma da aferesi (117 F, 408 M) di cui 42 (8%) sottoposte a CQ. Abbiamo calcolato la central line (CL) ed i valori del limite superiore di controllo (UCL) e del limite inferiore di controllo (LCL) utilizzando la media±2 DS dei parametri di qualità. Le cellule residue sono risultate: RBC (0.1±0.02×109/L), PLT (11±7×109), r-WBC (0.16±0.13 ×106). Il FVIII prima del congelamento e dopo 3 mesi di conservazione a -30°C è risultato 105±18 UI/dL e 88±17 UI/dL, con una riduzione statisticamente significativa del 16%. I dati per il FVIII sono stati riportati sulla carta di controllo per le variabili (x-s charts).Conclusioni: Dall'analisi della carta di controllo delle variabili per il FVIII è emerso un andamento variabile dovuta, in primis, all' impatto del genoma sul proteoma umano ma anche alla tecnica di raccolta del plasma da aferesi e dal gruppo sanguigno del donatore. I dati sono risultati distribuiti intorno alla CL e nessun dato è risultato consecutivamente, per più punti, al di sotto oppure al di sopra della CL dimostrando che il processo di raccolta e conservazione del plasma è "in controllo" e non è stato necessario intraprendere alcuna azione correttiva.

EP223

Patient Blood Managment: importanza di Ret-He come marker di anemia da carenza di ferro latente in pazienti chirurgici.

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Introduzione:I globuli rossi (RBC) vengono spesso trasfusi in modo inappropriato nei pazienti con anemia da carenza di ferro. Il parametro biochimico comunemente utilizzato per identificare la carenza di ferro è la ferritina sierica che presenta un'alta specificità ma una bassa sensibilità, poiché aumenta anche nei processi infiammatori. L'emoglobina reticolocitaria (misurata come RET-He) è una misura dell'emoglobina media contenuta nei reticolociti, i globuli rossi immaturi appena rilasciati dal midollo osseo. Questo studio valuta il Ret-He come marker di anemia da carenza di ferro latente nella valutazione preoperatoria di pazienti chirurgici.Materiale e metodiE' stato eseguito uno studio prospettico su 100 pazienti chirurgici. E' stata valutata l'emoglobina (Hb), la conta dei reticolociti (Ret) e Ret-He con analizzatore Sysmex XN-1000 (Dasit). Sono stati valutati anche i seguenti parametri biochimici: ferritina, sideremia, saturazione della transferrina e la proteina C reattiva (PCR). In accordo con i dati della letteratura, i pazienti con valori di Ret-He ≤29 pg sono stati sottoposti a sei settimane di trattamento con terapia orale di ferro e monitorati a fine terapia.Risultati: Dei 100 pazienti, 40 presentavano anemia: 22 (55%) uomini con valori di Hb <13 g/dL e 18 (45%) donne con valori di Hb < 12 g/dL con valori di ferritina (media 265 ±22 μg/L), sideremia (119±32 μg/dL) e transferrina (172±22 μg/L) nella norma. 31/40 (77,5%) pazienti anemici avevano RET-He ≥ 29 pg mentre 9/40 (22.5%) avevano un valore medio di Hb 9 g/dL e Ret-He ≤ 29 pg (valore medio 21±7 pg). I nove pazienti, dopo sei settimane di terapia orale con ferro, presentavano un aumento di Hb (valore medio 12 g/dL) ed un aumento di Ret-He (valore medio 36 pg).Conclusione:Dall'analisi dei dati è emerso che dei 40 pazienti con anemia, 9 presentavano una anemia da carenza di ferro con valori di Ret-He ≤ 29 pg e parametri biochimici del metabolismo del ferro nella norma. I 9 pazienti presentavano valori di PCR elevati compatibili con un processo infiammatorio. Ret-He potrebbe essere incorporato come marker surrogato per la diagnosi di anemia da carenza di ferro per una corretta applicazione del patient blood managment (PBM).

EP224

Incidental detection of the Hb anti-Lepore variant by Capillary electrophoresis

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Anti-Lepore hemoglobins (Hbs) are rare βδfusion variants that arise from non-homologous crossover during meiosis. Lepore (δβ) variants produce a thalassemia-like disorder, whereas individuals with anti-Lepore (βδ) hemoglobin typically are hematological normal. A 20 years-old woman of foreign nationality came to our laboratory for the prenatal screening for thalassemia and hemoglobinopathies. Blood test showed mild anemia with normal values for the red cell indices MCV, MCH and MCHC. The hemoglobin variant test performed in HPLC technology (PremierHb9210 Resolution by Trinity Biotech), with "quick scan" program showed a normal pattern, with HbA2 of 2.3%, and absence of Hb variants. The Hb test performed with SEBIA Capillarys 2 instrument, installed in our laboratory to evaluate performances, confirmed the HbA2 value (2.4%) but surprisingly showed an evident Hb variant for the presence of two additional peaks in HbF zone and HbC zone. For further investigation of this discrepancy the sample was also
subsequently tested for HbA1c with Sebia Capillarys instrument. An HbA1c value of 38 mmoli/mol (5.3%), also confirmed with HbA1c immunological method Nihon Kohden Chemi-4100K, was obtained, with an atypical profile due to the presence of a presumptive Hb Variant confirming the result obtained with Sebia Hb Capillarys test.DNA analysis was performed for the final diagnosis and confirmation of the Hb variant and revealed the presence of Anti-Lepore Hb in heterozygosis. This case underlines the importance of a high-resolution method for the screening of Hb variants (and quantification HbA1c) to avoid the possible miss-interpretation.

EP225

Digital Morphology performance assessment in Hematology: automated hemocytometry vs optical microscopy vs flow cytometry.

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Background

Complete Blood Count with Differential (CBC diff) performed by an automated cell counter frequently needs a review in patients with immature leucocyte populations in peripheral blood. In these patients, Optical microscopy (OM) or flow cytometric (FC) differential counting are advised, with the first still considered as the gold standard. However, the OM WBC differential count, has some economical and diagnostic limitations, like inter-observer variability. Technological innovation has allowed the development of Automated Information Systems (AIS) and several studies reported good performance of AIS cell classification vs OM, but to our knowledge no investigation has been performed to compare AIS and OM with FC. The aim of this study is to assess AIS performance in immature granulocytes and blast classification vs OM vs FC, in 50 patients belonging to the Hematology section of UOC Laboratory Medicine, San Martino Hospital IRCCS in Genoa.

Methods

Fifty samples (40 haematological+10 healthy patients) were analyzed on Beckman Coulter® DxH 900 System hemocytometer/System Manage Software and the Beckman Coulter® CYTOMICS FC500/software CytoDiff CXP flow cytometer to address concentration and differentiation of leukocyte population. Blood smear sample were prepared with DxH SlideMaker Stainer II and analyzed on both CellaVision® DM1200 /CellaVision DM Software (DM1200) and Leica® DM2000 LED optical microscope. A Bland-Altman test and correlation and regression analysis were developed on Stata 11 Software.

Results

A strength positive correlation has been found between AIS pre and post-classification and FC absolute value of blast and IG (r2=0.99 and 0.96; r2=0.83 and 0.86, respectively) with no statistical difference between the two methods for both the leucocyte immature populations (blast AIS pre and post classification vs FC p=0.95, p=0.303: IG AIS pre and post classification vs FC p=0.755; p=0.72). Result were confirmed by Bland-Altman test.

Conclusions

Both automatic gating FC and AIS can be used as first level for WBC differential count in troubleshooting patients. However, for leukocyte differentiation incongruities or complicated cases these must be followed by OM check and/or multiparametric flow cytometric analysis.

EP226

Found the needle in a haystack: the first case of BIA-ALCL in University-Hospital of Padova

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We present the case of a 51-year-old woman admitted to the breast surgery Department of the Veneto Oncologic Institute (IOV) for suspected left periprosthetic hematoma. The patient's clinical history recorded a previous infiltrating lobular carcinoma of the left breast staged G2 (diagnosed in January 2010) treated with radical mastectomy and unilateral left prosthesis implantation (May 2011). From August 2021, the woman complained about the presence of a periprosthetic flap, not associated with fever or other signs of inflammation, therefore she underwent a revision of the pocket in December 2021. During this procedure, a cold periprosthetic seroma was aspirated and it was sent for flow and immunohistochemical analysis to the laboratory of Padova.Flow cytometry revealed the presence of a lymphoid population with an abnormal phenotype that raised the suspicion of Breast Implant-Associated Anaplastic Large Cell Lymphoma (BIA-ALCL). This population was about 74% of total cellularity and showed increased physical parameters, an aberrant expression of CD30, a strong positivity for CD2 and a partial one for CD4, loss of expression of the pan-leukocyte antigen CD45 and of CD3, CD5, CD7, T-associated antigens.The morphological evaluation of the yellow-amber periprosthetic fluid was performed by human digital cell morphology Cella Vision DM96 (Sysmex, Kobe, Japan) and showed the presence of large cells with anaplastic morphology. Those cells presented a high nucleus/cytoplasm ratio with an abundant basophilic and granular cytoplasm, pleomorphic nuclei with vesicular or dense chromatin and prominent nucleoli. In some cases the cytoplasmic vacuoles were so abundant and confluent to give the neoplastic cells a signet ring appearance1.Immunohistochemistry demonstrated that all neoplastic cells were CD30 positive and ALK-negative, as well as positive for perforin-1, EMA and negative for PAX5 and EBER. Moreover, they expressed an incomplete cytotoxic T-cell phenotype. By integrating the clinical-anamnestic data with the laboratory data, a diagnosis of BIA-ALCL was made. It is a rare type of non-Hodgkin lymphoma, first described in 1997 with an estimated prevalence of 1 per 30,000 women with breast implants2. Both saline- and silicone-filled implants have been implicated, with a median interval from the time of

the implant to the lymphoma of about 10 years3.It usually presents as an accumulation of fluid between the implant itself and the surrounding fibrous capsule. If the neoplastic cells are confined to the seroma fluid and the patients receive surgical excision4, BIA-ALCL is mostly an indolent disease with an excellent prognosis. If the seroma is associated with lymphadenopathy and invasion through the capsule, systemic chemotherapy is recommended.

Since this lymphoma was recognized by the WHO in 20164, only few cases of BIA-ALCL have been described. However, thanks to the diagnostic workup introduced by the NCCN guidelines published in 20195, and applied by the University-Hospital of Padova, the diagnosis of a still little known and extremely rare lymphoma was possible.

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EP227

Un caso clinico di idrope fetale idiopatica e il contributo del Laboratorio Generale alla definizione della diagnosi eziologica

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L'idrope fetale è una grave condizione medica, contraddistinta dall'accumulo di liquido nei tessuti sottocutanei e nelle cavità sierose del feto. Ne esistono due sottotipi: non-immune e immune. Il sottotipo non-immune è il più comune, può derivare da condizioni cardiovascolari, anomalie cromosomiche, infezioni, malformazioni urinarie o polmonari, ernia diaframmatica o grave anemia. L'idrope fetale immune dipende quasi sempre da un'incompatibilità sanguigna materno-fetale per il fattore Rh. Il caso riguarda una paziente di 37 anni (gruppo A Rh+), alla quale sono stati riscontrati, alla 30° settimana di gravidanza: polidramnios, idrotorace e intestino iperecogeno fetale. La paziente è stata ricoverata presso il Dipartimento di Medicina e Diagnosi Fetale dell'Azienda Ospedaliero-Universitaria Careggi per approfondimenti diagnostici. Tramite guida ecografica, sono stati aspirati liquido pleurico da entrambi gli emitoraci, ascitico e amniotico. I campioni prelevati sono stati inviati, oltre che all'Anatomia Patologica per l'esame citologico, al Laboratorio di Microbiologia e Virologia per gli esami infettivi e al Laboratorio delle Malattie Metaboliche dell'Ospedale Pediatrico Meyer per il dosaggio di oligosaccaridi e mucopolisaccaridi, anche al Laboratorio Generale per la valutazione di: alfa-fetoproteina su liquido amniotico; conta linfocitaria, proteine totali, albumina e creatinina sui tre liquidi. I risultati degli esami non hanno fatto luce sulla causa dell'idrope fetale. Il feto è deceduto in utero pochi giorni dopo la procedura diagnostica invasiva. È stata offerta alla coppia la consulenza genetica ed è stata effettuata l'analisi dell'esoma clinico sul DNA fetale da liquido amniotico e sul DNA di entrambi i membri della coppia: tramite il sequenziamento dei geni associati a idrope fetale attualmente noti, non sono state individuate varianti di significato chiaramente patogenetico. Nonostante in questo caso non sia stata identificata la diagnosi eziologica, viene messo in luce come il Laboratorio Generale possa contribuire all'iter diagnostico delle patologie fetali mediante l'analisi di campioni biologici di varia natura. A tal proposito, in particolare nei casi a eziologia ignota, sarebbe auspicabile un coinvolgimento sistematico del Laboratorio Generale.

EP228

An N-glycosylation hot spot in immunoglobulin κ light chains is associated with AL amyloidosis

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Immunoglobulin light chain (AL) amyloidosis is caused by a small, low proliferating B cell/plasma cell clone secreting a patient-unique, unstable, toxic light chain (LC). The pathogenicity of LCs is encrypted in their sequence, yet molecular determinants of amyloidogenesis are poorly understood.

Higher rates of N-glycosylation among clonal κ LCs from patients with AL amyloidosis compared to other monoclonal gammopathies indicate that this post-translational modification is associated with a higher risk of developing AL amyloidosis.

In the present study, we exploited LC sequence information from previously published amyloidogenic and control clonal LC and from a series of 220 newly sequenced patients with AL amyloidosis or multiple myeloma followed at our Institutions to derive and validate sequence and spatial features of N-glycosylation, combining bioinformatics with biochemical, proteomics, structural and genetic analyses.

Using sequence-based, in silico prediction of N-glycosylation, we found a peculiar pattern of N-glycosylation in amyloidogenic κ LCs, with ≈75% of the N-glycosylation sites laying in the FR3, particularly within the E strand, and consisting mainly of the NFT sequon (≈50%), setting them apart with respect to non-amyloidogenic clonal LCs. Biochemical and proteomic analyses confirmed sequence-based N-glycosylation predictions in our cohort of patients. Based on genomic, genetic, and structural analyses, the occurrence of mutations within selected regions of IGKV genes (progenitor glycosylation sites) during somatic hypermutation, rather than ultra-rare genomic variants, are likely to explain the N-glycosylation hotspot in amyloidogenic κ LCs at specific solvent-exposed surfaces.

Finally, based on currently available amyloidogenic and control, clonal LC sequences, we showed that using the presence of a putative N-glycosylation site specifically within the FR3 region (rather than at any position) improved the classification of a clonal κ LC as potentially amyloidogenic.

Our data further support a potential role of N-glycosylation in determining the pathogenic behavior of a subset of amyloidogenic LCs and may help refine current N-glycosylation-based prognostic assessments for patients with monoclonal gammopathies.

EP229

Prevenzione nella trasmissione del West Nile Virus mediante la trasfusione di emocomponenti labili : studio preliminare sui donatori residenti nella Regione Campania, nel Sud-Italia

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Introduzione:La febbre del Nilo occidentale è una zoonosi virale causata dal West Nile virus (WNV), un arbovirus a RNA a singolo filamento trasmesso principalmente dalle zanzare. Ulteriori mezzi di infezione, sebbene molto rari, possono essere il trapianto di organi, le trasfusioni di sangue o la trasmissione dalla madre al feto durante la gravidanza. L'infezione nei mammiferi è generalmente asintomatica, tuttavia le persone possono sviluppare una sintomatologia simil-influenzale o, più raramente, sintomi neurologici. A differenza di alcune regioni dell'Italia centro-settentrionale, sono disponibili pochi dati sulla diffusione del WNV in Campania. In questo lavoro abbiamo studiato la presenza di WNV in 312 donatori volontari selezionati in due aree, una paludosa e una non paludosa, della Campania a rischio di zoonosi.Materiali e Metodi: In questo studio sono stati selezionati 312 donatori volontari di sangue intero selezionati da area paludosa Costa Domiziana (140 donatori) e area non paludosa Alto Casertano (172 donatori). WNV IgG e IgM sono stati testati mediante ELISA. A tutti i donatori è stata eseguita la ricerca dell' RNA virale mediante biologia molecolare (RNA-WNV NAT).Risultati: Tra i donatori selezionati, tre, residenti nella Costa Domiziana, hanno mostrato livelli di IgG debolmente positivi. Altri due, residenti nelle zone dell'Alto Casertano, hanno mostrato scarsa reattività collocandosi nella zona grigia. Di questi cinque individui WNV IgG-positivi, i livelli di anticorpi WNV IgM sono risultati alti solo in un donatore. Per tale donatore è risultata positiva anche la ricerca dell' RNA virale.Conclusioni: Il donatore è stato sospeso per la donazione di sangue intero per 28 giorni, sono stati eliminati gli emocomponenti destinati all'uso clinico ed è stato seguito il test di conferma su secondo prelievo. Questi risultati preliminari suggeriscono che la Campania, pur essendo un'area a potenziale rischio zoonotico, può essere attualmente classificata come area a basso rischio di trasmissione del virus del Nilo occidentale.

EP230

TARGETED NEXT-GENERATION SEQUENCING APPROACH TO ASSESS DIFFERENTIAL DIAGNOSIS OF LYSOSOMAL STORAGE DISEASES

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Gangliosidosis GM1 is a lysosomal storage disease caused by mutations in the GLB1 gene, coding for the beta-galactosidase-1 enzyme. Differential diagnosis with Krabbe's disease is recommended, which is associated with mutations in the GALC gene, encoding the lysosomal enzyme galactocerebrosidase. Both diseases are autosomal recessive. In January 2022, an 8-month-old patient affected from cytomegalovirus (CMV) infection was analyzed, who showed signs of leukodystrophy on brain RMN. Suspicion of lysosomal disease was proposed, and the enzymatic activity of beta-galactosidase-1 was found to be bordeline. In order to reach the definitive diagnosis of Gangliosidosis GM1, genetic test for lysosomal storage diseases was performed in emergency, by NGS sequencing of 46 genes encoding different lysosomal enzymes. Molecular confirmation is essential to get the patient access to a life-saving gene therapy trial. Molecular analysis did not reveal the presence of variants with clinical significance in the GLB1 gene. However, a heterozygous mutation (c.302_308dup) has been found in the GALC gene, described as associated with Krabbe disease. NGS technologies are generally not certified to detect possible macro-duplications/deletions. However, the calculation of the diagnostic index (ID), obtained by normalizing the number of paired reads of the gene regions of our interest compared to a double-dose internal control and three normal control subjets, was found to be 0.5 from exon 11 to exon 17 of GALC gene. This result suggested the presence of a heterozygous macrodeletion, including exons 11-17, subsequently confirmed by Array-CGH. The activity of the enzyme galactocerebrosidase was subsequently found to be absent. The NGS approach allowed differential diagnosis for the patient, came to our attention with suspicion of Gangliosidosis GM1 but definitely diagnosed as suffering from Krabbe disease. Unfortunately, for the simultaneous presence of the CMV infection the child could not access the gene therapy trial for Krabbe's disease.

EP231

Alterations of haematological parameters in patients COVID 19 and correlation between the different variants.

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Background

SARS-CoV-2, which appeared in December 2019 in Wuhan, China (1) in the first pandemic wave (from February 24 to June 11, 2020) infected more than 236,000 people, in the second wave (from September 14 to December 31, 2020) the number of infected was more than 1,822,000 (2). During the first wave, in our P.O., the number of deceased, as well as hospitalizations in intensive care were higher. Purpose of the work

In our study we evaluated haematological alterations in hospitalized COVID 19 patients and, often in critical condition, for a hyperinflammatory state secondary to cytokine storm related to alterations of CD4/CD8 T lymphocytes.

Materials and methods

In the period between March and December 2020, 160 patients (97 males, 63 females average age 63.33 years + / -10.2) were enrolled in the COVID department of the P.O. S.Giuliano, because they were positive for the molecular swab in RT-PCR for SARS CoV-2. For each patient, blood count with smear, routine blood chemistry tests and in particular c-reactive protein, ferritin, ves, LDH, procalcitonin, troponin, fibrinogen, D-Dimer were performed.

Results

In COVID 19 patients with more severe symptoms, there was an increase in the value of white blood cells with an average of 20.0+/-5.0X103/microlitre (increase in neutrophils and monocytes, reduction in lymphocytes with NLR>5.92). Cytofluorimetric analysis has shown that CD3 T lymphocytes are significantly reduced. Patients with more severe syntoms showed a significant increase in C-Reactive Protein (10.6+/-7.5mg/dl), Troponin (433+/-400pg/ml), Myoglobin (3700+/-3000ng/ml),Fibrinogen(800+/-500mg/dl),D-Dimer(800-5800ng/ml),Ferritin(950+/-450ng/ml),LDH (850+/-250U/L))

Discussions and conclusions

From our study it is evident that the hematological parameters associated with specific biomarkers of inflammation are useful to identify and differentiate covid positive patients with more severe and critical pathology even in the second pandemic wave. The severity of the pathology is positively correlated with the percentage values of neutrophils and NLR and negatively with the percentage of lymphocytes.

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EP232

Determination of HbA1C in J Calabria hemoglobin variant

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INTRODUCTION The determination of HbA1C is a key tool in the diagnosis and follow-up of diabetic patients. Since such determination is performed on adult hemoglobin, the presence of certain hemoglobin variants could alter the measured value and make it no longer reliable.CASE DISCUSSION The sample came to our attention for the measurement of HbA1C. The patient was a male, aged 71 and was born in Southern Italy. His glucose levels were suggestive of prediabetes and his red blood cells parameters were low (MCV 66 fL, MCH 21.0 pg, MCHC 31.6 g/dL). The sample was firstly tested with D100 Biorad (HPLC) which detected a value of 42 mmol/mol of HbA1C; it also showed an unknown peak (14.81%, RT 22.41) that eluted next to the HbA0 peak (69.70%). According to acceptability recommendations, an unknow peak greater than 10% should not be reported. We therefore tested the sample with Variant II Biorad dual kit (HPLC): the method detected a value of 40 mmol/mol of HbA1C; the unknown peak seemed to coelute with HbA0 peak (82.1%, RT 1.703) and no particular alteration was shown. HbA2 was above reference values (3.9%, RT 2.871). The sample was at last tested with Sebia Capillarys for the evaluation of the hemoglobin variant: the capillary electrophoresis was able to detect a single well separated peak that eluted in Z12 zone and had a percentage of 18%. The capillary electrophoresis also confirmed a higher HbA2 (4.2%). The sample was also tested with Capillarys HbA1C, which confirmed a value of 40 mmol/mol of HbA1C.A molecular testing was also performed, detecting the presence of Hb J-Calabria variant, an hemoglobin variant affecting β-chains, and -(α)20.5 deletion, which lowers the percentage of Hb variant if compared to the cases described in literature.CONCLUSIONS In this case, thanks to capillary electrophoresis, it has been possible to confirm the presence of an hemoglobin variant and to rule out the occurrence of any interference. When available, different methods should be complementarily used in order to confirm the data measured; for our sample, these three different approaches gave overlapping results. A definitive diagnosis can only be performed by molecular testing.

EP233

Tamponi nasofaringei e test sierologici per COVID-19: cosa è cambiato dall'avvio della campagna vaccinale

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Dopo più di due anni, la pandemia determinata dalla diffusione del virus SARS-CoV-2 rimane un tema di straordinaria rilevanza per la salute pubblica. Numerosi sforzi sono stati fatti a livello regionale e nazionale per rafforzare il coordinamento e la pianificazione di misure atte a ridurre picchi pandemici in particolare prima dell'inizio delle stagioni autunno-inverno in cui è prevista un'aumentata co-circolazione di altri patogeni respiratori. Documentare i risultati dell'attività di sorveglianza sanitaria relativi all'andamento dei contagi e della copertura vaccinale potrebbe fornire spunti per la pianificazione. Nel Centro Diagnostico Italiano sono stati raccolti i dati relativi all'analisi molecolare dei tamponi nasofaringei (TNF) effettuati tra aprile 2020 e maggio 2022 e i valori di carica anticorpale di soggetti sottoposti a screening tra gennaio 2021 e maggio 2022. Su 80557 TNF analizzati, 9358 sono risultati positivi con una prevalenza variabile. Dati di prevalenza superiori al 20% si sono registrati in particolari periodi: aprile-maggio, novembre-dicembre 2020 e gennaio 2022. GI dati mostrano una maggiore incidenza di positività nei soggetti di età compresa tra i 20 e i 60 anche a seguito del completamento del ciclo vaccinale. In particolare, nei soggetti appartenenti alla fascia d'età compresa tra 20 e 40 anni si è osservata una positività superiore al 45% da maggio 2021. L'analisi dei risultati dei sierologici nella stessa fascia d'età e nello stesso periodo mostra invece una sostanziale uniformità di copertura della carica anticorpale con valori medi > 4000 BAU/mL.Complessivamente, il numero di TNF positivi evidenzia che il numero di persone che contrae il virus aumenta all'aumentare del rischio di esposizione. I soggetti tra i 20 e i 40 anni sembrano risentire maggiormente dell'andamento dei contagi suggerendo una minore protezione dall'infezione del vaccino. La campagna vaccinale infatti è risultata molto efficace per tenere sotto controllo i ricoveri e la pressione sulle strutture ospedaliere, pur non impedendo la diffusione del virus. Attivare un programma di monitoraggio e sorveglianza sanitaria personalizzato per specifiche categorie di soggetti età dipendente, potrebbe essere uno strumento utile a prevedere e ridurre picchi pandemici.

EP234

CONSIDERATIONS FOR DESIGN, DEVELOPMENT AND ANALYTICAL VALIDATION OF A BLENDED NUCLEAR-MITOCONDRIAL NEXT GENERATION SEQUENCING (NGS) METHOD FOR THE DIAGNOSIS OF MITOCHONDRIAL DISEASES

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INTRODUCTION: Mitochondrial diseases (MDs) are a group of rare disorders (5-12/100.000), due to both mitochondrial DNA (mtDNA) and nuclear genome (nDNA) alterations, affecting the oxidative phosphorylation (OXPHOS) and consequently impairing ATP production. MDs, commonly, show multi-organ involvement, resulting in heterogeneous clinical phenotype, mainly characterized by neurological, ophthalmological, cardiological, reproductive and endocrine features. Because of the twofold genetic control on the "mitochondrial phenotype" and the large number of nuclear involved genes, the molecular diagnostic approach by Sanger method is very complex and many patients still lack a genetic diagnosis. The aim of the study is the design, and analytical validation of a "blended" nuclear-mitocondrial Next Generation Sequencing (NGS) method for the simultaneous identification of genetic variants both in whole mtDNA and in numerous nuclear genes included in a clinic exome panel.METHODS AND RESULTS: Six DNA samples (12 in duplicate), from different tissues [blood, buccal swab, Formalin-Fixed Paraffin-Embedded tissue section (FFPE), fresh tissue and tissue fixed to a slide] were chosen for the "one-step" validation experiments. Four were positive controls for mtDNA variants, detected by Sanger sequencing, and two were negative controls. Being mtDNA in greater amount than nDNA, we used diluted 1:300 and 1:900 mitochondrial probes, to test the correct dilution to use for mtDNA probes. These dilutions are fundamental to obtain a more balanced coverage among mtDNA and nDNA. Our results, revealed a balanced (155X for nDNA and 3000X for mtDNA) coverage by using 1:300 diluted mitochondrial probes. Furthermore, our NGS sequencing confirmed the mutations previously found in the Sanger mtDNA analysis and highlighted pathogenetic variants in nDNA.CONCLUSIONS: This validation experiments proved to be important for the implementation of a blended nuclear-mitochondrial NGS analysis to the MDs diagnosis. This approach represents a powerful means for the simultaneous analysis, in a single analytical session, of a large number of nuclear genes and the whole mitochondrial genome, compared to first-generation techniques.

EP235

Valutazione delle prestazioni di un analizzatore ematologico POCT: l'esperienza del Laboratorio Generale di Careggi

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Introduzione

In accordo con le linee guida internazionali (documento CLSI EP09-A2), questo studio ha lo scopo di comparare i risultati dei parametri emocromocitometrici forniti dalla strumentazione POCT Sight OLO® e dall'analizzatore ematologico di riferimento Sysmex XN-9100™, ai fini della valutazione delle performance dei due strumenti.

Metodi

Campioni di sangue venoso (N=38), pervenuti presso il Laboratorio Generale per l'analisi emocromocitometrica di routine, sono stati parallelamente processati con il sistema XN-9100™, presente presso il Laboratorio, e con l'analizzatore ematologico POCT Sight OLO®. I risultati sono stati confrontati attraverso l'analisi di regressione Passing-Bablok e il test di Bland-Altman. La significatività di tale test è stata valutata tramite test di Wilcoxon. Lo studio ha incluso campioni sia normali che patologici, al fine di valutare le prestazioni del sistema Sight OLO® anche nei casi con risultati prossimi ai valori critici decisionali. Inoltre, sono stati confrontati gli allarmi analitici strumentali relativi a eventuali anomalie distributive, numeriche e morfologiche degli elementi cellulari.

Risultati

È stata osservata un'elevata correlazione ($r \geq 0,99$) per i parametri WBC, RBC, PLT, HGB, HCT, neutrofili, linfociti e monociti; una moderata correlazione per MCV, RDW, MCH ed eosinofili ($0,90 < r < 0,99$), e una bassa correlazione per MCHC e basofili. La valutazione della concordanza tra i due metodi ha mostrato valori di bias <10% per tutti i parametri ad eccezione di linfociti, monociti e basofili; un'alta significatività statistica è stata osservata per i più importanti parametri emocromocitometrici. La comparazione degli allarmi analitici ha mostrato un parziale accordo tra i due strumenti (concordanza completa 21% e parziale 12%); il confronto rispetto al referto finale, dopo revisione effettuata dallo specialista di laboratorio, ha mostrato una buona performance dello strumento Sight OLO® relativamente agli allarmi morfologici.

Discussione

Da un punto di vista analitico, il confronto dei due metodi dimostra che il sistema POCT Sight OLO® fornisce risultati emocromocitometrici comparabili con quelli ottenuti tramite il sistema Sysmex XN-9100™ in un ampio range di intervalli di misura, compresi campioni altamente patologici.

EP236

Detection of SARS-CoV-2 neutralizing antibodies: reliability of serological tests

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Serological assays allow to explore the synthesis of antibodies against SARS-CoV-2 proteins. The gained knowledge finds applications in sero-epidemiology studies, to develop accurate forecasts of protective immunity spreading within a population. Indeed, last February, the European Centre for Disease prevention and Control (ECDC) recommended the use of serological tests for population surveys, in the context of epidemiological studies. The up-to-date reference method to determine the amount of protective antibodies is the in vitro neutralization test, which is expensive, time-consuming and requires biosafety level 3 laboratories. The current study aims at highlighting the correlation between the results of the reference method and four different serological assays. Three immunoassays (Elecsys® Anti SARS-CoV-2 S, MAGLUMI® SARS-CoV-2 S-RBD IgG and MAGLUMI® 2019-nCoV IgG test) and an ELISA surrogate viral neutralization test (EUROIMMUN® SARS-CoV-2 NeutraLISA assay) have been studied on 83 patients, previously infected by SARS-CoV-2. The correlation between each serological test and the reference method is reported, as well as the predictive performances to distinguish serum samples with neutralizing antibody titers higher than 160. All the assays achieved good performances in terms of correlation with the reference method, as well as concerning the predictive ability over NAb titers > 160. The best correlation (Spearman coefficient = 0.784) and predictive features (area under curve = 0.921) have been observed for EUROIMMUN® SARS-CoV-2 NeutraLISA assay, as displayed by ROC curve analyses.

EP237
HUS or PTT: case report

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Case Report
Female patient age 56 aa. with autonomous walking inability, for postpartum neurological deficits, received at the P.S. of our P.O. for strong chest compression and previous Covid-19 infection treated at home. At the entrance, examinations performed with an emergency profile showed alterations in cardiac and pancreatic markers, severe renal failure, as well as significant alterations in blood count and coagulation. Therefore, hematological, nephrological, cardiological and surgical advice and II level examinations for diagnostic classification are requested: blood count with smear for schistocytes, direct and indirect coombs test ADAMTS 13 Routine hematochemical profile + C3 and C4 Complete Coagulation tests .
Results
Blood count: severe thrombocytopenia (10x 10 3micronL) Hb (7.5 gr/dl) MCV 80 Fl Reticulocytes >2.5 Accentuated neutrophilia >90% Coagulation: PT 10.9 sec INR 1.01 PTT 65.2 sec (no corrected by mixing test) PTT ratio 1. 98% D-Dimer 2741ng/mL Myoglobin>12000 Troponin 68.5 pg/ml LDH 864 U/L Amylase 666 U/L Lipase398 Ui/L Phosphorus 9 mg/dl Creatinine >2 mg/dl Direct bilirubin >2 mg/dL C3 70 mg/dl C4 14 mg/dl.
Discussion and Conclusion
The patient arrived at the P.S. with an extremely critical and complex clinical picture, such as to require multi-specialistic consultations and very difficult to frame from a diagnostic point of view, given the multiple organ involvement. Widespread ischemic damage due to microthrombosis in arterioles, thrombocytopenia due to platelet trapping, microangiopathic hemolytic anemia due to red blood cell fragmentation.SARS-COV2 patients have an increased risk of arterial and venous thrombosis. Elevated levels of D-dimer are associated with increased thrombosis and mortality. Multiple pathways likely contribute to thrombosis in COVID-19 patients.When COVID-19 patients experience cytokine storms, interleukin-8 and TNFα stimulate VWF release from vascular endothelial cells, while interleukin-6 inhibits both production of ADAMTS13 and its interaction with VWF, resulting in localized severe deficiency of ADAMTS13 activity. The presence at the smear of schizocytes, increased LDH, platelets in progressive decrease, and so Hb, D-Dimer in increase, led to the request for diagnostic completion with research of ADAMTS 13 (40%) and LAC (1.65). The patient was transfused with platelet concentrates before a certain diagnosis. This picture directed us towards the diagnosis of Moscovitz, but the positive direct coombs test (score + +--) and renal involvement, high phosphorus, altered C3 and C4, the presence of pancreatitis, directed towards an HUS atypical. Thanks to the dialogue and the active collaboration between pathologists and clinicians, the patient was subjected to life-saving plasma exchange, but the diagnostic doubt still remains.

EP238
HIGH-THROUGHPUT SEQUENCING DEPICTS THE MOLECULAR EPIDEMIOLOGY OF GALACTOSEMIA IN A SUD ITALIAN POPULATION

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Galactosemia includes a group of rare genetic metabolic disorders, characterized by the body's inability to convert galactose into glucose, due to mutations in the genes GALT, GALK1 and GALE, coding for the enzymes galactose 1-phosphate uridyl transferase, galactokinase and UDP-glucose4-epimerase, respectively. GALT is the most frequently mutated gene (70%). Newborn Screening (NBS) for Galactosemia has been active at CEINGE since 2020, by measuring total galactose on neonatal blood spots and subsequently the enzymatic activity of galactose 1-phosphate uridyl transferase. In the presence of high values of galactose and reduction of enzymatic activity, genetic testing is required to reach definitive diagnosis. The aim of our work is to verify the diagnostic suspicion of Galactosemia in 28 newborns, tested positive for NBS, using Next Generation Sequencing (NGS) methods. We also analyzed 4 proband results affected by Galactosemia in the pre-screening era. The genetic test to search mutations in the three genes associated with Galactosemia was carried out in 32 probands by NGS. Molecular analysis identified 1 patient suffering from classical galactosemia and 8 suffering from Galactosemia Duarte, among 28 newborns showing positive screening results. Nine newborns were found to be carriers and 10 negatives. Among the positives, 2 newborns have mutations in the GALE gene. The 4 probands with a clinical diagnosis of Galactosemia showed two mutations in the GALT gene, each. Globally, NGS, including the "minor" genes, identified 13 affected and 9 carriers. Two mutations have been identified in the GALE gene. The 10 genotype-negative probands stopped follow-up. However, clinical follow-up continues in heterozygous newborns (carrier). Genetic test for Galactosemia is a fundamental tool to confirm or rule out the diagnostic suspicion of Galactosemia and to target the appropriate therapy. Parallel sequencing of the 3 genes improved the molecular diagnosis of Galactosemia.

EP239

Plasma iperimmune in pazienti SarsCov2 con Distress Respiratorio Acuto (ARDS) lieve: valutazione degli indici infiammatori.

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Premessa
Il virus SarsCov2 induce una tempesta citochinica che genera uno stato infiammatorio sistemico associato ad ipercoagulabilità.
Scopo del lavoro è stata la valutazione di indici infiammatori (PCR, Ferritina, LDH, D-Dimero, Fibrinogeno) e di danno polmonare (HRCT Lung score) in pazienti SarsCov2 con ARDS lieve (PO2/FiO2 200-300 mmHg) prima e dopo trattamento con terapia standard e plasma iperimmune.
Metodi
Presso il SIT di Aversa, secondo i protocolli del C.N.S. (n.2228, n.1296/2020) nel 2021 sono state raccolte 20 unità di plasma iperimmune da donatori convalescenti (titolo anticorpi neutralizzanti ≥1:160) mediante aferesi, ed inattivate (Intercept Blood System).
Lo studio ha coinvolto 40 pazienti (52,5% F, 47,5%M) ricoverati presso U.O. Sub-intensiva del Covid Hospital di Maddaloni di cui 20 trattati con terapia standard e antivirale (gruppo TS) ed i restanti 20 con terapia standard e plasma iperimmune (3 sacche) (gruppo TP). L'analisi statistica è stata eseguita con MedCalc 19.
Risultati
Al ricovero differenze significative sono state rilevate solo per il fibrinogeno TP 714 Me vs TS 914 Me (p=0,0006), dovuta ad una prevalenza di donne nel TS (60%), che in età avanzata possono avere livelli di fibrinogeno più alti rispetto ai maschi.
Alla dimissione, nel TP si è evidenziata una riduzione significativa degli indici infiammatori (p<0,0001) con normalizzazione dei valori, ed un miglioramento del danno polmonare (HRCT Lung Score TP 11 Me vs TS 14 Me p=0,0013). Nel TS si è osservata una riduzione significativa di tutti gli indici, ma non una normalizzazione dei valori. Il D-Dimero invece è risultato aumentato (TP 213,5 Me vs TS 1052,500 Me p <0,0001) per l'istaurarsi di uno stato infiammatorio cronico polmonare in linea con un HRCT Lung score invariato (15 Me vs 14 Me p=0,990).
Conclusioni
Il plasma iperimmune si è mostrato essere un ottimo coadiuvante alla terapia standard per l'ARDS. Diversi studi mostrano che esso può agire sulle citochine, regolando l'espressione delle cellule CD4+ e CD8+ e stimolando la formazione di cellule B memory. Ulteriori studi serviranno per comprendere gli effetti immunomodulatori e regolativi dei meccanismi coagulativi e infiammatori del plasma iperimmune nel Covid-19 rispetto all'effetto placebo e alla sola infusione di plasmasafe.

EP240

A capillary electrophoresis system for HbA1c measurement and variants analysis: role and relevance of separative methods for HbA1c in the presence of hemoglobin variants, a case report

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IntroductionHbA1c is a widely used biomarker for long-term glycemic control. However, hemoglobin variants may interfere with its measurement by separative methods, potentially leading to erroneous results. On the other hand, HbA1c analysis may lead to the unexpected finding of previously unknown hemoglobinopathies. A separative measurement method for HbA1c also capable of hemoglobin variants analysis could therfore be of analytical and clinical relevance. Case reportIn June 2022, HbA1c was requested for a diabetic female patient from western Africa in our Laboratory. The biomarker was measured by HPLC (BioRad D-100), with a result of 62 mmol/mol and a flag for the possible presence of a variant hemoglobin: consequentely, HbA1c was not reported. The hemoglobin fractions of the patient were previously studied in another Laboratory with a HPLC method and homozigosity for HbS was suggested. Complete blood count showed slightly microcytic red blood cells (mean corpuscolar volume 79.2 fL) with no anemia (hemoglobin 135 g/L).The sample was further analyzed with Sebia Capillarys 3 TERA HbA1c method. The electropherogram clearly showed the absence of HbA0 (and, accordingly, a result of HbA1c was not reported by the instrument software) and the pattern was identified as atypical. Therefore, the sample was re-analyzed with Capillarys 3 Hb test mode for hemoglobin variants analysis, with the following results: HbF 38.6%, HbS 59.2% and HbA2 2.2%. A presumptive diagnosis of a double beta gene defect was therefore supposed, more probably compound heterozigosity for HbS and hereditary persitence of HbF. Molecular studies are ongoing to confirm this hypothesis.Eventually, no erroneous HbA1c was reported to the patient and an alternative and more appropriate biomarker of glycemic control could be suggested (serum fructosamine). ConclusionsOur case highlights the importance of ruling out any possible interference affecting the HbA1c measurement that could compromise its interpretation, posing a risk for erroneous clinical management of diabetes mellitus. At the same time, the availability of both high resolution HbA1c and hemoglobin variants analysis on the same instrument could lead to a rapid presumptive identification of previously unknown or misdiagnosed hemoglobinopathies.

EP241

Tipizzazione molecolare eritrocitaria estesa: impatto sull'alloimmunizzazione in pazienti mielodisplastici

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Introduzione: L'alloimmunizzazione nei Mielodisplastici (MDS) è un problema significativo, in quanto necessitano di un supporto trasfusionale cronico. Per contrastare ciò, sono state introdotte terapie trasfusionali con unità di emazie con fenotipo molecolare esteso. Scopo del nostro studio è stato quello di valutare l'incidenza di allo/auto-immunizzazione in pazienti trasfusi con tale procedura.
Metodi: 79 pazienti con MDS trasfusi nel SIT Asl Caserta da Marzo 2014 a maggio 2022, sono stati trattati con emazie con/senza fenotipo esteso (42 senza fenotipo esteso e 37 con fenotipo esteso). La caratterizzazione è stata effettuata sui donatori in sierologia, (Neo-Iris; Immucor), e sui pazienti (Molecular BeadChip; Immucor) per la determinazione dei sistemi gruppo-ematici (Rh, Kell, Kidd , Duffy, MNS, Dombrock, Lutheran, Landsteiner-Wiener, Diego, Colton e Scianna).
Risultati: Dei 22 pazienti risultati alloimmunizzati, 10 (52%) lo erano prima di iniziare la terapia trasfusionale. Dei 42 pazienti trattati con unità di RBC senza fenotipo esteso, 10 (24,3%) hanno sviluppato allo-anticorpi nello specifico (2 Anti-Fya, 2 anti-Jkb, 1 anti-S, 2 anti-e, 1 anti-D, 2 anti-C). Dei 37 pazienti con terapia trasfusionale estesa, 5 (11,8%) sono risultati alloimmunizzati (1 anti-Kpa,1 anti-E, 1 anti-C, 2 anti-e). 9 pazienti con alloimmunizzazione Rh presentavano varianti alleliche RH. Sono stati inoltre rilevati autoanticorpi in 4 pazienti (3 trattati senza fenotipo esteso, ed 1 con fenotipo esteso). Dei 42 pazienti, 22 risultavano immunizzati, mentre 59 non lo erano.
Conclusioni: La tipizzazione eritrocitaria estesa ha permesso di ottenere un ottimo risultato sul decremento di auto/alloimmunizzazione, permettendo una terapia specifica e perfettamente compatibile. La genotipizzazione del DNA svolge un supporto fondamentale in medicina trasfusionale per i pazienti che necessitano di una terapia costante; tale approccio riscontra successo in ambito clinico anche per procedure automatizzate sempre più produttive, rapide e specifiche permettendo una trasfusione sartoriale e maggiormente sicura.

EP242

DEALING WITH PSEUDOGENES IN MOLECULAR DIAGNOSTICS IN THE NEXT-GENERATION SEQUENCING ERA: THE EXAMPLE OF GAUCHER DISEASE

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Gaucher disease (GD) is a lysosomal storage disease, inherited as autosomal recessive trait, due to glucocerebrosidase's enzyme deficiency, encoded by the GBA gene. Molecular analysis of GD is difficult because of a highly homologous pseudogene (GBAP1), which causes homologous recombinations, also. We performed genetic test of GD in two patients, who already had a clinical diagnosis of GD, because of hepatosplenomegaly, anemia, thrombocytopenia and reduced glucocerebrosidase activity. Molecular analysis, essential for gaining access to gene therapy, was performed by Next Generation Sequencing (NGS) method, using the panel of genes for lysosomal diseases.Molecular test showed that proband N1 was homozygous for the mutation c.1448T>C in the GBA gene, known as associated to GD. We confirmed the mutation by using a Long-PCR, which selects the GBA gene, and subsequent Nested PCR. Proband N2 carried the heterozygous mutation c.1226 A>G, subsequently confirmed by Long PCR - Nested PCR. The presence of a single heterozygous mutation does not confirm the clinical diagnosis. A complex allele is known, due to the recombination between GBA and GBAP1 genes, in the regions between exons 9-11, which may not be identified by NGS, since the probes are specific for the GBA gene, exclusively. To verify the possible presence of the complex allele, we co-amplified by Nested-PCR the exon 10, specific of GBA, and exon 3, which has many different nucleotides compared to GBAP1, to make sure the pseudogene was not improperly analyzed. By using this approach, we identified in Patient N2 the second mutation, which corresponds to the complex allele resulting from the recombination between GBA and GBAP1 genes.Conclusions: Although NGS is revolutionizing molecular diagnostics, there are still situations, such as the diagnosis of GD, that can only be definitively resolved by integrating the Sanger method with NGS.

EP243

Determinazione di auto e allo-anticorpi anti piastrine in pazienti affetti da Covid 19

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Introduzione: Diversi studi hanno dimostrato che il COVID-19, nelle forme più gravi è associato a trobocitopenia ed anomalie sia morfologiche che funzionali delle piastrine. Gli stessi indicano che le IgG di tali pazienti, attivando il recettore FcγRIIA, inducono attivazione piastrinica e morte cellulare programmata con rilascio di vescicole extracellulari, probabilmente responsabili di auto e allo-anticorpi. Scopo del nostro studio è stato quello di rilevare auto/o alloimmunizzazioni piastriniche in pazienti affetti da Covid 19.
Metodi: Da Marzo 2021 a Febbraio 2022 sono stati analizzati 36 pazienti ricoverati nel Covid Center di Maddaloni (CE) affetti da trombocitopenia. I campioni sono giunti presso il SIT Asl Caserta per esami pre-trasfusionali per trasfusione piastrinica. E' stata eseguita la ricerca di auto e allo-anticorpi anti-piastrine mediante Test di aderenza delle Piastrine in fase solida (Capture P Immucor) e Test in ELISA (Pak-Auto e Pk-Lx).
Risultati: Dei 36 campioni testati, 22 sono risultati positivi alla ricerca di anticorpi-anti PLT, di questi 13 al test indiretto, 5 al test diretto, e 4 ad entrambi. Sono state inoltre analizzate le specificità anticorpali, quelle maggiormente coinvolte sono risultate verso le glicoproteine IIb/IIIa e Ia/IIa. Di tutti i pazienti, solo 7 sono stati trasfusi, senza incremento della conta piastrinica.
Conclusioni: La piastrinopenia potrebbe essere attribuita a: (1) microambiente ematopoietico alterato a causa di tempesta citochinica; (2) il virus potrebbe infettare direttamente i megacariociti attraverso ACE2, CD13 e CD66a; (3) gli anticorpi antivirali reagiscono con L'integrina piastrinica GPIIb/IIIa. La Trombocitopenia rilevata risulta secondaria a COVID-19, non essendo riferite malattie autoimmuni sottostanti, ne ulteriori cause. Il recupero della conta piastrinica dopo trattamento con IVIG e steroidi, è un presupposto per cui tali pazienti possano aver avuto distruzione piastrinica auto/allo-immune indotta dall'infezione, ciò è in relazione anche allo scarso ricorso alla pratica trasfusionale piastrinica.

EP244

Indicatori biochimici di trauma cranico nell'umor vitreo: studio pilota

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Una valutazione prognostica e affidabile della gravità ed entità del trauma cranico (TBI,Trauma Brain Injury) è essenziale per il corretto e pronto management del paziente.La scala clinica attualmente utilizzata è la Glasgow Coma Scale,che però ha dei limiti legati alla contemporanea presenza di lesioni extracraniche ed è influenzata da manovre di primo soccorso, tali per cui spesso in acuto è attribuito un punteggio superiore rispetto a quanto poi accade ad una seconda valutazione.Da anni i Ricercatori hanno posto l'attenzione a biomarcatori sierici che possano dare un riscontro oggettivo all'entità del TBI,anche in riferimento all'evoluzione dello stesso. Alcuni di questi marcatori sembrano avere una correlazione tra entità del TBI e caratteristiche cliniche/radiologiche dello stesso;in particolare si tratta di proteine dette "delle fase acuta": IL-6, PCR e PCT. Tuttavia, resta scarsa la conoscenza sul significato da dare ai diversi marcatori e sulla loro capacità di diagnosticare o classificare varie lesioni traumatiche (incluso un eventuale pattern di concentrazioni di biomarcatori qualora la lesione interessasse diverse sedi anatomiche .Per quanto riguarda le analisi post-mortem,rari sono i contributi su questo tema,anche per le difficoltà tecniche di utilizzo della matrice sierica.Essendo ben noto il fenomeno di mirroring della concentrazione di analiti nell'umor vitreo rispetto alle loro concentrazioni sieriche,gli Autori hanno condotto come "proof of concept",un'analisi retrospettiva su 20 campioni di umor vitreo da casi autoptici di soggetti deceduti per TBI versus 10 casi autoptici controllo (decesso non-TBI), valutando un pannello di marcatori legati al danno neuronale (S100,NSE,Copeptina,BDNF e GFAP) e uno legato a proteine di infiammazione (IL-6,Ferritina,LDH,PCR,PCT,Glucosio,N-Gal).I risultati preliminari,nonostante la scarsa numerosità del campione, indicano un aumento significativo dei livelli di LDH, S100 e Ferritina nei soggetti deceduti per TBI.Inoltre, un modello logistico costruito con questi marcatori e con NSE (che mostra differenze fra i due gruppi,pur non essendo significativa, p=0.052),porta ad una ROC=0.929 (Sens=0.889;Spec=0.714),incoraggiando ulteriori ricerche sull'utilizzo sierico di tali marcatori come strumento supplementare nella comune pratica clinica di gestione del TBI.

EP245

PARAMETRI EMATOLOGICI DI RICERCA COME AUSILIO ALLA DIAGNOSI PRECOCE DELL'INFEZIONE DA SARS-COV-2

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Il SARS-CoV-2 (Sindrome Acuta Respiratoria Severa-Coronavirus 2) è l'agente patogeno che ha causato quella che è stata poi definita dal WHO pandemia di COVID-19: una malattia virale altamente contagiosa, responsabile di una risposta immunitaria dell'ospite iperattivata e disregolata.Le varianti di SARS-CoV-2 destano molte preoccupazioni a causa dell'elevata capacità di mutare e di fugare sia l'immunità innata che quella da vaccino.Obiettivo dello studio è valutare il potenziale contributo alla diagnosi precoce di COVID-19 dei parametri di ricerca della formula leucocitaria forniti dalla strumentazione ematologica Sysmex XN-9100TM (linfociti ad alta fluorescenza o HFLC, parametri posizionali delle diverse popolazioni leucocitarie e conteggio dei granulociti immaturi), al fine di identificare tempestivamente casi positivi già durante il triage.Sono stati considerati 350 pazienti sospetti COVID-19, presentatisi presso il Pronto Soccorso dell'AOU-Careggi durante la prima ondata (Aprile 2020); di questi 92 sono stati identificati come SARS-CoV-2 positivi in base alla complessiva valutazione clinica del paziente oltre che all'RT-PCR di accesso ("physicians' gestalt"). Una prima analisi è stata effettuata confrontando i casi positivi con i non-casi mediante Mann-Whitney. Le curve ROC sono state utilizzate per valutare la capacità diagnostica dei biomarcatori.I risultati relativi alla prima ondata mostrano leucopenia e aumento dei seguenti parametri: HFLC, Linfociti secernenti (in percentuale e in valore assoluto) e la complessità dei Monociti, ciascuno con ROC>0.7.L'analisi di regressione logistica è in elaborazione, come l'analisi degli stessi parametri sulla popolazione attualmente esposta a SARS-CoV-2, quindi nel periodo successivo all'introduzione del vaccino, con l'obiettivo futuro di confrontare i risultati della prima ondata con quelli relativi alle infezioni attuali (in cui un alto tasso di vaccinazione e di immunità naturale potrebbero variare i parametri analizzati).In conclusione, data l'estrema facilità con cui il virus, mutando, evade i meccanismi dell'immunità innata e di quella da vaccino, i risultati ottenuti intendono sottolineare l'importanza di trovare parametri che aiutino il clinico a identificare tempestivamente l'infezione da COVID-19.

EP246

Comparison between ELISA and automated ECLIA for Interleukin-6 determination during COVID-19 pandemic

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Background: The severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) is a highly contagious illness associated with a hyperactivated and dysregulated host immune humoral response. In detail, there is a cytokine storm which may take to the release of interleukin IL-6 as a critical mediator for respiratory failure, shock and multiorgan dysfunction. Such dysregulation may act as a target for therapeutics and, in this view, a blockade of IL-6 function by an anti-IL-6 receptor antibody (tocilizumab) has been described to be effective for the treatment of the inflammatory process COVID-19-related. Timing of administration of therapy was reported in literature to have a critical role in benefit for patients; thus, the aim of the present study is to compare two different methods for the IL-6 assessment: the Human IL-6 ELISA Kit (Invitrogen) and the Elecsys IL-6 (Roche).
Methods: IL-6 levels of 128 COVID-19 patients, who were consequently admitted to the Emergency and Medicine Department of AOU Careggi (Hospital in Florence -Italy) between April and May 2020, were assessed by using the two above mentioned methods and were analysed through Passing-Bablok regression fit and Bland-Altman plot.
Results: The analyses showed that the two methods correlate, but do not agree in terms of numeric results. In particular, further investigation were performed on the Bland-Altman results, showing that the maximum number of samples for which the differences between the two methods is close to "0" ($p > 0.05$) (which means a good overlap between the two methods) is 49 ($p=0.07$), and among them, 40 samples showed a complete agreement of results ($p=0.95$). These results can be attributed to the different methods' linearities: 3.1–200 pg/mL for ELISA and 1.5#5000 pg/mL for ECLIA, which could be extended to 50 000 pg/mL.
Conclusions: Although a small percentage of data overlapping in a certain range, still a high correlation among the two methods can be found; given the overall analytical performance of the ECLIA, it can be considered more adequate for different reasons: i) it is available on a fully automated platform h24, ii) it uses of a small sample volume, iii) it is low cost and no-time consuming and iiii) the different timing for measuring IL-6 is much attractive.

EP247

The toxicological examination of the keratin matrix in the management of the evaluation of parental capacity

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Since June 2019 the Juvenile Court has entered into an agreement with the USL of Umbria 2 for the evaluation of parenting skills.
Also at the request of the court of minors, toxicological tests on hair are also carried out in minors. The situations in which the magistrate requests this test are varied: drug dealing, brawls, reports of violence, situations of juvenile hardship that require insertion into the community, on the children of drug addicts
Patients who join to this protocol are used to be subjected 3 or 4 times par year to a keratin matrix withdrawal (hair, axillary hair, pubic hair) to detect any substances of abuse (opiates, cannabinoids, amphetamines, cocaine).
Now thanks to this agreement the procedure of urine screening is finally passed away and users are no more supposed to go to SERD twice or more times a week for urine collection. Once the protocol is completed, depending on the hair test result and the psychologists reports, the SERD doctor finally writes a final report which will be sent to the magistrate.
Starting from 2019 until now 150 were totally patients chased on, these ones in 4 years performed 316 toxicological exams.Adults were 112, with 221 accesses, (an average of 1.9), meanwhile minors were 38, with 70 accesses (an average of 2.1).
Abuse substances were discovered in 79 patients, overall 142 positive cases:
-42 of them, for an amount of 65 positive cases were for cannabinoids (24 only once time, 14 twice, 1 three times, 2 five times). Of 42 people 16 are minors, which 7 of them were positive several times.
-46 of them, for an amount of 65 positive cases were for cocaine (35 only once time, 9 twice, 1 five times, 1 nine times). Of 46 people 8 are minors, which one of them was positive several times.
-8 of them, for an amount of 12 positive cases were for opiates (4 only once time, 2 twice, 1 four times). Of 8 people one minor was positive several times.
High is the percentage of individuals, mostly adults, who at the first feedback of positivity left the protocol.
From the patients was taken a urine sample too, for the toxicological screening. The positives ones were 72, while positives from the hair testing where 142.
This fact confirms the importance of utilizing this matrix instead of using urines to certificate the condition of abuse substances assumption over the time. The use of the hair test ensures an assessment that is not susceptible to adulteration, allows the detection of substances of abuse even in the case of occasional use, limits the costs of personnel in charge of sampling and diagnostics.

EP248

Distal lower limbs myopathy as first and predominant manifestation in two siblings with POLG1 mutation

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Introduction

The DNA polymerase γ 1 (POLG1) nuclear encodes the mitochondrial (mt) DNA polymerase's catalytic subunit, responsible for mtDNA replication and repair. Mutations in POLG1 results in mtDNA depletion and accumulation of multiple mtDNA deletions in postmitotic tissues such as the nervous system, muscles, and liver [1]. Within the wide spectrum of clinical phenotypes related to POLG1 mutations, the myopathic involvement include progressive external ophthalmoplegia, proximal limb myopathy, often associated with ataxia and/or axonal sensorimotor peripheral neuropathy [2].

Methods

We observed two siblings (39 and 43 years) with a similar disease history, both evaluated with a standardized protocol.

Results

M/39 performed regular physical activity until the age of 25 years, when he begins to suffer from slowly progressive muscle weakness in the distal muscles mainly involving the anterior compartment. He referred the onset of mild dyspnea at the same age and at 32 years of dysphagia. At the observation he presented mild bilateral ptosis, rhinolalia, severe bilateral distal hyposthenia of lower limbs with bilateral foot-drop.
Laboratory test showed an increase of plasmatic CK levels (589 IU/L, nv: 38-204 IU/L) and of lactate after physical exercise (39.5 mg/dL, nv: 5-22 mg/dL). Brain MRI was normal whereas legs MRI showed fibroadipose substitution and atrophy in all muscles except those in the deep compartment in association to changes suggestive of edema in some muscles. Muscle biopsy showed a mitochondrial myopathy, and the analysis of mitochondrial respiratory chain complex activity was normal.
In both patients genetic investigation revealed the presence of heterozygous mutation in POLG1 gene c.1327>T(p.Arg443Cys).

Conclusions

This report highlights that the diagnostic framework of adults with hereditary neurological diseases associated to mitochondrial dysfunction is difficult for the clinical and genetic heterogeneity [2]. A prevalent distal myopathy

was previously described only in two patients with POLG1-related mutation but involving the upper limbs [3]. Our findings underlined that this clinical manifestation should be taken in account in the differential diagnosis.

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EP249

Severe neonatal direct hyperbilirubinemia: a novel pathogenic G6PD variant in an Italian infant patient

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Introduction. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common X-linked enzyme defect, widespread in the Mediterranean area. This genetic defect can cause hemolytic anaemia and neonatal direct hyperbilirubinemia affects. Variants G6PD gene are classified from I to V according to WHO classification. In this work we described a 2325 grams male infant who was born at 36+5 weeks of gestational age to 29-years-old Caucasian mother. Neonatal jaundice occurred two hours after birth with peak serum total bilirubin was 24 mg/dL at 60 hours of life.Methods. G6PD activity was performed on the proband and his mother by a commercially available kit (Trinity Biotech, Jamestown, NY) in association with Siemens ADVIA Chemistry XPT system. Fot the molecular analysis, genomic DNA were extracted from blood samples using the QIAamp DNA Blood Kit (Qiagen, Germany) and Sanger Sequencing of full gene was performed in association with ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) and SeqScape Software v2.5 for data interpretation. Finally, the genetic analysis of the UDP-glucuronosyltransferase (UGT1A1) gene was also performed.Result. Child's mother showed normal G6PD activity, while infant's G6PD activity was undetectable. The sequencing analysis of the G6PD gene showed, in both subjects, the presence of an unreported variant (c.1361G>C, NM_001042351.3) (p.Arg454Pro). This variant is considered as novel, since absent in the main databases. In addition, the molecular analysis of the UGT1A1 gene showed the Gilbert's disease in the infant.Discussion. Basing on infant's clinical picture and the substitution in the 454 position of arginine in proline, we hypothesize a Class I variant classification. The child's bilirubinemia, hematologic profile and lactate dehydrogenase levels were followed during the first year of life. At 4 months of age, the child received his fourth RBC transfusion at another hospital, following hemolysis due to an episode of urinary infection. Peripheral blood smear performed at 1 year of age, under healthy conditions, revealed no erythroblasts, anisopoikilocytosis, or stomatocytes. The clinical course was also characterized by severe hyperbilirubinemia, but, surprisingly, this was not so much indirect bilirubin jaundice as cholestatic jaundice.

We could speculate that the hemolysis induced by our novel G6PD deficiency variant may be associated with an alteration of the complex interactions between red blood cell metabolism, iron recycling, and macrophage immunoregulatory functions. Further observations of the clinical course in other patients with the same genetic mutation variant we identified are needed to confirm our hypotheses.

EP250

Detection of MET exon 14 skipping using a single comprehensive genomic profiling: optimization of Non Small Cell Lung Cancers molecular diagnosis and clinical management

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Introduction. The exon 14 skipping of the MET gene (METex14) occurs in about 3% of patients with Non Small Cell Lung Cancers (NSCLCs) that are consequently eligible for personalized molecular therapy. Multiple approaches are available for METex14 analysis but relevant limitations emerged from both DNA-based and RNA-based workflows. We reported two novel MET variants causing the exon 14 skipping describing the advantages of the simultaneous evaluation of DNA and RNA in a single comprehensive molecular workflow.

Methods: DNA and RNA were simultaneously extracted from Formalin-Fixed Paraffin-Embedded (FFPE) samples using the AllPrep®DNA/RNA FFPE kit (QIAGEN). A pan-cancer analysis was performed using the TruSight Oncology 500-High Throughput DNA (Illumina) kit that analyzes both DNA and RNA identifying single nucleotide variants, insertions/deletions (indels) and copy number variations in 523 genes as well as fusions and splicing variants in 55 genes. Tumor Mutational Burden and Microsatellite Instability were also evaluated. Next Generation Sequencing (NGS) was performed on the NovaSeq6000 platform (Illumina). Output data were analysed on the Clinical Genomics Workspace platform by PierianDx tool. Orthogonal confirmatory tests were performed using the AmoyDx® MET Mutation Detection Kit (Amoy Diagnostics) for the targeted analysis of fusions events and Sanger sequencing for the DNA-level evaluation.

Results: Our molecular strategy effectively identified two novel MET indels variants from DNA sequencing: c.2942-23_2943del24 and c.2942-16_2942-1del. Both variants accounted at the 5' of the exon 14 and cause the exon 14 skipping as simultaneously predicted from NGS-based RNA analysis. NGS results were confirmed using the orthogonal tests.

Discussion: The simultaneous evaluation of DNA and RNA provided a complete characterization of METex14

skipping events: the effect of the genomic variant identified from DNA sequencing is directly proved by RNA sequencing, avoiding further tests with a lower turnaround time. It represents an effective strategy for new MET exon 14 skipping variants characterization. Such approach is an example of high-performance molecular diagnostic strategy with a great improvement of routine practice and management of patients with NSCLC.

EP251

VALUTAZIONE DEL KIT VitaPCR™ GEN2 PER RICERCA RNA VIRALE SARS-CoV-2 IN URGENZA

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Premessa: Nella pandemia da SARS CoV-2, vi è la necessità di disporre di un test diagnostico rapido ed attendibile da impiegarsi nei casi urgenti. I test antigenici sono poco sensibili soprattutto a basse cariche virali, vi è pertanto necessità di disporre di un test molecolare che permetta una rapida diagnosi. Questo studio è volto a valutare il nuovo kit per ricerca RNA virale VitaPCR™ SARS-CoV-2 Gen2 (Credo2), un test rRT-PCR rapido (<25 min) da impiegarsi per I pazienti con accesso al Pronto Soccorso e per lo screening dei donatori di sangue.Materiali and Metodi: Il kit VitaPCR™ Gen2 rileva, con LoD pari a 1 copia/mL, su tamponi naso-faringei (TNF) sia l'RNA specifico del SARS CoV-2 che l'RNA del virus SARS-like universale (che comprende SARS-CoV-2, SARS-CoV e coronavirus SARS-like del pipistrello) entrambi si trovano sulle regioni del gene N. Il nuovo kit comprende inoltre un RNA a singolo filamento artificiale che funge da controllo interno del processo della rRT-PCR e inoltre permette la conservazione dello specifico tampone fino a 7 gg a 4-8°C.L'accuratezza diagnostica è stata determinata in 66 TNF rispetto ai metodi rRT-PCR in uso per la diagnostica molecolare in urgenza. In particolare sono stati analizzati: 26 TNF sul sistema Liaison MdX Diasorin, con tempi di analisi <2 h, con rilevazione geni S (Ct: 13.5–33.9) e ORF1ab (Ct: 13.2 – 31.6), 22 TNF sul sistema GeneXpert , con tempi di analisi < 1h, con rilevazione geni E (Ct: 12.7–40.0) e N2 (Ct: 13.2 – 41.0) e 18 TNF con la versione precedente del kit VitaPCR™ SARS-CoV-2 (Credo1), con rilevazione delle sequenze SARS-CoV-2 (Ct: 15.0-34.0) e SARS-like (Ct:15.0-33.0) del gene N.Risultati: Una concordanza del 100% è stata ottenuta tra Credo 2 e Liaison Mdx e tra Credo2 e Credo1. Una minore concordanza (86.4%) è stata ottenuta con il sistema GeneXpert, per n.2 casi di negatività in TNF debolmente positivi con Ct >40.0 per gene E >41.0 per gene N2 ed un terzo TNF con Ct 33.1 per E e 35.5 per N2.Conclusioni: Il nuovo kit VitaPCR™ Gen2, migliorato rispetto al precedente per maggior sensibilità analitica e stabilità del campione, permette un'accurata diagnostica molecolare per la ricerca RNA virale SARS-CoV-2 in TNF con elevata carica virale (Ct <30 su sistema Cepheid), mostrandosi una valida alternativa di diagnostica molecolare rapida, da impiegarsi nelle patologie tempo-dipendenti, grazie ai tempi di analisi inferiori ai 25 minuti. La presente valutazione preliminare, eseguita su TNF conservati in UTM (materiale non validato dal produttore), dovrebbe essere estesa a TNF trattati secondo le indicazioni per verificare la possibilità di miglioramento della concordanza tra sistemi.

EP252

A comprehensive cancer genome profiling highlighted the possible role of BRCA genes in Lung Cancer precision medicine: a pilot study

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Background: Since the discovery of driver oncogenic alterations in advanced lung cancer (LC), the therapeutic treatment landscape has grown exponentially and genomic profiling has been proposed as standard care. Among all the LC driver oncogenic variants (OVs), the therapeutic relevance of BRCA1/2 (BRCA) genes OVs remain poorly defined.

Aim: Our aim is to investigate in a clinical setting the prevalence of germline and somatic BRCA mutations in a cohort of patients with adenocarcinoma of lung through a comprehensive cancer genome profiling.

Methods: DNA was extracted from FFPE slides using the AllPrep®DNA/RNA FFPE kit (QIAGEN). A pan-cancer analysis was performed using the TruSight Oncology 500-High Throughput DNA kit that allows alterations in 523 genes including BRCA, Tumor Mutational Burden, and Microsatellite Instability. Next Generation Sequencing was performed on the NovaSeq6000 platform (Illumina). Output data were analysed on the Clinical Genomics Workspace platform by PierianDx tool.

Results: We enrolled 134 patients with advanced adenocarcinoma of lung. BRCA1 OVs were reported in 17 of the 134 patients (12.7%). Among them, 8 patients carried OVs (5.97%) and 9 (6.7%) presented copy number variations (CNVs). BRCA1 OVs occurred in 3 patients (2.23%), 2 of these were of suggestive germline origin. Concerning BRCA2, we found 5 of the 134 patients carring OVs (3.73%), including 2 suggestive germline mutations. Both BRCA1/2 variants were frameshift and non-sense mutations. We also found 9 of the 134 patients carried a whole BRCA2 gene deletion, and a patient with a BRCA1 gene deletion harboring also a BRCA2 gene deletion. BRCA VUS were detected in 13 of the 134 patients (9.7%): 3 gene variants in BRCA1; 7 gene variants in BRCA2; 1 BRCA1 gene amplification; 2 BRCA2 gene amplification.

Conclusion: The predictive role of BRCA1 OVs in LC treatment decisions is still limited due to the lack of pre-clinical and clinical studies. Our data show a possible involvement of BRCA1/2 genes in LC, with a higher incidence of BRCA2 than BRCA1 mutations. Finally, given the well-known correlation between BRCA and Homologous Recombination Deficiency (HRD), it would be interesting to investigate about the role of HRD in LC.

EP253

Use into clinical practice of a high-throughput targeted next-generation sequencing assay for comprehensive genomic profiling of metastatic colorectal cancer: Italian center cohort study

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Introduction: Timely and accurate identification of molecular alterations in solid tumors is essential for proper management of patients with metastatic colorectal cancer (mCRC). This has created a need for rapid, scalable comprehensive genomic profiling (CGP) systems that detect an increasing number of therapeutically-relevant variant types and molecular signatures. Detection of KRAS, NRAS, and BRAF mutations in tumor tissue of patients with mCRC is currently used to predict resistance to treatment with EGFR antibodies. The main aim of this study is to determine the mutation frequencies of KRAS, NRAS and BRAF genes from patients with mCRC in a referral center and compare it to international patterns.

Materials and Methods: From FPG500 (approval number 3837), a monocentric, interventional prospective study of CPG in 10 cancer types launched in January 2022, 58 mCRC cases were selected. DNA from tumor tissue were profiled on the TSO500 HT panel and sequenced on an Illumina Novaseq 6000 instrument. In order to estimate the mutation frequencies of most common actionable mutations in mCRC, we compared RAS/ BRAF mutation frequencies observed and expected using TCGA colorectal cancer dataset from cBioPortal platform. The expected mutation frequencies in KRAS, NRAS and BRAF are 53,8%, 7,7% e 7,7% respectively. The correlation between mutation frequencies observed vs expected was evaluated by two-tiled binomal exact test and statistical analyses were performed using R software. P values <5 was statistically significant.

Results: Based on tumor tissue DNA analysis, 53% of patients harbored a KRAS mutation (31/58; p=1; 95%(CI):[39,9%-66,7%]), 5% had a positive NRAS mutation (3/58; p= 0,626; 95%(CI):[1,1%-14,4%])and 10% harbored a BRAF mutation (6/58; p= 0,454; 95%(CI): [3,9%- 21,2%]). No significative difference between the frequencies observed in our cohort vs Pan-Cancer TCGA was observed.

Discussion: The observed KRAS,NRAS and BRAF mutation rates in our cohort (53%, 5% and 10%, respectively) conformed with what is reported in the literature, TCGA dataset and also were highly concordant for recurrent alteration in mCRC. Additionally, because of high concordance between results obtained, we believe that CGP solution could be proposed for routine use in precision oncology.

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EP254
Validazione del Kit "quantILab Glycated Albumin" su Alinity c

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Premessa: L'albumina glicata (AG) è ritenuta un valido marker di controllo addizionale nel paziente diabetico ed in tulle quelle condizioni in cui l'emoglobina glicata non è utilizzabile. Scopo del lavoro è stato quello di validare il metodo per la determinazione di AG "quantILab Glycated Albumin" (GlyAlb Werfen) sul sistema Alinity c (Abbott) in uso presso ASST-Valcamonica.Materiali and Metodi: Lo studio di precisione è stato effettuato secondo il protocollo del Clinical and Laboratory Standard Institute (CLSI) EP5-A3 impiegando i 2 livelli (Low e High) del materiale di controllo (QC) SeraChem Glycated Albumin; i dati sono stati confrontati con quelli dichiarati dal produttore su sistema Ilab Taurus (Werfen), ovvero CV≤2.5% e ≤2.0% per QC Low e High, rispettivamente. La comparazione di GA% Alinity c vs Ilab Taurus è stata effettuata su n. 40 campioni con concentrazione di GA tra 8.0 e 43.6% analizzati in duplicato in 3 diverse sedute analitiche secondo il protocollo CLSI EP9-A3; è stato considerato accettabile un bias <3.0%. Lo studio di linearità è stato effettuato secondo il protocollo CLSI EP-6A mediante analisi in duplicato di 7 diversi campioni a diverse concentrazioni di GA%, con un recupero accettabile entro il range 95-105%. La stabilità dei reagenti e della calibrazione è stata valutata mediante mantenimento del reagente a bordo strumento e valutando albumina, GA e GA% su n. 3 campioni (QC Low, QC High e calibratore) analizzati a T0 e dopo 7, 8 14, 24 e 31 giorni, impiegando quale criterio di accettabilità un recupero pari a ±5% vs T0.Risultati: Lo studio di precisione ha evidenziato una ripetibilità, espressa come CV%, per GlyAlb di 0.7% a 6.8 g/L e 0.6% a 17.3 g/L e per GA% di 0.6% a 14.9% e 0.7% a 33.4% ed una riproducibilità (CV % totale) di 1.0% a 6.8 g/L e 0.8% a 17.3 g/L per GlyAlb e di 0.8% a 14.9% e 0.9% a 33.4% per GA%. La regressione di Passing e Bablok nel confronto con Ilab Taurus ha mostrato: GA% Alinity c= 1.0000 * GA% Taurus -0.4000%, con bias medio % pari a -2.2%. Il test GA% su Alinity c si è mostrato lineare tra 8.0 e 87.6%, con y = 1.0011 x – 1.1572 x, R^2= 0.9992. I reagenti per albumina e GA si sono mostrati stabili nello scomparto refrigerato dello strumento per 30 gg, mentre la calibrazione è stabile per 7 gg.Conclusioni: Il metodo "quantILab Glycated Albumin" implementato sul sistema Alinity c mostra prestazioni analitiche di riproducibilità, bias e linearità accettabili rendendolo idoneo all'impiego nella diagnostica diabetologica, in particolare in ambiti organizzativi in cui sia necessaria una elevata produttività strumentale.

EP255
Monitoring of a personalized therapeutic program in haematology by innovative TGA/Chemometrics test

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Thermogravimetry coupled with multiparametric analysis has been applied to detect hereditary hemolytic disorders. The innovative screening method was able to highlight differences in blood samples from anemic patients and healthy donors detecting different condition of hemolysis and anemia [1]. In this study, the ability of the new approach to monitor effects of the transfusion therapy as changes in blood samples composition is tested. The advantage of this method consists of a quick identification of patients with negative response to transfusion allowing a prompt change in the therapeutic program. Blood samples collected from healthy individuals and the sickle cell anemia patients under transfusion treatment were analyzed by thermogravimetric balance. The resulting TG and DTG curves were compared in order to highlight a different thermal behavior of blood samples collected from patients before and after therapeutic treatment and respect to those of healthy donors. The multiparametric approach based on Principal Component Analysis was used to study the possible correlation among the profiles of the three populations according to a different amount of water content and corpuscular fraction. Results were found to be in accordance with complete blood count, reticulocytes count and hemoglobin profile analysis: the TG/Chemometrics method was able to recognize patients who respond to therapy proving the ability of the proposed test to monitoring therapy effect using few microliters of whole non pretreated blood.

[1] R. Risoluti, S. Materazzi, F. Sorrentino, L. Maffei, P. Caprari. Thermogravimetric analysis coupled with chemometrics as a powerful predictive tool for β-thalassemia screening. Talanta 2016;159:425-32.

EP256

DEVELOPMENT OF A PROCEDURE FOR DETERMINATION AND VERIFICATION OF REFERENCE INTERVALS BY INDIRECT METHODS

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INTRODUCTION. Reference intervals (RIs) are an essential tool for interpreting laboratory test results, and ISO 15189 requires their periodic review. Determining and re-evaluating RIs with conventional methods is a challenging task because, on the one hand, a "healthy" population must be defined a priori and, on the other hand, there is a risk of obtaining unrepresentative RIs due to the small sample size, especially if the recruited subjects have to be divided into several classes. For this reason, "indirect" methods have been proposed that allow RIs to be determined in a cheaper way by using data obtained during routine measurements. In this work, we present a method developed with the dual aim of reducing the proportion, and hence the impact, of pathological results in the laboratory database and automating some steps of data processing.
METHODS AND RESULTS. The procedure, which was developed using three-year data for ferritin, total protein, ALT and creatinine, included the following steps: extraction of a .csv file from the LIS; implementation of an automated workflow using the software Knime, which performed the conversion of the .csv file into an Excel file and formatting it for further processing; remixing the dataset to reduce the proportion of possible pathological results (by selecting the wards of origin, using single-access subjects, statistically removing outliers, selecting based on other tests requested at the same time); processing the data using the R programme (refineR package); comparing the generated RIs with those proposed by the manufacturer and/or obtained from the donor population.
CONCLUSIONS. The use of indirect methods is a promising solution for the establishment and revision of reference intervals, as it makes use of data already available in the laboratory and avoids the planning of ad hoc tests that are difficult to perform. However, in order to obtain reliable and truly representative data from a "normal" population, the procedure for processing the raw data from LIS must be carefully defined and implemented to obtain a dataset that is more suitable for statistical processing in specialised computer applications.

EP257

The gut microbiome affects the regression of oncolytic adenovirus-treated melanoma in a syngeneic mouse model

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In the last decade, cancer immunotherapy has achieved tremendous results, however the outcome of immune checkpoint inhibitors (ICIs) therapy has many genetic and environmental sources of variability. Several studies demonstrated the influence of gut microbiome on immunotherapy outcomes and in particular commensal Bifidobacterium was identified as a positive regulator of antitumor immunity in vivo, promoting anti-PD-L1 efficacy. Recently, novel strategies of active immunotherapy, based on oncolytic viruses (OVs) are achieving preclinical and clinical success, with a relevant contribution to the treatment of several types of tumors.We hypothesized that modulation of gut microbiota could also affect OVs therapeutic efficacy and, therefore, decided to investigate whether the effect of OVs on tumor progression could be altered by manipulation of the intestinal microbial community.To address this question, we pre-treated a group of C57BL/6J mice with an oral administration of vancomycin and, subsequently, inoculated subcutaneously syngeneic B16.OVA melanoma cells to the groups. We then treated both groups with an intratumorally injection of oncolytic adenovirus Ad5-CpG and we observed that its antitumoral effect was extremely reduced in mice pre-treated with vancomycin, showing a faster tumor progression and less tumor-infiltrating lymphocytes (TILs), compared to the control group. To confirm that alteration of the intestinal microbiota was involved in this phenomenon, we performed the rescue of the group pre-treated with vancomycin, by cohousing with a Ad5-CpG-treated control group. Successively, we combined the adjuvant activity of virus with a probiotic containing Bifidobacterium spp. that increased the response to adenoviral therapy, reducing tumor-infiltrating T-regs and stimulating an enrichment of bacterial genera belonging to Firmicutes phylum. Using a bioinformatic tool Homologous Evaluation Xenopeptides (HEX) we identified specific Bifidobacterium-derived

peptides highly similar to melanoma epitopes. As confirmed by IFN-γ ELISPOT assay, these peptides are able to trigger a robust CTL-response. Our data indicate that gut microbiota affects the immune responses elicited by oncolytic adenovirus Ad-CpG and Bifidobacterium vaccination optimized its antitumor activity in melanoma, because of a possible mechanism of molecular mimicry between Bifidobacterium-derived peptides and melanoma epitopes.

EP258

Evaluation of the use of VitaPCRTM SARS-CoV-2 Assay for the confirmation of samples tested positive with LIAISON® SARS-CoV-2 Ag assay

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Background

At the Urbino hospital, all patients tested with antigen tests for SARS-CoV-2, if positive, are confirmed with a molecular test. Among the molecular tests in use in our laboratory, VitaPCR™ SARS-CoV-2 Assay is the fastest and easiest to use, as it takes about half an hour from preparation to the result and does not require a biosecurity laboratory, because performed from inactivated samples.

Aim of the study

Given the rapidity of VitaPCR™ SARS-CoV-2 Assay, it was decided to use it for the confirmation of samples found positive to LIAISON® SARS-CoV-2 Ag test. This last test is performed by dedicated tubes with inactivating buffer based on detergent, therefore it does not interfere in the processes of extraction and amplification of nucleic acids.

Materials and methods

For evaluation, 48 samples tested positive with LIAISON® SARS-CoV-2 Ag (Diasorin) test were analyzed, coming from various departments of the Urbino hospital, and confirmed by Simplexa™ COVID-19 Direct (Diasorin) molecular test. From the tubes of all the samples, 200 uL were taken and transferred to dedicated tubes for analysis with VitaPCR™ SARS-CoV-2 Assay (Menarini) to be analyzed, after eliminating 2.2 mL of buffer.

Results

VitaPCR™ SARS-CoV-2 Assay was able to detect SARS-CoV-2 RNA in all 48 tested antigenically positive samples. In particular, even in all positive samples with low values of TCID50/mL (<1000), 11 out of 48, results with Ct<28.8 were found, although not maintaining a direct correspondence between quantity of antigen and quantity of detected RNA.

Conclusions

The results obtained, despite requiring an increase in the number of cases, demonstrate the potential use of VitaPCR™ SARS-CoV-2 Assay for the confirmation of positive samples directly from the inactivated buffer used for LIAISON® SARS-CoV-2 Ag assay. VitaPCR™ instrument, given the speed, simplicity of use and the possibility of use outside biosafety laboratories, it is an excellent diagnostic tool to support the confirmation procedures of samples tested positive for the SARS-CoV-2 N antigen.

EP259

On site detection of seized illicit drugs by MicroNIR and Chemometrics

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The main concern of forensic scientists is to develop analytical methods with high specificity and accuracy able to provide real time results to support law enforcement. In this work, a new analytical platform based on MicroNIR spectroscopy and chemometrics was developed for the screening of illicit drugs. The advantage of this approach is the use of a miniaturized and portable spectrophotometer entirely controlled by a laptop through a wireless interface. The MicroNIR weights less than 250 g and requires only few seconds to collect spectra on samples which do not need any pre-treatment. It allows to perform measurements in contactless mode (until to 1,5 cm) avoiding any contamination and ensuring the integrity of the samples that could be investigated by further analytical techniques. The platform was developed to detect and quantify psychotropic compound in seized materials. Spectra were collected in reflectance mode on samples prior characterized by chromatography techniques and were used to develop a Partial Least Squares regression (PLSr) models. The coupling with Chemometric tools ensured the automation of the platform that provided results with high precision and accuracy. Outcomes demonstrate that the MicroNIR spectrophotometer is a promising click-on device and could be used by trained personnel that carry out on the field.

EP260

GESTIONE DELLA DIAGNOSTICA DEI LIQUIDI BIOLOGICI IN REGIME DI URGENZA

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Nel Laboratorio di Urgenza del DAMEDLAB dell'AOU Federico II afferiscono circa 1000 richieste l'anno di analisi di urgenza su liquidi biologici e liquidi di drenaggio di diversa origine. Nel 2022 è stata effettuata una revisione dei pannelli di richiesta condivisa con i vari Reparti afferenti allo scopo di: a) guidare i Richiedenti in una richiesta appropriata e completa di informazioni diagnostiche, b) individuare nel Laboratorio il percorso diagnostico specifico per la richiesta, c) redigere un referto con definizione diagnostica di probabilità.Oggetto della riorganizzazione sono state: Modalità di raccolta dei campioni; indicazioni sull'invio, prenotazione in order entry degli esami, individuazione delle metodiche corrette da utilizzare, strutturazione di un referto completo di tutte le informazioni inerenti il paziente, del tipo di esame effettuato, delle idonee unità di misura e di riferimento, dell'interpretazione dei risultati con definizione diagnostica di probabilità.Riportiamo gli elaborati dei modelli di richiesta, la tipologia del referto e le principali problematiche diagnostiche giunte in quest'anno alla nostra osservazione.Benchè la gestione dell'interfaccia informatica sia stata molto complessa, le maggiori problematiche sono state rappresentate dalla difficolta di automatizzazione di tali esami: l'unicità di ogni richiesta impone a monte del processo automatizzato il giusto inquadramento clinico da condividere con i Clinici e con i vari settori operativi del Laboratorio.

EP261
ESAME DEL LIQUIDO SINOVIALE: DOSAGGIO CALPROTECTINA

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L'esame del liquido sinoviale rappresenta una risorsa molto informativa e specifica per ortopedici e/o reumatologi di facile reperibilità, basso costo, semplice esecuzione, purtroppo ancora sottoutilizzata anche in ambito specialistico. Presso il Laboratorio di Urgenza del DAMEDLAB Federico II nel 2022 sono stati inviati circa 50 campioni al mese di liquido sinoviale prelevato principalmente a livello della sinovia articolare del ginocchio, anche, polso, caviglia. Il 60% è rappresentato da controlli per revisioni postoperatorie di versamenti di varia natura, nel restante 40% si tratta di articolazioni non trattate chirurgicamente di pazienti in fase diagnostica. Da circa un anno nel laboratorio si segue un protocollo operativo che prevede eventuale pretrattamento del campione iperviscoso, conta leucocitaria con formula percentuale di elementi mononucleati e polimorfonucleati, dosaggio proteine totali, osservazione microscopica, dosaggio calprotectina. Riportiamo i risultati di 50 campioni analizzati negli ultimi mesi con particolare riferimento alla correlazione tra i valori di calprotectina riscontrati, la formula leucocitaria e l'orientamento clinico-diagnostico allo scopo di approfondire il razionale di utilizzazione di tale test soprattutto nella discriminazione tra artriti settiche e forme infiammatorie non infettive.

EP262
BRCA status in epithelial ovarian cancer (EOC): comparison of two different Next Generation Sequencing-Based Tumor Testing

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Introduction: BRCA1/2 (BRCA) mutation status has become one of the most important parameters for treatment decision in patients with epithelial ovarian cancer (EOC). Treatment of EOC with poly (ADP-ribose) polymerase inhibitors (PARPi) has resulted in improved survival for individuals with a germline or somatic BRCA PV. Tumour genetic testing is particularly important for EOC, as 23–43% of BRCA1/2 PVs are somatic and would be missed by standard germline (i.e. blood) genetic testing. The aim of this study was to evaluate the reliability of tumor BRCA testing results starting from FFPE using two different Next Generation Sequencing (NGS)-based tumor testing.
Materials and Methods: 150 DNA from tumor tissue were profiled by a capture-based library preparation comprensive genome profiling (TSO500HT, Illumina) and an amplicon-based library preparation Devyser BRCA CE-IVD (Devyser, Stockholm, Sweden). NGS was performed on the NovaSeq6000 (Illumina) and a Miseq (Illumina) instruments, respectively. Output data were analysed on the Clinical Genomics Workspace platform by PierianDx tool and Amplicon Suite tool (Smartseq), respectively.
Results: All the 150 FFPE tumor samples were considered adequate for the NGS testing, according to the tumor cell content (more than 20%) and the DNA yield (more than 5ng/uL). Among the 150 tumor samples successfully sequenced, 35 (23.4%) cases harbored a pathogenic/ likely pathogenic (P/LP) variant and 15 (10%) cases showed a variant of uncertain clinical significance (VUS). Only two cases (1.3%) showed a discordance between the 2 NGS approach.
Discussion: Assessment of BRCA1/2 molecular status has become part of the standard of care in the management of patients with EOC. This study showed that it is possible to highlight BRCA status using both targeted testing and comprensive genome profiling starting from FFPE samples.

EP263

A personalized virtual gene panel from clinical exome sequencing identifies an Italian novel disease-causing variant in the LDLR gene

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Introduction: The rapid advances in NGS technologies have decreased the cost of sequencing per base pair, improved accuracy, and greatly increased the speed of generating sequence data. Exome sequencing has been rapidly applied to variant discovery in research settings and recent increases in accuracy have enabled development of clinical exome sequencing (CES) for mutation identification in patients with suspected genetic diseases. One the main challenge of CES test is the rate of variant interpretation, owing to the huge amount of sequence data produced. Consequently, identifying an efficient and appropriate strategies to investigate variants from CES analyses for clinical interpretation can have functional benefit in clinical diagnostic laboratories. Detailed phenotypic information along with CES give opportunity to develop personalised testing strategies through virtual gene panels.Herein, we describe an effective variant analysis, making, which identified a novel variant in LDLR gene in a patient with a suspected diagnosis of Familial Hypercholesterolemia (FH).
Material and Method: Exome sequencing was performed with the Clinical Exome Solution kit on NextSeq 500 system. (.BAM) and (.VCF) files were analysed via Sophia DDM® software v.4.2. using a virtual panel of 47 genes out of those included in the capture kit and associated with FH, based on Human Phenotype Ontology (HPO) and customised selection of gene specified by clinicians associated with clinical phenotype of patient.
Results: Using the FH virtual panel designed for this peculiar patient, NGS analysis highlighted the frameshift variant c.1171del (p.Ala391Profs*22) accounted in LDLR gene. This is a novel variant, since it is not previously described in literature or reported in the main mutational database nor in the population frequency databases. In addition, among more than 200 alleles routinely analysed by NGS-based molecular screening, we did not identify the two variants in other FH or healthy individuals.
Discussion: Thanks to the detailed phenotypic information, it was possible to employ an appropriate filtering strategy in analysing and interpreting the results of testing. In our study, the use of “ad hoc” virtual panel enabled the identification of novel variant in LDLR gene associated with FH reducing greatly the number of variants needing examination. Our results emphasize the usefulness of CES filtered by a virtual gene panel, based of phenotype, to reduce interpretative variant workload and preserve diagnostic yield and potentially maintain a deliverable timeframe for clinical laboratories.

EP264

IMPLEMENTATION OF A POCT (POINT-OF-CARE) SYSTEM FOR THE DETECTION OF THE ANTIGEN OF THE SARS-COV2 VIRUS IN EMERGENCY DEPARTMENT AND FOR THE PRE-HOSPITALIZATION OF THE HOSPITALS OF THE COMPANY USL TOSCANA SUD EST

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Introduction
Following the release of the regional circulars recommending the execution of antigen tests for the diagnosis of Sars-Cov2 infection for all patients entering the emergency Department and in case of pre-hospitalization, it was decided to implement a system of POCT (Point-Of-Care) instruments in hospitals throughout the South-East USL area.
Materials and methods
The managers of the POCT network have provided each hospital with third generation tests capable of guaranteeing a sensitivity of over 95% and a specificity of 100%.The necessary skills for the staff were then determined and training sessions were planned to cover all the staff concerned thanks to the involvement of the specialized staff of the supplier company and the previously trained laboratory staff.Following the training of over 300 operators, its effectiveness was verified through online self-assessment questionnaires and each user was enabled to use the tool with their own identification. The insertion of the identification code for the individual operators, it was possible to monitor the correct performance of the pre-analytical procedures and to implement any corrective measures where necessary.
Results
All this has ensured, in addition to an optimal collaboration between the laboratory, the supplier company and the emergency room, a high level of data security related to a very low rate of invalid or discordant results. This resulted in a considerable time and cost saving when compared with the need to carry out repeated tests or confirmations with molecular tests.
Conclusions
This organization has allowed not only a supply of tests with superior performances, but to have a complete traceability of the results following the connection of the instruments to the systems of the hospitals and in the ability to apply the criteria necessary for an aware training of the operators, in accordance with what is reported in the ISO 9001/2015 standard.

EP265

RETRAINING FORMATIVO SULL'ESECUZIONE DEGLI EMOGAS CAPILLARI BASATO SULLA COMPETENZA E CONSAPEVOLEZZA: UN QUESTIONARIO CONOSCITIVO

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Introduzione
Il prelievo capillare pur essendo largamente utilizzato in più ambiti come nei reparti di neonatologia e in area urgenza/emergenza, presenta molti aspetti poco conosciuti dell'applicazione del sangue capillare quali: gli errori preanalitici, la non applicabilità per alcuni parametri e le sedi di prelievo da evitare. Lo scopo del lavoro è stato quello di eseguire tra il personale medico, infermieristico e ostetrico del reparto di neonatologia un retraining formativo per il prelievo capillare, sulla consapevolezza delle conoscenze degli operatori facendo emergere tutte le criticità sull'utilizzo di tale modalità.
Materiali e metodi
Un questionario di 23 domande è stato somministrato a 61 operatori tra infermieri, medici e ostetriche dei reparti di neonatologia in modalità web attraverso piattaforma "google". Le domande del questionario indagavano le conoscenze riguardanti la fase preanalitica, analitica e post analitica dell'emogas capillare . Ad ogni risposta è stato assegnato un punteggio di 1. La popolazione statistica è stata divisa in 3 classi in base al punteggio ottenuto. Gli operatori con punteggio da 17 a 23, non necessitavano di retraining, operatori con punteggio da 9 a 16, necessitavano di un retraining parziale, operatori con punteggio da 0 a 8, necessitavano di un retraining completo.
Risultati
I risultati hanno mostrato che il 75% (46 operatori) aveva eseguito un ottimo test e non necessitavano di retraining, il 25% (15) operatori aveva eseguito il test in maniera sufficiente e necessitava di un retraining parziale, nessun operatore aveva eseguito il test insufficiente. Le fonti di potenziali errori riguardavano le sedi del prelievo, l'utilità del massaggio del tallone del paziente pediatrico prima del prelievo e l'emolisi del campione capillare.
Conclusioni
I risultati dimostrano come la conoscenza e la consapevolezza di una corretta esecuzione dei prelievi capillari sono fattori chiave per avere un analisi accurata e di qualità. Il punto 7.3 della norma ISO 9001:2015 affronta proprio queste problematiche legate alla consapevolezza degli operatori attraverso un processo che mira a rendere gli operatori consapevoli del loro lavoro e delle loro responsabilità indispensabili nell'ambito della qualità analitica.

EP266

Integrated network of the ECMU (physical, chemical and morphological urine examination) of the Azienda USL Toscana Sud Est

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INTRODUCTION
Following the Sars-Cov2 pandemic of the last 2 years, telemedicine has become fundamental for the territorial assistance of patients and the consultation of professionals among them to carry out assistance services in a very complex territory such as that of the Azienda USL Toscana Sud Est (TSE). Currently, the middleware, on the network, allows for the integration of all data from the physical, chemical and morphological urine examination (ECMU), allowing the transmission of data and images between computer-connected laboratories. This work illustrates the organizational architecture of an analytical integration network managed in telemedicine and teleconsulting applied to the ECMU and consisting of all the laboratories of the Azienda TSE.
MATERIALS AND METHODS
The integrated network of the ECMU includes the territorial laboratories of the Azienda TSE, such as the hub centers of Arezzo, Grosseto, Nottola and Campostaggia with the spoke centers of Cortona, Bibbiena, Sansepolcro, Valdarno, Massa Marittima, Orbetello, Pitigliano, Abbadia San Salvatore , Castel del Piano. The instrumentation used by the laboratories is Aution Max 4030 (Menarini) and Pochet Chem UA for the chemical-physical examination, Sedimax Contrust (Menarini) and the Director Web (Menarini) management software.
RESULTS
The integrated network of the ECMU consists of all the Azienda TSE laboratories, which are connected to each other electronically through the middleware Director Web, which allows the sharing of rules, chemical-physical analysis data and images of the urinary sediment. Through the Director Web management software it is possible, in fact, for hub laboratories not only to consult and validate the ECMU carried out by the spoke centers in the area, but to receive and give "second opinion" advice between the network laboratories.
CONCLUSIONS
The use of the same analytical systems and the middleware system common throughout the Azienda TSE allows to obtain harmonized and standardized results throughout the territory with shared criteria in the integrated network of the ECMU, in fact, the collaboration of specialists is indirectly promoted with time much faster

diagnosis and a less rigid and leaner organization of work, uniform throughout the territory.

EP267

Dynamics of T-cell responses to the BNT162b2 mRNA vaccine in a healthcare cohort for up to 9 months after the administration.

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The cellular responses to the BNT162b2 vaccine and their correlation with antibody titer and gender determinants are critical to assess. We aimed to evaluate the cellular response kinetics, correlating it with gender and antibody titer.Peripheral Blood Mononuclear Cells (PBMC) were stimulated with SARS-CoV-2 Spike protein, and the IFN-γ was evaluated by Elispot assay at 5 different time-points, for up to 9 months after the BNT162b2 vaccine. 107 healthcare workers were divided into 4 age groups: <40 and >40 years for men, based on the gradual decline of testosterone with aging; <48 and >48 years for women based on the decrease of estrogen with menopause. Furthermore, seropositive individuals were analyzed at baseline to avoid confounding factors caused by the previous infection with SARS-CoV-2. We also analyzed pre-pandemic samples as controls to consider the cross-reactivity toward other coronaviruses. Among seronegative at baseline, the T-cell response against S-protein changed from a median of 2 spot forming cells (SFC)/400.000 PBMC (Interquartile range, IQR, 0-17) before vaccination to a median of 42 (17.5-78.0) after the second dose. Then, it progressively decreased to 13 (0-34) at 6 months after vaccination and 11 (0-31) after 9 months. At all timepoints, the differences were statistically significant compared to baseline values. Moreover, the results obtained after the second dose were significantly higher than those observed at any other time point. Indeed, a significant correlation was observed between T-cell response and anti-S antibody titer ($p<0.001$), previously analyzed, even if it was weak in magnitude ($r=0.314$). Natural seropositive showed higher T cell response at baseline than seronegative ones (median: 2 vs. 29, $p=0.003$), even if there were no significant differences in response at later time points. Our data suggest that T-cell reactiveness is poorly impacted by sex and age, whereas age was significantly associated with anti-S antibody titer. T-cell response declines 9 months later administration, indicating a waning of immune response over time. So, the positive correlation with the antibody titer confirms a linkage between different arms of adaptive responses and potentially converts future vaccinations into a tailored process.

EP268

INTEGRATED ANALYTICAL NETWORKS OF THE SOUTH EAST TUSCAN USL: MANAGEMENT OF CQI AND VEQ ON 13 LABORATORIES

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Introduction

The use of CQI and EQA are the basis for controlling the analytical performance of an analytical method/ system. Even more complex is reaching the analytical goals (CV, ET, Moving and Fixed Average etc.) in multi-laboratory and multi-instrumental analytical networks even if on analytical lines of homogeneous instrumental groups (hematology, coagulation, clinical chemistry and immunometry). In this project we illustrate a management method of the global CQI and of the structured and shared EQA indicators.

Materials and methods

For the concentration and dissemination of the control data, management software of the supplier companies organized in networks were used: SISTEMA 24.7 (Randox), Caresphere (Dasit), Acusera (Werfen). In the evaluation, instrumental alignment is sought, the alignment between batches of reagents and calibrators, and everything necessary for analytical harmonization. The results of the VEQ of the various providers are summarized on ad hoc indicator reports for shared evaluation and for the identification of data that are not accepted, aberrant and not presented. In addition to dedicated software concentrators, Excel and Access (Microsoft) and SSPP (IBM version 20.0) were used to analyze the results.

Results

The CQI data of all the centers were collected in the software, the point alarms were managed locally by the operators dedicated to the instrumentation. The analyzes on the CV% calculated weekly, the ET, and the moving average of each center, were used to evaluate the deviation from the shared analytical goal. For the instrumental alignment, the moving average was used for each analyte on all the centers on a fortnightly basis, any alarm values caused by excessive deviation from the accepted value were grounds for studying the reasons for interference. The planned interventions on the deviations are represented in order of frequency by: Different control lot, error in target configuration, instrument maintenance, different reagent lot.

Conclusions

The evaluation of the analytical quality with a system built for integrated analytical networks of Hematology and Coagulation, Clinical Chemistry and Immunometry, becomes an indispensable tool for the management of the analytical flow. The organizational complexity of the multi-laboratory cannot ignore the use of strictly analytical indicators based on structured and shared algorithms.

EP269

Confronto tra Brescia-Covid Respiratory Severity Scale (BCRSS)/ Algorithm e livelli di Procalcitonina plasmatica in pazienti con malattia da Covid-19

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Obiettivo di questo studio è determinare se esiste una relazione tra il sistema di punteggio clinico Brescia-Covid Respiratory Severity Scale (BCRSS)/ Algorithm e i livelli di Procalcitonina plasmatica nel determinare la gravità della malattia da COVID-19.

Materiali e metodi: 722 pazienti con malattia da COVID-19 da moderati a gravi ricoverati presso l'Ospedale di Rovereto da Gennaio a Dicembre 2021. Tali pazienti sono stati classificati secondo il sistema di punteggio clinico BCRSS in 3 gruppi e ne sono stati testati i valori di Procalcitonina plasmatica entro 3-5 giorni dal loro ricovero.

Risultati:I risultati evidenziano che la concentrazione di 0.5 ng/mL di Procalcitonina plasmatica rappresenta il valore decisionale ottimale per la migliore prognosi.

Conclusioni: Lo studio dimostra che un aumento della concentrazione di Procalcitonina plasmatica (<0.5 ng/mL) è associato alla necessità di ventilazione invasiva e a una maggior durata della ventilazione meccanica.

EP270

Monoclonal gammopathy in COVID-19 pregnant patients

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Monoclonal gammopathies (MG) are a group of disorders ranging from the benign to the malignant plasma cell dyscrasias to the lymphoproliferative disorders, typically characterized by the presence of a monoclonal component (MC) at serum protein electrophoresis (SPE). This condition has been also observed in association with a spectrum of acute and chronic inflammatory diseases, including viral infections.

We selected for this study pregnant patients hospitalized from 2018 at the Department of Gynecology of the AOU Federico II of Naples (that include from March 2020 a section dedicated to pregnant patients with Sars-Cov2 infection), who presented a MC at the SPE (Capillarys 2, Sebia), at prenatal checks.

Our population has involved 20 pregnant patients (average age of 35yo, from 22 to 47), 8 of which tested positive for COVID-19 by real-time reverse-transcriptase polymerase chain reaction of nasopharyngeal swabs. It should be considered that 3 patients came in the pre-pandemic period.

The MC characterization revealed: 7 patients with IgG k ranging from 1 to 10 g/L; 5 with IgG k<3 g/L; 1 with IgM k 7,7 g/L; 5 with IgG k and IgG lambda<2 g/L; 1 with 2 IgG lambda 6,7 g/L and 2,0 g/L; 1 with 2 IgA k 9,6 g/L and 1,3 g/L.

At hospitalization, 2 patients already had a diagnosis of MGUS, while for the others the MC presence was an occasional finding.

It should be noted that 2 COVID-19 patients underwent therapy with casirivimab and imdevimab and one non COVID-19 patient to anti-D immunoprophylaxis after having already identified the MC.

For 5 patients MC was detected during the second trimester and 4 of these had Sars-Cov2 infection; in the other 15 at the end of pregnancy.

We observed that in the pregnant population, where in the pre COVID-19 era in our laboratory the MGUS percentage was of 0.3/year, the incidence increased to 0.6/year since February 2020.

The presence of these MGUS must be investigated over time to understand the transience of the MC linked to viral infection. Previous studies hypothesized that the presence of MC during the inflammatory phase could reflect the degree of immune system hyperactivation in patients with severe COVID-19 disease. Further studies are needed to evaluate its frequency in pregnancy, long-term evolution, and prognostic role in this clinical setting.

EP271

Serum sodium level of 98 mmol/L: a true case of severe hyponatremia in a complex patient

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Introduction:

Hyponatremia, defined as a serum sodium concentration <135 mmol/L, is the most common alteration of body fluids and electrolyte

balance found in clinical practice. It can lead to a broad spectrum of symptoms and is associated with increased mortality, morbidity

and length of hospital stay1. Collaboration between clinicians and laboratory is essential for a correct classification and choice of an

adequate therapy.

Case:

A 42-year-old man had been treated with haloperidol, olanzapine, delorazepam, flurazepam and biperiden because of a diagnosis of

schizophrenia and seizures before admission to our hospital for head trauma in seizure and unquantified diarrhea. At admission physical

examination revelead a bronchitis and the blood chemistry tests were within their reference ranges except for natremia (98 mmol/L) and serum osmolality (231 mOsm/Kg). In the first hours of hospitalization the patient was alert, the only peculiarity was a diuresis of 1.5 L after catheterization and a total daily diuresis of 8 L; within two hours the patient had another seizure and gets severe neurological deterioration with GCS of 3, then taken to the ICU. The therapy was initially an infusion of physiological with 2 ampoules of NaCl, after his condition worsened he was intubated, Levetiracetam for the seizure, Augmentin for bronchitis and Desmopressin because a suspect of diabetes insipidus for his high diuresis. It continued with a gradual correction of the natremia until it was brought back to normal.

Upon

awakening, the patient reveals that he is a potomaniac, drinking about 7 liters of water a day. The patient was reintroduced into his high care community after all clinical-laboratory evaluations and correction of his therapy.

Discussion:

The cause of this multifactorial hyponatremia wasn't easy to understand because this unexpected potomania, the diarrhea and the mix

of drugs that had hyponatremia as unwanted effect. Hyponatremia is a dysionia that must be diagnosed properly and corrected slowly

especially to avoid iatrogenic complications such as cerebral edema and osmotic demyelination syndrome. For this reason it is necessary to have a diversified instrumental setting in the laboratory (indirect ISE, direct ISE, Osmometer) to diagnose it quickly.

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EP272

Leucemia Acuta a Promielociti: quando lo striscio periferico diventa un limite

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La leucemia Acuta a Promielociti (LAP) rappresenta il 5-15% di tutte le leucemie acute mieloidi. Marcatore genetico della LAP è il gene PML/RARα, la cui fusione si può riconoscere con FISH e RT-PCR, test non immediati e non disponibili ovunque. Una tempestiva diagnosi morfologica è cruciale per la sopravvivenza del paziente con LAP per avviare la terapia con Acido trans retinoico e ridurre significativamente la mortalità. Nella LAP “classica” i promielociti hanno nucleo rotondeggiante, bilobato, inciso, reniforme e citoplasma con numerosi granuli grossolani azurofili. Marcatore morfologico della LAP sono i corpi di Auer multipli, talora così numerosi da raccogliersi in fasci (faggots). Nella variante microgranulare (LAPv) i blasti hanno nucleo irregolare, ampio citoplasma poco granulato e rarissimi o assenti corpi di Auer. Negli ultimi 2 anni, presso il nostro Laboratorio sono stati diagnosticati all’esordio 6 casi di LAP: Donna (D) di 56 anni (a) con iperleucosi (WBC: 41,89x109/L) e 95,5% di blasti (LAPv) Uomo (U) di 83a con leucopenia (WBC: 3,08x109/L,) e 16,5% di blasti con Auer Bambina di 10a con WBC:14,56x109/L e con 70% di blasti (LAPv) U di 44° con leucocitosi (WBC: 58,01x109/L) e 76,7% di blasti (LAPv) U di 66a con leucopenia (WBC: 0.48x109/L) e 4.3% di blasti ipergranulari. D di 73a con leucopenia (WBC: 0.77x109/), positiva a Sars-Cov-2 e assenza di blasti.Nella nostra esperienza, i promielociti patologici, anche non tipici, sono stati riconosciuti se presenti in alta percentuale (%) e con un numero di WBC >3.00x109/L. La difficoltà c’è stata con WBC< 1.00 x109/L in cui sono stati allestiti più strisci per raggiungere il numero di cellule raccomandato e i tempi di valutazione sono stati estremamente lunghi (in media 2 ore). Nel caso con assenza di blasti, solo l’analisi molecolare da aspirato midollare dopo 4 giorni ha dato riscontro di positività. Sebbene siano descritte caratteristiche che distinguono la LAP, la sua diagnosi morfologica periferica è ancora difficile, per la sua rarità e i diversi aspetti di presentazione. E’ indispensabile investire nella formazione continua di patologi in grado di riconoscerla. Nei casi con leucopenia, utile potrebbe essere la preparazione di vetrini allestiti con arricchimento di sangue periferico.

EP273

Protocollo di Convalida delle strumentazioni di laboratorio del CQB – Campania Centro dell'A.O.R.N. "A.Cardarelli" di Napoli per lo screening siero virologico dei donatori di sangue

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INTRODUZIONE

Presso il Centro di Qualificazione Biologica del Dipartimento Campania Centro dell'AORN A.Cardarelli di Napoli si eseguono lo screening siero-virologicoe il test NAT su tutti i campioni delle unità di sangue donate,come disciplinato dal D.Lgs 300/2015 emanato dal Ministero della Salute in tema di "Disposizioni relative ai requisiti di qualità e sicurezza del sangue e degli emocomponenti".A seguito di gare espletate nel 2021,è stata introdotta una nuova strumentazione analitica,ALINITY s della ditta ABBOTT per la determinazione qualitativa dell'HBsAg,HCVAb,HIV1/2 Combo,Treponema Ab. In linea con quanto prescritto dalla normativa in materia di produzione di emocomponenti,a garanzia della qualità e sicurezza del sangue da trasfondere,la strumentazione deve essere qualificata. Scopo del lavoro è l'illustrazione del protocollo operativo di convalida dell'apparecchiatura,descrivendo le prove di convalida effettuate e la verifica dei requisiti di qualificazione secondo quanto stabilito dalle Linee Guida del CNS inerenti i percorsi di convalida.

MATERIALI E METODI

La convalida della strumentazione consiste nell'allestimento di prove documentate comprovanti che i requisiti dichiarati dal Produttore possono essere soddisfatti .Le prove di convalida sono state condotte sull'Analizzatore ALINITY s della Ditta ABBOTT la cui tecnologia è basata sul dosaggio immunologico chemiluminescente a cattura di microparticelle(CMIA) per la rilevazione di antigeni e anticorpi specifici nello screening dei donatori.Per la verifica dei parametri"Precisione nella serie,Precisione tra le serie,Accuratezza e Sensibilità analitica (solo per gli antigeni HBsAg e p24 HIV) sono state utilizzate preparazioni di riferimento a matrice di plasma umano fornite dal CNCF(Centro Nazionale Controllo e Valutazione dei Farmaci).Le preparazioni,previa opportuna diluizione,sono state impiegate per allestire un Campione Positivo Debole(CPD) vicino al valore di cut-off e un Campione Positivo Forte(CPF) almeno 10 volte superiore al valore di cut-off. Per la Precisione nella serie il CPD, il CPF e il Campione Negativo sono stati dosati in 20 repliche nella stessa seduta analitica.Per la Precisione tra le serie il CPD,il CPF e il Campione Negativo sono stati dosati in 4 repliche in 5 diverse sedute analitiche.Per l'Accuratezza Diagnostica sono stati analizzati 10 campioni positivi e 10 campioni negativi.Per la sensibilità analitica è stato analizzato in 2 sedute indipendenti un pannello di 9 campioni costituito da diluizioni del campione positivo in matrice negativa con concentrazione 3x,1.5x,0.75x il valore di sensibilità dichiarato dal Produttore.Sono stati analizzati inoltre 200 campioni negativi e 20 campioni positivi.I risultati sono stati registrati sui moduli forniti dal CNS che calcolano il CV%,la DS,la retta di regressione per il calcolo della sensibilità analitica .Il Report di convalida ha fornito l'evidenza delle attività analitiche effettuate e i criteri di accettabilità oltre che una descrizione del processo,il numero dei lotti utilizzati e gli operatori coinvolti.

RISULTATI E CONCLUSIONI

L'accuratezza del metodo è risultata del 100% per tutti gli analiti.La precisione nella serie e tra le serie sono risultate congruenti a quelle dichiarate dal produttore.La sensibilità analitica ha dato esito positivo confermandole specifiche stabilite : HBsAg 0.02 UI/mL(dichiarata ≤0.04 UI/mL),p24 HIV 0.40 UI/mL(dichiarata 1 UI/mL).Il report di convalida ha fornito l'evidenza delle attività svolte mediante il confronto con i criteri di accettabilità stabiliti dal CNS.I risultati ottenuti hanno consentito di convalidare il nuovo analizzatore.In conclusione l'attività svolta rappresenta un'opportunità di miglioramento dei livelli di qualità e sicurezza e non solo un adempimento di legge.

EP274
DETERMINATION OF NACU (NUCLEIC ACID CONTAINING UNITS) AS A QUALITY INDEX OF THE SAMPLE FOR THE HEMOCHROMOCYTOMETRIC EXAMINATION

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INTRODUCTIONThe ADVIA 2120i system is a state-of-the-art automatic blood cell counter employing flow cytometry and light scattering (1), which allows the determination of cells with higher absorption values than platelets, but with lower dispersion values than cross-linked platelets and called NACU (nucleic acid containing units). This parameter could be an indicator of the presence of bacteria, fungi, parasites or of degraded cells or cell fragments, for example in samples stored for a long time or in hemolyzed samples.MATERIALS AND METHODSThe determination of NACU was performed in samples taken in K2 EDTA and examined with ADVIA 2120i. For the evaluation of reticulocytes, the reagent dedicated to the staining of nucleic acids (Oxazine 750) was used and the processing with the instrument software (version 6.10.9) allows the identification of both cross-linked platelets and NACU in the cytogram produced. The number of NACUs was determined in 127 samples, received in the laboratory within 1 h of collection (t0). A part of the samples (n = 70) was divided into 2 aliquots and stored one at room temperature and one at + 2-8 ° C. The NACU determination was repeated after 4 hours (t + 4h) and 12 hours (t + 12h). The NACUs were also determined in 40 samples taken from peripheral sampling points and received approximately 4h after sampling (external t + 4h) to verify the possible influence of the transport conditions of the tubes. In addition, the effect of hemolysis was evaluated by determining the NACUs in 31 samples both immediately and after a freeze / thaw cycle.RESULTSThe results obtained show a significant variation in the number of NACU in the samples stored at room temperature already after 4 hours (average values 57.9 with 95% C.I. 54.4-61.5 and 72.3 with 95% C.I. 60.4- 84.2; p = 0.0163) and even greater after 12 hours (mean value 81.5 with 95% C.I. 68.3-94.8; p = 0.0002). Even for samples from peripheral sampling points, the NACU value proved to be at the limit of significance when compared with the values obtained in samples analyzed immediately after sampling (average value 61.7 with 95% C.I. 58.2-65.2 ; p = 0.0485), but comparable to the values obtained in samples stored for 4 hours at room temperature (p = 0.1903). There was a significant increase (mean values 36.8.9 with 95% C.I. 27.3-46.3 and 254.9 with 95% C.I. 160.4-349.5; p = 0.0001) in the number of NACU in samples measured just after collection and after haemolysis.CONCLUSIONSIn our experience, the NACU parameter demonstrates a significant variability as a function of time and of the storage conditions of the sample and is closely related to the presence of hemolysis. The evaluation of this index, after an adequate definition of the reference parameters, could be useful as an indicator of the pre-analytical quality of the sample in the execution of the blood count.BIBLIOGRAPHY Instrumental report Siemens ADVIA 2120, G Zini (Hematology in progress.it 2011)

EP275

TAT performances in TLA merging Emergency and routine requests with no process differences

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IntroductionStarting from January 2021 Baggiovara laboratory's Corelab has a Total Laboratory Automation (TLA) for 9 millions of tests/year. TLA integrates sorting, centrifugation, aliquoting, storage with the analytics (hematology, coagulation, clinical chemistry, immunometry), both for routine and emergency requests with no process differences. Every operation is managed by a software system. Intra-laboratory turnaround time (TAT) is a key indicator of clinical laboratory performance. Laboratory tests are also an important contributor to decision in Emergency Department (ED) by reducing the length of stay (LOS) and improving patient outcomes. The aim of this study is to evaluate TAT for local ED within 24 hours on a tipical day.MethodsTLA consists of 2 automation lines Power Express (Beckman Coulter) with mirror configuration. Each one has 4 centrifuges, decapping, aliquoting, storage. Along any automation multiple analyzers are located: 2 workcell DxH 900-2S (Beckman Coulter), 2 ACL TOP LAS (Werfen), 1 AU 5800 and 1 DxC 700 (Beckman Coulter), 2 DxI 800 (Beckman Coulter), 1 Architect (Abbott), 1 Liaison (Diasorin). All samples are placed on TLA, both routine and urgency. We evalueted TAT ≤ 1 h, >1-≤ 1.10 h, >1.10-≤ 1.20 h, >1.20-≤ 1.30 h, >1.30-≤ 2 h, >2 h.ResultsA total of 81 request were ordered by local ED: 62 (76.5%) ≤1h, 6 (7.4%) >1-≤1.10, 6 (7.4%) >1.10-≤1.20, 2 (2.5%) >1.20-≤1.30, 3 (3.7%) >1.30-≤2, 2 (2.5%) >2 h. Among 62 request with TAT ≤1h, 1 had a TAT <30' (1.6%), 35 had a TAT >30'-≤45' (56.5%), 26 had a TAT >45'-≤1h (41.9%). A total of 207 tubes corresponding to 81 requests: 153 (74%) ≤1h, 19 (9.2%) >1-≤1.10, 18 (8.7%) >1.10-≤1.20, 5 (2.4%) >1.20-≤1.30, 8 (3.8%) >1.30-≤2, 4 (1.9%) >2 h. TAT >2h in two requests (four tubes) and TAT >1.30-≤2 (8 tubes) was due to microscopic review for strumental/morfological alarms.DiscussionTLA affects the core laboratory by creating more efficient and stable management of workflow and less reporting time has impact on ED LOS. In our one-day verification only two reports had a TAT >2 h and three had a TAT >1.30'-≤2 h. In these five reports was necessary a microscopic review for hematological results validation and a contact with ED doctor resulted important to share prolonged TAT.

EP276

ROLE OF PLATELET PARAMETERS AND TPO SERUM LEVELS IN THE DIFFERENTIAL DIAGNOSIS OF THROMBOCYTOPENIA

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Background. The differential diagnosis of an isolated thrombocytopenia may be challenging, especially when it comes to differentiate immune thrombocytopenia (ITP) and an isolated thrombocytopenia in the context of bone marrow failure (myelodysplastic syndromes (MDS), acute myeloid leukemia (LAM), aplastic anemia (AA), post-chemotherapy (post-CHT)).Aim. The aim of this study was to compare platelet indices and TPO serum levels in patients with thrombocytopenia of different etiologies: ITP, LAM/MDS, AA/post-CHT.Methods. Blood samples of patients with thrombocytopenia (platelet count < 100x109/L) were collected. Immature platelet fraction (IPF), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet large cell ratio (P-LCR) were obtained using Sysmex XN-1000 hemocytometer (Sysmex Corporation). TPO serum levels were measured using a quantitative sandwich ELISA (R&D Systems). Microsoft Excel software was used for statistical analysis. A p-value of less than 0.05 was considered statistically significant.Results. We evaluated 50 ITP patients, 22 LAM/MDS, 12 AA/post-CHT. Mean platelet count was not statistically different in the three groups. While TPO serum levels were available for all patients, platelet indices were not determinable, due to technical issues, in 10 patients for IPF and 36 patients for MPV, PDW, PCT, P-LCR. Patients with AA/post-CHT had higher TPO serum levels if compared with ITP and LAM/MDS groups (1415.6pg/mL vs 97.4 pg/mL vs 219.3 pg/mL, p<0.001). Compared with AA/post-CHT, ITP patients showed higher IPF (p=0.021), MPV (p=0.041), PDW (p=0.017) and P-LCR (p=0.0071).Compared with LAM/MDS, ITP patients showed higher IPF (p=0.0045), MPV (p=0.033), PDW (p=0.00185) and P-LCR (p=0.013), lower TPO (p<0.001).Multivariate analysis confirmed that IPF and TPO can be used to discriminate between the two different etiologies (IPF: p-value = 0.0185, c.i 0.8647-0.9868; TPO: p-value = 0.004, c.i 1.002-1.011). The other platelet indices were not included into this analysis due to the lack of a consistent number of data.Conclusions. Platelet indices and TPO serum levels may be useful and not invasive tools that can help in the differential diagnosis of an isolated thrombocytopenia.

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