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Riassunti Sessioni Scientifiche

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Legenda:

SP	Sessione Plenaria
SS	Sessione Parallela
CO	Comunicazione Orale
CC	Casi Clinici
CJ	Sessione Young

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

SS01 - La necessità delle competenze statistiche e informatiche in Medicina di Laboratorio

SS01-01

THE ROLE OF STATISTICS IN LABORATORY MEDICINE

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Statistics plays a crucial role in many areas of Laboratory Medicine. The knowledge and the correct use of the statistical methods allow us to deal with data variability, to organize and summarize information, to make inference and communicate meaningful experimental results.
In the laboratory, specific statistical methods are applied for the calculation of biological variability and the definition of analytical performance specifications, verification of analytical and diagnostic performances, analytical method validation, evaluation of commutability of control materials, method comparison, instrument alignment verification and the calculation of reference intervals by direct or indirect methods.
Furthermore, experimental protocols have been designed to evaluate the impact of pre-analytical and analytical variables on laboratory results.
Continuous quality monitoring, by quality control charts and measures based on patient results, is crucial to rapidly identify errors or instrumental drift, hence avoiding clinical risks for patients and minimizing waste of economic resources.
The crisis of reproducibility, reported in many areas of scientific research, requires considerable statistical skills: laboratorians should not only deepen their statistical knowledge (methods, software, interpretation), but also being able to document, and hence potentially reproduce, each step of the experimental study, from design, to data collection, data treatment (data cleaning, coding, missing data), analysis, interpretation and presentation. In the era of Big Data and Machine Learning, these skills are even more important in the field of Laboratory Medicine, where massive amounts of data are produced each day.
Statistics should not be considered as a magical tool but as a necessary competence for the Laboratory Professionals: it allows the researchers to analyze and quantitatively describe the world, making them better and proficient professionals.

SS01-02

INFORMATICS AND THE CLINICAL LAB: PRESENT AND FUTURE

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The clinical laboratory is at the forefront of patient care, and clinicians rely on the laboratory to obtain vital information to support the clinical decision.
The laboratory's current success depends on its ability to deliver rapid and quality results to an increasing number of patients. Information technology "Art and science of transforming data into useful information"(1) is one of the key pillars of the clinical laboratory as it makes a significant contribution to the implementation of laboratory modernization processes and the introduction of new diagnostic technologies (molecular medicine, genomics) allowing the best use of resources, but it is still a critical component today. Information systems, historically born for the efficient management of large volumes of data, have to face new organizational challenges that require a redesign of their architecture. The increase in workloads, the production of rapid and accurate results and the integration with electronic medical records require new IT tools capable of satisfying increasingly autonomous and interconnected patients (homo digitus or e-patients) (2) also providing a different communication model.
Although great strides have been made, traditional information systems, such as laboratory information systems (LIS) essential for managing the flow of information between healthcare professionals, patients and laboratories and designed for the optimization of laboratory operations, have capabilities significantly lower than the ones of current hardware and software technologies. On the other hand, the complexity of the information produced by clinical laboratories has increased over time with a rapid expansion due to the use of new technologies and therefore it is necessary to rethink Information Technology and digital tools not only as methods of investigation or an essential component of instrumental techniques but also, or above all, as tools for the processing, integration and synthesis of data obtained with different methods and techniques.
Over the years, following the consolidation of the diagnostics sector and the introduction of complex analytical platforms, companies have begun to offer complete IT platforms as innovative and secure solutions capable of improving laboratory operations. (3)
Clinical middleware(4) and cloud-based systems are two rapidly growing segments in this field. Each independently, helps to improve data usage and interoperability, together they are expanding the middleware used to date. An appropriate use of such complex technological systems requires the development of personnel skills.
So, the laboratories will need qualified personnel to support the entire range of information systems and their use. Therefore, the computer literacy of all staff is necessary as the correct use of computer systems is now an integral part of the analytical activity.

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4 Healthcare Middleware Market by Type: Communication (RPC, Message Oriented Middleware), Platform (Web, Portal, Database), Integration (ESB, BPM, BAM), Application (Clinical), Deployments Model (On-premise, Cloud), End User: Global Forecast to 2023. Northbrook, Ill

SS01-03

EVALUATION OF THE COMPARABILITY OF TEST RESULTS: PITFALLS AND CAVEATS FOR GUARANTEEING DATA RELIABILITY

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The current organization of clinical laboratories is focused on the consolidation of analysis and on providing reliable results in the shortest possible time, close to the place of patients care. In this scenario, multiple examination procedures (EP)s may be used in different settings and locations within the same healthcare system. Further, to avoid delay of results due to instruments failure, more than one EP for the determination of the same measurand can be implemented within the same lab. In this context, the level of results comparability of the same measurand determined on several EPs, performed on both identical and different methodology/technology, is a fundamental aspect to be evaluated and monitored in the quality assurance procedures, in order to ensure harmonization and interchangeability of results, in space and time, and avoid misinterpretations in the clinical-decision-making process. The importance of this verification is further endorsed by ISO 15189:2012 for laboratory accreditation. Despite several papers are available on literature for evaluating test results comparability, only a few guidelines (e.g. Clinical and Laboratory Standards Institute EP31-A-IR), have been developed for dealing with this problem with a structured approach. Moreover, a pragmatic approach has to be evaluated due to the cost restraints and lack of time available to laboratory professionals to perform this activity.
In a recently published paper, a pragmatic protocol was proposed for evaluating the comparability of test results, with the aim of overcoming laboratory issues arising in medical laboratories during this process (1). This proposal provides a step-by-step guidance on how to assess results comparability in different scenarios. Up to four experimental designs are presented to meet laboratories' needs. The four designs utilize replicates of samples, pools, or internal quality controls material at two or more levels for determining instrumental bias. The first design deal with comparability assessment using replicates of single samples (or pools), while quality control data are considered in the second design. It is suggested to test at least two or three concentration levels. In the third design, a series of samples with levels ranging within ±25% of two or three concentrations defined by the laboratory are used. Differently, in the fourth design, patients' specimens, collected in a wide range of values, are used. In all designs, the bias between EPs are estimated and evaluated. The definition of acceptability criteria for bias are based on the criteria defined at the 1st EFLM Strategic Conference held in Milan on November 24–25, 2014. Samples selection, statistical analyses and sample size calculations, and results reporting are included in the protocol.
In conclusion, the assessment of test results comparability is of utmost importance for medical laboratories. However, some pitfalls and caveats in this process exist and therefore, the experimental design should be carefully planned to reduce time and costs and achieve reliable results.

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SS02 - Big Data ed intelligenza artificiale: quali prospettive per la Medicina di Laboratorio?

SS02-01

WHAT IS MEANT BY BIG DATA IN LABORATORY MEDICINE?

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Among the so-called "disruptive technologies", Big Data (BD) and Artificial Intelligence (AI) are those with the fastest growth and the main impact on the healthcare ecosystem (1). This mostly because of the increasing computing capacity, the development of complex algorithms and the increasing availability of digital data (2). Clinical laboratories of course represent the largest producers of Big Health Data and are consequently involved in this rapid transformation process.
Using AI, it is possible to extract clinically relevant information hidden in huge amounts of data, and just to give some example AI allow to find patterns, to analyze cell images, to discover inefficiencies, to predict future results based on historical trends.
However, despite the growing body of literature, few examples exist of AI implemented into routine clinical practice. The definition of AI as "*the ability of a machine to perform tasks and actions typical of human intelligence*", doesn't mean that this is a simple solution. On the contrary, it is a very complex process, called Data Mining (3) that, even with respect to classical statistical methodologies, is continuously developing and requires greater effort to be consolidated in clinical laboratories.
In this process, after data collection, the first step is Data Cleaning, which is essential to guarantee the cleanliness and correctness of the data.
The other step, Data Analysis, is based on Machine learning (ML) or Deep Learning, an advanced ML technique that uses artificial neural networks models inspired by the functioning of animal brain neurons. The characteristics of ML are that it *"can learn from experience without being explicitly programmed*" and that it is based on learning algorithms classified as supervised or unsupervised depending on the availability of coded reference systems.
On the basis of the clinical problem to be solved, different algorithms can be used but only at the end of a training, verification and validation process, it will be possible to identify the one that will present the best performances in terms of Bias-Variance trade-off. Therefore the control

and verification of outcomes obtained with data mining becomes a fundamental aspect in which laboratory professionals play a crucial role.
Moreover there are still several obstacles to overcome, including poor data quality and harmonization, fragmentation of sources and the issue of data accessibility as it is necessary to manage data guaranteeing patient privacy.
The complexity of the AI approach in laboratory medicine is therefore evident, even considering that the methodologies described here are not part of the training background of laboratory professionals. However the knowledge about BD and AI must not appear like a black box for laboratory professionals but, in order to enhance their role in this matter, it becomes all the more necessary to integrate medical/biological skills with those of data scientists (4). Only if we manage to achieve this goal we will be able to fully exploit the potential of AI applied to laboratory medicine, making the fear and insecurity linked to the inevitable change that awaits us also disappear

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SS02-02

THE VALUE OF DIAGNOSTIC LABORATORY INFORMATION IN HEALTH POLICIES FOR CHRONIC KIDNEY DISEASE PREVENTION

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Kidney disease (KD) is a public health burden with both acute kidney injury (AKI) and chronic kidney disease (CKD) having substantial impact on mortality and morbidity and thus on global health expenditure. Recent data show that 1 in 3 people is at risk of CKD and there is an yearly 6% increase of patients starting dialysis (stage 5) accounting for a total cost of € 1.98 billion per year (conservative estimate). Two tests according to KDIGO 2012 guidelines, estimated Glomerular Filtration Rate-eGFR (CKD-EPI equation) and Albuminuria (urine Albumin to Creatinine Ratio; ACR) allow the staging of CKD into 5 categories. It has been demonstrated that an early diagnosis of stages 3A and 3B, and hence a closer follow-up and ad hoc treatments, can slow the progression to dialysis of five years. Our project, “Ulysses”, uses an instrument of Business Intelligence aiming at identifying early asymptomatic patients affected by CKD and thus to prevent or slow the progression towards end-stage renal disease (ESRD). The algorithm, by using laboratory parameters, has the final aim to compute a risk score and generate an early warning. Based upon the personalized risk stratification, nephrologists are able to identify patients who are at high risk of CKD, and thereby implement personalised care and follow up. This automated early diagnosis allows to elude the delayed nephrology intervention typical in asymptomatic patients which are the large majority. In addition, the model allows a comprehensive epidemiologic mapping to find clusters in different geographical areas. Our preliminary study was conducted on 170,000 subjects using the following parameters: eGFR, proteinuria/24h, albuminuria/24, ACR, PCR, and glycated haemoglobin. Algorithm accurately identified at risk patients generating an early warning for clinicians. An incidence rate of 7% was found for CKD in our cohort of subjects. Our study suggests that the use of algorithms on Big Data not only could improve the clinical laboratory information and the identification of large-scale patients with kidney disease at early stage, but also could drive the health policy in terms of prevention.

SS02-CO01

DEVELOPMENT, EVALUATION, AND VALIDATION OF MACHINE LEARNING MODELS FOR COVID-19 DETECTION BASED ON COMPLETE BLOOD COUNT TEST FROM 1,624 PATIENTS

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The amplification of viral RNA by the reverse transcription polymerase chain reaction (rRT-PCR) test is the current gold standard for the confirmation of an infection from coronavirus (COVID-19), the worldwide pandemic that, in the first eight months post-outbreak, has caused almost 730.000 deaths. However, this test presents known limits, such as a long turnaround time, false-negative rates as high as 15–20%, expensive equipment, and a lack of trained personnel. Thus, there is a need for alternative, faster, cheaper, and more accessible tests.
We developed a number of machine learning (ML) classification models (Logistic Regression, Naive Bayes, K-Nearest Neighbors, Random Forest and Support Vector Machines) based on the routine complete blood count (CBC) data, from 1,624 patients (52% COVID-19 positive) on admission to the Emergency Department (ED) at the San Raffaele Hospital (OSR) from February 19, to May 31, 2020. This dataset is composed by 21 features including age, gender, suffering from COVID-

specific symptoms at triage, COVID-19 positivity, and CBC data. The ML models were trained using a training set comprising 80% of the cases (after model selection and hyperparameter optimization) and were evaluated on a test set comprising the remaining cases (20% of the cases). An external dataset (from 58 patients admitted at the ED of the Istituto Ortopedico Galeazzi of Milan, March 5 - May 26, 2020) and an internal dataset (data from 54 patients randomly chosen admitted in OSR in 2018), were used for the validation.
All models showed a reported accuracy that is consistently higher than 85%. They achieved good performances in symptomatic patients (with both the sensitivity and specificity at approximately 80%) and performed even better in terms of specificity in asymptomatic patients (100% specificity), although the sensitivity was as low as 50%.
Our study demonstrates that ML can be applied to CBC as both an adjunct and alternative method to rRT-PCR for the fast and cost-effective identification of COVID-19-positive patients. This is especially useful in developing countries, or in countries facing an extraordinary increase in contagions, as they can suffer from shortages of rRT-PCR reagents and specialized laboratories.

SS04 - Marcatori cardiaci nelle patologie extra-cardiache

SS04-01
MARKERS OF CARDIOVASCULAR DISEASE IN METABOLIC DISEASE

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Type 2 diabetes mellitus (T2DM), often associated with obesity, is the most widespread and progressive metabolic condition, affecting approximately 8.5% of the world population. Individuals with type 2 diabetes have a significantly higher risk of developing chronic conditions such as cardiovascular disease (CVD) therefore, it is important to establish strategies to combat diabetes and its associated chronic conditions. The current literature has identified several biomarkers that play a key role in the pathogenesis of CVD, many of which are linked to the increase in oxidative stress characteristic of type 2 diabetes. These biomarkers derive from seven main cellular pathways; NF-κB, Keap1-Nrf2, protein kinase-C, macrophage activation, arachidonic acid mobilization, endothelial dysfunction and advanced glycation end products.
In addition to inflammation and the increase in advanced glycation products, the lipid profile also plays a very important role in the determinism of cardiovascular disease in the diabetic patients.
In this context, the approaches that used advanced mass spectrometry methods proved to be very promising in terms of defining accurate markers.

SS07 - La gestione del rischio: quali strumenti e quali attori

SS07-01
THE ROLE OF HTA IN LABORATORY MEDICINE BETWEEN INNOVATION, EFFICIENCY AND APPROPRIATENESS

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Health Technology Assessment (HTA) is defined as the comprehensive, systematic and multidisciplinary analysis of the medical, social, ethical, and economic implications, both direct and indirect, of development, diffusion, and use of health technology.
More specifically, in the field of diagnostics technologies it relates to the study of:
- performance: the sensitivity and specificity of diagnostic tests; compliance with manufacturing specifications; reliability; ease of use and maintenance;
- clinical safety: the judgment on the acceptability of a risk associated with the use of a technology in a specific situation;
- efficacy: the benefit granted by the use of a technology in ideal conditions (clinical efficacy) and in general routine conditions (effectiveness);
- cost-effectiveness: on a micro-economic level, it concerns costs, tariffs and reimbursement methods; at a macroeconomic level, however, it refers to the consequences that new technologies may have on healthcare costs at a national level or on the allocation of resources between different health programs or between different health sectors;
- social, legal, ethical, political impacts.

HTA methodology has been adopted to evaluate the most suitable technologies and organizational solutions to implement a highly complex, automate and performant system, called "Corelab", at the Laboratory Medicine of the Bambino Gesù Pediatric Hospital, in order to always guarantee high standards of safety, efficacy and efficiency, with the aim of continuously improving the quality of the services provided.
Decision-oriented HTA (Do-HTA) method, which involves the integration of the EUnetHTA CoreModel and the Analytic Hierarchy Process, was applied to assess the best technological and organizational solution. It is an analytical instrument for the identification of the main evaluation criteria leading to the attribution of their performances.
28 professionals from different fields have been involved in the study to develop:
- Context analysis and clinical-technological needs elicitation
- Definition of the evaluation scheme according to Do-HTA method, taking into consideration the dimensions identified for the evaluation of the health technology under assessment: Safety, Clinical Effectiveness, Economic Aspects, Technical Characteristics, Organizational Aspects. Such scheme consisted in a

decision tree made up of the aforementioned 5 domains, 17 first level KPIs and 30 second level KPIs
- Definition of the weight system
- Evaluation of the solutions proposed by manufacturers, by computing the performance of each proposed solution according to the Do-HTA method.

The comparison between the two technological and organizational solutions proposed by the participating companies, highlighted a substantial qualitative equivalence, confirming the high technological level of both proposals.
The adoption of the HTA theory and in particular of the DoHTA methodology, has made it possible to evaluate in depth and in detail the various solutions proposed, allowing to effectively and consciously support the Departments responsible for the assessment and the decision. It also allowed the sharing of the evaluation process with all the professionals involved, in a truly multidisciplinary perspective, resulting in a global and shared evaluation of the value of the technology under scrutiny, in a structured and transparent way.

SS07-02
CLINICAL DECISION SUPPORT SYSTEMS AND CLINICAL RISK MANAGEMENT IN LABORATORY MEDICINE

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The "brain-to-brain-loop" seminal concept, originally introduced by Lundberg in 1981 is now considered as a continuum from step one (physician's order) through various steps (ordering, collection, identification, transportation, preparation, analysis, reporting) to the final step, ensuring that appropriate information is adequately defined and interpreted by clinicians. Quality in laboratory testing must be guaranteed in all steps of this total testing process (TTP), from pre-pre- analytical phase to post-post- analytical phase. The integration of artificial intelligence into the health care system is creating new potential in the clinical risk management, to improve patient safety outcomes and the quality of care. Clinical Decision Support System (CDS) is a computer-based information system used to integrate clinical and patient information and provide support for decision - making in patient care. Up to now, studies have primarily focused on the AI performance at the clinical level, such as for disease diagnosis and management or clinical risk and medication errors assessment. Even if the use of this approach in laboratory medicine has usually been applied in test selection (request appropriateness) and result interpretation as crucial steps of the whole diagnostic process, CDS could be applied in each phase of the TTP combining a variety of tools, IT and web based. According to the Agency of Healthcare Research and Quality the improvement of patients' outcome using CDS should be achieved by CDS Five right approach (right information, person, CDS format, channel, and workflow).
Several applications in clinical laboratory could be already identified, from management of quality indicators to notification of critical results, through information and interpretative comments in laboratory reports. The challenge is to consider all these examples as elements of the puzzle to create patient-centered diagnostic pathways with laboratory as a key player.
The more evolution of digital and communication technologies increases, the more the variety of possible application will grow, thus maximizing patient safety.
CDS is going to be an invaluable tool in spreading and supporting evidence-based knowledge into clinical practice.

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SS08 - Anemie: dalla tecnologia al processo decisionale clinico

SS08-01
ROLE OF TECHNOLOGY IN DIAGNOSIS AND MONITORING OF ANEMIA

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The cell blood counting is one of the most requested laboratory tests since it can provide relevant information about different clinical conditions. As a result of the evolution and improvement of technology, the latest generation of hematology analyzers are able to provide new parameters, useful in the diagnosis and monitoring of anaemia.
In addition to the traditional parameters of the CBC count, depending on the technology used, the nucleated red blood cell count (NRBC), the RBC distribution width (RDW), the fragment RBC count (FRBC), the immature reticulocyte fraction (IRF), the mean reticulocyte hemoglobin content (CHr or Ret-He), the percentage of hypochromic (HYPO-He) and hyperchromic (HYPER-He) red blood cells, may be available.
Since various and severe pathological conditions, such as inefficient erythropoiesis (thalassemic and myelodiplastic syndromes) or primary abnormalities of hematopoiesis (leukemia) are associated with NRBCs presence in peripheral blood, identification and correct count of these is mandatory for its clinical relevance.
The erythrocyte anisocytosis index (RDW) has proved useful in the morphological anemias classification (1); despite there can be a wide distribution of RDW values within a single disease that can affect its usefulness

during a differential diagnosis, however its importance as a general marker of abnormality has been maintained (2). Schistocytes are circulating FRBCs formed as a consequence of mechanical damage. They can be found in a patient's peripheral blood in the occurrence of various diseases. Among which, schistocytes in microangiopathies need immediate diagnosis and treatment, thus the identification and quantification of them represents a crucial diagnostic criterion (3).
The IRF parameter is an early and sensitive index of marrow erythropoietic activity. It is useful in distinguishing between anemias characterized by increased marrow erythropoiesis (such as in acquired hemolytic anemias or the loss of blood), from anemias due to reduced marrow activity (chronic renal disease), as well as situations such as acute infections and myelodysplastic disorders (4). Other uses include therapy monitoring of nutritional anemias (B12, folates, and iron) since the increase in IRF precedes the increase in total reticulocyte count by several days.
The CHr, which directly reflects the hemoglobin synthesis in marrow precursors, gives a measure of the iron availability adequacy. Its reduction indicates iron-deficient erythropoiesis, even in conditions in which traditional biochemical markers result inadequate (for istance, during inflammation or chronic disease anemia). It is also useful for monitoring early response to intravenous iron therapy (4).
The percentage of hypochromic RBC is a very sensitive parameter in detecting functional iron deficiency, such as in patients with chronic renal failure. Hyperchromic RBCs are of diagnostic utility in hereditary spherocytosis and indicators of severity of this disease. These parameters are also used in differential diagnosis of microcytic anemia, for thalassemia screening and in distinguishing iron deficiency anemia from anemia of chronic disease(5).

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SS08-02

DIAGNOSIS OF HEMOGLOBINOPATHIES IN THE BASIC LABORATORY

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Hemoglobinopathies represent a large group of quantitative and / or qualitative genetic disorders (more than a thousand to date) (1), caused by mutations in the genes responsible for the synthesis of globin chains. They can be classified, based on the genes involved and the type of defect, in thalassemias (α, β, δ / β) and hemoglobin variants (α, β, δ) (2).
Hemoglobinopathies represent an increasingly emerging problem for global health policy, with 7% of the world population carrying a hemoglobin disorder and 300,000-500,000 children born each year with a severe homozygous form of these diseases (83% sickle cell disease, 17% thalassemias) (3). These diseases are increasingly diagnosed also in Italy, where, in addition to the cases of hemoglobinopathy already present in the national territory, there are also numerous cases due to massive migratory flows, international adoptions and the presence of multi-ethnic couples (4). In fact, with immigration, the frequency of several hemoglobin defects already present in the Italian population, has increased, such as Hb S, Hb C, Hb D Punjab (D Los Angeles), Hb E and Hb O Arabia as well as several common and novel beta thalassemia defects.
Laboratory Medicine plays a central role in the prevention and diagnosis of thalassemias and hemoglobin variants. In particular, its role is indispensable in family screening, in couples of childbearing age and is irreplaceable at birth, because the newborn, even if a carrier of hemoglobin defects, in most cases does not show any evident clinical sign. The early diagnosis in the affected newborn in many cases allows to foresee risks, establish adequate prophylaxis and prevent complications.
Laboratory diagnostics of hemoglobinopathies is based on the careful interpretation of basic tests, primarily the blood count cell and in particular: MCV and MCH parameters, for the assessment of the presence of microcytosis and hypochromia, typically present in thalassemia carriers; the RDW parameter for the evaluation of anisocytosis, characteristic of hereditary anemias; the reticulocytes count for the differential diagnosis with acquired anemias. Equally important is the evaluation of the morphological characteristics of the erythrocytes on optical microscope on peripheral blood smear.
For a differential diagnosis of hemoglobinopathies it is essential to analyze, together with the blood count cell, also the martial profile (sideremia and ferritinemia) and the hemoglobin pattern, which allows: (i) to quantify the hemoglobin HbA2, essential for evaluating the presence of a ✓ thalassemia trait (but not only); (ii) to identify the main hemoglobin variants.
It should be emphasized that the evaluation of the hemoglobin structure is to be considered equivalent to a genetic test in the identification of hemoglobin variants, even if genetic analysis is still necessary in order to characterize the variant from a molecular point of view (5).

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SS08-CO05

WRONG BLOOD IN TUBE: A SIBIOC PROJECT FOR A PERSISTENT PROBLEM

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Background. The identification error (IE) rate still ranges between 0.04–1% also generating adverse events. Although delta-check was largely used to prevent IE, criteria to define it are still not harmonized and outcome not assessed. Moreover multi-analytes delta-check seems to be more effective.In this context, “Haematology” and “Clinical Risk” SIBioC working groups launched a project aiming to develop a multi-cell blood count (CBC) parameters delta-check (MDC) to identify as many IE as possible. Aim of this work is to describe the project and preliminary results. Methods. The project consists of four phases: I) collection of CBC results (January-June 2019) from 15 Italian laboratories, different for organization, volume, geographical area and haematological analysers to create an original dataset (OD) containing an appropriate number of consecutive results for the same patient; II) pilot study on a smaller dataset (SD) to: a) create an artificial mix-up dataset (MD) by casual resampling of the SD containing IE and b) identify the best statistical model to find a MDC; III) identification of the most accurate MDC on OD; IV) testing the MDC in the involved labs and verification of its effectiveness in the IE detection. Results. The SD included 2367 pair of consecutive results for the same patient (age of patients: 0-100 years; the most of repetitions were by 5 days). The casual resampling of SD allowed creating a MD with 2000 pair of patient-mixed consecutive results. Variability of the pair of consecutive results was wide in MD and low in SD for about all parameters (except for MCHC and WBC), and it also depends on the considered unit of measure. When one of the most frequent used delta-check alert (ΔMCV=7fL) was applied to detect IE in MD, the method accuracy was low (AUC=0.542). On the contrary, testing of a multivariate model, obtained by a stepwise logistic analysis, allowed to obtain a more accurate MDC in IE detection (AUC=0.931, sensitivity=91,6%, specificity 4%) Conclusions. MDC may offer a practical strategy to identify IE prior to test reporting, improving patient safety. However a good planning of project workflow, selection of methodology, computer tools and competence of professional involved are key elements to reach the objectives.

SS09 - Sessione Young Scientists: Media e Medicina di Laboratorio: un’alleanza possibile?

SS09-CO06

MOLECULAR CHARACTERIZATION OF TUMOR-DERIVED EXOSOMES: A WINDOW OF OPPORTUNITY FOR MINIMAL RESIDUAL DISEASE MANAGEMENT IN HEMATOLOGICAL MALIGNANCIES

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B cell lymphoproliferative diseases, including Chronic Lymphocytic Leukaemia (CLL), are often characterized by varying patterns of clinical behavior and treatment responses. The advancement in the discovery of new treatments have led to improved progression-free survival and overall survival, but complete response rates remain disappointingly low and often characterized by undetectable MRD (uMRD). In recent years, liquid biopsy tests have gained attention as a valuable alternative to traditional cancer diagnostics. Indeed, despite the standard techniques, such as PET/TC and tissue biopsy, represent the gold standard in cancer diagnosis, the analysis of tumor circulome is a promising tool for early detection and follow up of cancer patients. To this end, in terms of disease staging and treatment regimen design, there is an urgent need to develop novel diagnostic tools which allow for a non-invasive approach to early detection of disease and for individualized disease state monitoring. Representing a molecular footprint of the cell of origin, exosomes have been recently taken into consideration in order to design a reliable liquid biopsy for non-invasive monitoring of tumor evolution and recurrence, and useful to evaluate the therapy response. We successfully validated the screening of random peptide libraries as a method to identify peptide binders

for the idiotypic determinants of the immunoglobulin B Cell Receptor (IgBCRs). Recently, we demonstrated that tumor B cell-derived exosomes express the IgBCR of their parental B-cells, thus constituting a personal "barcode" of tumor clones which can be subsequently targeted by so-called “Idpeptides” (Iaccino E. et al. Molecular Cancer 2017). Moreover, in these studies the analysis of tumor-derived exosomes (TDEs) allowed an earlier detection of tumor growth compared to the conventional measure of serum paraprotein. In translating this research to the clinic, our work describes the design of an innovative technological platform allowing for a comprehensive TDEs characterization. The identified Id-peptides has been used to develop a multicolor flow cytometric protocol for TDEs detection allowing a multiplexed approach in the isolation and enrichment of TDEs subpopulations, aimed at the molecular analysis of TDEs contains.

SP01 - Gestione del’emergenza da COVID-19

SP01-01
BIOLOGY AND CLINICS OF SARS-COV-2 INFECTION

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Coronavirus disease 2019 (COVID-19) can be considered the third coronavirus outbreak during the past 20 years, after severe acute respiratory syndrome in 2002-2003 and Middle East respiratory syndrome in 2012 [1]. The two former outbreaks caused altogether less than 2000 deaths, while COVID-19 has already affected several millions of people worldwide, causing hundreds of thousands deaths. The International Committee on Taxonomy of Viruses has endorsed that COVID-19 is caused a beta coronavirus, belonging to the same family of viruses causing common cold, and defined severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This is an enveloped virus with positive-sense, single-stranded RNA genome, encoding four leading structural proteins, i.e., Spike (containing the receptor-binding domain through which the virus binds to its natural receptor at the surface of host cells), envelope protein, membrane protein and nucleocapsid protein. The genome of SARS-CoV-2 contains additional genes, encoding accessory proteins including RNA-dependent RNA polymerase. SARS-CoV-2 spread from person to person through small droplets from nose or mouth, especially when an infected person coughs or sneezes. Contagion may also be possible also during aerosol-generating procedures or transporting infected material from surfaces to eyes, nose and mouth. The cell receptor is angiotensin converting enzyme 2, expressed at the surface of many human cells (respiratory epithelia, pneumocytes, cells of heart, kidney, gastrointestinal system, adipose tissue and testis). Binding of ACE2 and SARS-CoV-2 involves the spike protein, which needs to be “primed” by another protein at cell surface, such as Transmembrane Serine Protease 2 or others (e.g., furin). The clinical picture of COVID-19 is dominated by cough, fever and dyspnoea, reflecting interstitial pneumonia. Less frequently reported are gastrointestinal symptoms, while upper respiratory symptoms (sore throat, rhinorrhoea, chill and conjunctival congestion) seem infrequent. Myalgia, unusual headache, taste and olfactory disturbances are also described. COVID-19 is a gradually evolving pathology, characterized by five stages, sustained by different molecular and biological mechanisms. While remaining asymptomatic or mildly symptomatic in many subjects (up to 70%), in others the illness progresses towards a respiratory phase with interstitial pneumonia. An abnormal, almost exaggerated, immune response in some of patients justifies the progression towards a third phase, with lung and systemic hyper-inflammation and injury. In a percentage variable between 2-5% of patients, the disease progresses into a fourth critical phase, where hyper-inflammation triggers hemostasis activation, leading to intravascular coagulopathies such as pulmonary thrombosis, venous thromboembolism or disseminated intravascular coagulation. Patient treatment must hence be tailored according to stage or severity of disease. Cumulative worldwide mortality so far is 4-6%.

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SP01-02
SEROLOGICAL TESTING TO MANAGE COVID-19

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The natural history of serological assays for SARS-CoV2 antibodies is comparable to that of other biomarkers: after an initial and excessive emphasis on the diagnostic role of serological assays, a series of papers raised serious concerns on their clinical usefulness, finally leading to a more rational and evidence-based identification of the main areas of clinical use, linked to a better methodological validation. The multifaced rationale for serological testing in COVID-19 management should be summarized as follows: a) to improve knowledge on immunoresponse to SARS-CoV-2; b) to define and monitor the extent of virus spread; c) to screen particular populations and sub-populations at higher risk (e.g. healthcare workers) and define disease prevalence; d) to characterize efficacy of containment measures at local and global level; e) to screen convalescent sera for both therapeutic and prophylactic use; f) to combine the results of molecular tests (rRT-PCR) for a more accurate diagnosis (in “difficult patients”) ; g) to allow the diagnosis for later stages of infection (when the virus has been eliminated); and finally h) to identify individuals who have been infected but suffered only minor suymptoms (or asymptomatics) and did not seek medical attention. From a methodological view point, the main issues are represented by the type of test (laboratory-based, point-of-care and/or neutralizing activity); the target (spike

protein and/or S1/S2 subunits, nucleocapside, receptor binding protein, native antigen etc), and the immunoglobulin class or classes (IgA, IgG, IgM) recognized by the assay. Testing strategies aim to maximize specificity and thus positive predictive value (PPV), particularly as the overall prevalence is likely low; they should be summarized as: 1) Choosing a test with a very high specificity (95% or greater); 2) Focus testing with a high pre-test probability (e.g. history of COVID-19 illness) and 3) Employ an orthogonal testing algorithm (positive persons tested with a second test). The standardization/harmonization initiatives to improve the clinical comparability of results obtained by different assays are currently based on the optimization of the diagnostic thresholds (cut-offs), and the definition of the predictive positive and negative values (PPV/NPV) in relation to a different disease prevalence. In fact, epidemiological surveys have highlighted significant variations in seroprevalence ranging from 2-3% to 40-50% in different regional or national areas. The knowledge of antibody kinetics represents an essential issue to define the right timing for identifying the immune response and to better understand the duration of protection. This, in turn, plays a relevant role in evaluating the answer to therapies and vaccines, even if current research on the immunoresponse due to B and T lymphocytes may provide useful information for better understanding the host response to the “misterious” SARS-CoV- 2 virus.

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SP01-CO02

RUOLO DELLE PROTEINE DELLA FASE ACUTA NEL PREDIRE LA POSITIVITÀ PER SARS-COV-2

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La continua diffusione della pandemia da severe acute respiratory coronavirus 2 (SARS-CoV-2), responsabile della malattia da coronavirus 2019 (COVID-19), ha avuto un impatto devastante sui servizi sanitari e sui laboratori in Italia e in altre nazioni. Il volume di test fronteggiare i focolai può essere così ampio da richiedere l’utilizzo di strategie basate sul calcolo della probabilità pre-test, onde aumentarne l’efficienza. Poiché uno dei paradigmi di COVID-19 è un’infiammazione anomala ed eccessiva, caratterizzata dall’aumento in circolo delle proteine della fase acuta, abbiamo voluto valutare se la determinazione di alcune di esse all’accesso del paziente al pronto soccorso (PS) possa predire la positività del test molecolare.La popolazione è consistita in una serie di pazienti ammessi al PS dell’Azienda Ospedaliera Universitaria Integrata di Verona, con sospetto clinico di infezione da SARS-CoV-2. A tutti è stato prelevato un campione di sangue all’accesso, e eseguito un tampone oro- e naso-faringeo per ricerca di SARS-CoV-2 (Seegene AllplexTM2019-
nCoV Assay; Seegene, Seoul, South Korea). Nei campioni di siero è stata eseguita la determinazione di proteina C reattiva (PCR), procalcitonina (PCT) e interleuchina 6 (IL-6) su strumento MAGLUMI 800 (SNIBE, Shenzhen, China).La popolazione finale oggetto dello studio ha compreso 92 pazienti, 48 con tampone negativo (54±20 anni; 58% donne) e 44 con tampone positivo per SARS-CoV-2 (57±23 anni; 50% donne). La concentrazione di nessuna delle differenti proteine della fase acuta è apparsa significativamente differente tra le due popolazioni di pazienti con tampone positivo o negativo. In particolare, PCR: 20 mg/L (IQR, 3-75 mg/L) vs. 17 mg/L (IQR, 5-66 mg/L; p=0.198); PCT: 0.06 ng/mL (IQR, 0.01-0.11 ng/mL) vs. 0.05 ng/mL (IQR, 0.01-0.11 ng/mL; p=0.279); IL-6: 27 pg/mL (IQR, 12-65 pg/mL) vs. 24 pg/mL (IQR, 12-59 pg/mL; p=0.146). L’analisi dell’area sotto la curva (AUC) ha confermato l’inefficienza dei tre biomarcatori per predire la positività al tampone; PCR: 0.52 (95%CI, 0.40-0.64); PCT: 0.53 (95% CI, 0.41-0.65); IL-6: 0.51 (95%CI, 0.39-0.63). In conclusione, nei pazienti ammessi al PS con sospetto diagnostico di COVID-19, la determinazione di marcatori di flogosi non sembra

SP02 - Medicina di Laboratorio: la creazione del valore per la salute del cittadino

SP02-01

LABORATORY MEDICINE: CREATE VALUE FOR COMMUNITY HEALTH

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Clinical laboratories are at a cross-roads. The focus on automation, distorted economics which subordinated medical objectives to economic ones turning the laboratory into a mega-factory, has resulted in the devaluation of laboratory medicine which is considered a support service rather than a true clinical department. However, in addition to a series of papers and visionary opinions, the SARS-CoV-2 pandemia has highlighted the central role of clinical laboratories as a vital part of the continuum of patient care and disease prevention. In fact, in the case of COVID-19, as in many other diseases and medical conditions, the diagnosis is performed by a laboratory test (rRT-PCR), many biomarkers and "conventional" laboratory tests play a relevant role in disease monitoring and prognostication, and serological assays are used for epidemiological surveillance. Clinicians, patients, citizens and politicians have finally realized the importance of laboratory medicine and the need for implementing a more value-based patient centric model for delivering laboratory services,
Moving from the traditional to a value-based model of organizing medical laboratory practices requires a) a patient centric view; b) integration with other clinical services e.g.imaging); c) optimized test request and utilization; d) focus on all phases of testing cycle, not only on the intra-analytical; e) aggregation of "big data"; f) use of artificial intelligence to improve the value of laboratory information; and g) the promotion of precision and personalized medicine.
To improve outcomes for populations and individual patients requires laboratory professionals to increase their scope of practices influence outside the traditional laboratory model to diagnostic management throughout clinical care pathways, interdisciplinary collaborations to break down traditional practice silos, and outcomes research.
Diagnostic stewardship, teleconsultations, integration in diagnostic management teams, efforts in improving patient safety using appropriate quality indicators are valuable tools in order to create value and improve the visibility of laboratory professionals.

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SP02-CO03

THERAPEUTIC DRUG MONITORING OF B-LACTAM ANTIBIOTICS USING MICROSAMPLING DEVICES AND LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Therapeutic drug monitoring is a routinely laboratory practice to measure serum/plasma levels of various drugs with the aim of preserving an effective and safe trough concentration in patients through dosage adjustement. Microsampling techniques, such as dried blood spot (DBS) and volumetric absorptive microsampling (VAMS), can minimize sample volume needed, especially in newborns where it is recommended not to exceed a 2.5% of total blood volume/draw. Dried blood spot (DBS) sampling is a useful tool for pharmacokinetic studies but introduces additional variability in analyte quantification due to possible blood spot inhomogeneity and variability in haematocrit values. Volumetric absorptive microsampling (VAMS) potentially should overcome some of these issues due to the absorption of a fixed blood volume. Here, we analyzed the effect of pre-analytical variables such as hematocrit, blood spot volume and anticoagulants (K2- and K3 ethylenediaminetetraacetic acid (EDTA) and lithium heparin (LH)) on either a VAMS- or a DBS sampling – liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the simultaneous measurement of amoxicillin (AMX), ampicillin (AMP) and meropenem (MRP). LC–MS/MS was carried out on a Thermo Scientific TSQ Endura triple quadrupole mass spectrometer coupled to Dionex UltiMate 3000 UHPLC system equipped with a Phenomenex Luna Omega C18 column (50 x 2.1 mm; 1.6 µm particle size). After validation following the European guidelines, the effects on peak areas, linearity, precision and accuracy of the different preanalytical variables were investigated. Our results showed, among others, that blood spot volume and high hematocrit levels (>60%) significantly affected quantitative analysis of antibiotics in particular when DBS were used. In addition, our data suggest that TDM of β-lactams from DBS requires a careful choice of the anticoagulant used for calibration standard and quality control preparation that should be maintained constant throughout the study to reduce over- or under-estimation of β-lactam concentrations from real samples.

SP03 - Alterazioni dell'emostasi in corso di infezione da SARS-CoV-2 (COVID-19)

SP03-01

COAGULATION LABORATORY ROLE IN THE DIAGNOSIS AND MONITORING OF COAGULOPATHY DURING COVID-19

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Albeit a good knowledge has been gained on clinical features of COVID-19, less clear information has been provided on laboratory abnormalities and, especially, on the potential derangement of hemostasis tests. During COVID-19 disease, thrombotic complications and coagulopathy frequently occurs; however the characteristics of COVID-19 associated coagulopathy (CAC) are different from those seen in bacterial sepsis

induced coagulopathy (SIC) and in disseminated intravascular coagulation (DIC). Moreover venous and arterial thrombosis are more frequent in CAC compared to SIC and DIC. Laboratory features of CAC overlap somewhat also with antiphospholipid syndrome (APS), hematophagocitic syndrome (HPS), thrombotic microangiopathy and heparin induced thrombocytopenia (HIT). It is suggested that for all patients who are hospitalized for COVID-19, a minimum panel of hemostasis tests must be performed; this includes PT, aPTT, Fibrinogen, D-dimer. and platelet count. These tests are useful for interpreting the patient's hemostatic balance and the evolution of coagulopathy and to suggest an appropriate antithrombotic treatment. Inflammatory reactions and cytokines storms caused by severe infections have pleiotropic repercussions such as activation of the coagulation system through different procoagulant routes; sometimes, it can be imperceptible, but when this activation is very strong, it can be reflected in prolonged PT and APTT and thrombocytopenia due to development of DIC. D-dimer is commonly elevated in patients with COVID-19; D-dimer levels correlate with disease severity and are a reliable poor prognostic marker, especially if the values are higher than 6 times the cut-off (> 3 mg/L). Other parameters of the hemostatic system were studied; among these physiological inhibitors of coagulation (antithrombin, protein C and protein S), procoagulant factors (FII, V, VII, VIII, IX, X, XI and XII), but on these parameters no information useful for clinical monitoring has been obtained. Interesting insights into the pathogenesis of thrombotic complications in COVID-19 were instead provided by some studies in which ADAMTS-13 (A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13) was measured or in which viscoelastometry tests were performed. The coagulation laboratory plays an other important role in monitoring the efficacy and safety of UFH treatment; in the case of LMWHs, these usually do not require monitoring but, in the case of alterations in renal function, it is important to perform the chromogenic dosage of the anti-Xa activity in an optimal manner, particularly checking the timing of the sampling, the pre-analytical variables, calibration and reference ranges.

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SP03-CO04

REPORTING OF D-DIMER AND HARMONIZATION NEEDS: DATA FROM PARTICIPANTS TO THE EQA SCHEME OF THE CENTER OF BIOMEDICAL RESEARCH

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Introduction: D-Dimer assessment represents a cornerstone in the diagnostic approach of several thrombotic disorders. Recent literature has highlighted the role of D-Dimer also in the diagnostic pathway of COVID-19 and the importance of a harmonized reporting [D-dimer unit (DDU) or fibrinogen equivalent unit (FEU); unit of measure; cut-off] in order to guarantee the correct interpretation of the results1.
Purpose and Methods: Evaluation of D-Dimer data from 100 EQA participants and the inter-laboratory variability (CV%) of the last 7 years for the most used analytical systems: HemosIL HS, IL-HS (n=37±4), HemosIL HS-500, IL-HS500 (n=8±1), Sclavo Auto, SCLA (n=12±5), Siemens Innovance, INN (n=14±3), bioMérieux VIDAS, VID (n=10±3), Stago STA Liatest, STA (n=10±3).
Results: Concerning the results expression in DDU or FEU, there is a prevalence of FEU (55.1%) over DDU (44.9%), value confirmed in the last 7 years (average FEU = 55.6%), differently from data obtained in the survey conducted in 2014 at national level2.The units used are: ng/mL (67.7%), µg/L (29.0%) and mg/L (3.2%) for D-Dimer DDU; ng/mL (57.9%), µg/
mL (21.1%), µg/L (15.8%) and mg/L (5.3%) for D-Dimer FEU. Inter-laboratory variability (mean CV%±SD) calculated on a total of 68 control samples (range, µg/L):- D-Dimer, DDU = SCLA: 8.8±3.3 (82-170); 5.4±2.7% (402-1482); IL-HS: 12.8±6.1 (122-166); 6.4±2.7% (480-1705). - D-Dimer, FEU = IL-HS: 13.9±2.0 (192-340); 5.2±2.3 (1016-3158); IL-HS500: 5.1±2.6 (1430-3442); INN: 11.3±5.6 (191-330); 6.3±2.3% (1439-4521); VID: 6.9±3.5 (158-378); 5.4±2.3 (673-2018); STA: 7.1±6.6% (220-385); 5.1±2.0% (860-2600).
Discussion: This study demonstrates that the reporting of D-Dimer results does not comply with the 2014 SIBioC consensus document which recommended the use of µg/L FEU3, and highlights 8 different types of information. The CV% is lower for all diagnostic systems at pathological levels than the ones at concentrations near to the cut-off. Conclusion: Data reported in this study call for the harmonization of D-Dimer reporting in order to guarantee the correct interpretation of information, both of COVID-19 and all diseases already known for which this analyte has a clinical relevance.

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CC001

Il laboratorio di autoimmunità nella diagnosi di miopatia da statine

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Background. Statins are some of the most widely prescribed medications, and though generally well tolerated, can lead to a self-limited myopathy in a minority of patients. Recently, they have been associated with a necrotizing autoimmune myopathy (NAM). Statin-associated NAM is characterized by irritable myopathy on electromyography (EMG) and muscle necrosis with minimal inflammation on muscle biopsy and it is associated with the presence of anti 3-hydroxy-3-methylglutaryl-coenzim A reductase antibody (anti-HMGCR antibody).
Case Report. We describe a case of 51-year-old man who started atorvastatin because of his hypercholesterolemia complaining muscle weakness. Laboratory tests were all normal except for LDH and CK which were very elevated. LDH was 1533 U/L (208-379) and CK was 11500 U/L (10-171). The main autoimmunity test (ANA, ANCA, ASMA, LKM) were negative as the research of major myositis antibodies. Muscle biopsy and EMG showed a myopathic process with active inflammation. Due to these results we decided to perform QUANTA Flash HMGCR test (Werfen®). It is a semi-quantitative chemiluminescence immunoassay performed on the BIO-FLASH instrument for the determination of IgG autoantibodies against HMGCR antigen in human serum. The positive result confirmed the diagnosis of NAM. After this results the patient stopped the therapy with atorvastatin and started steroid therapy with improvement in symptoms and the decrease in CK and LDH.
Discussion. We want to underline the importance of autoimmunity laboratory in the diagnostic framework of this rare autoimmune disease. The detection of anti HMGCR antibodies plays a key role in this diagnosis and their early detection can avoid muscle biopsy (a more invasive exam for the patient). Though statin-associated NAM is a relatively rare entity, it is an important consideration in patients who continue to have CK elevation and weakness during or after discontinuation of statin therapy. Continued research is necessary to better define statin-specific and dose-dependent risk, as well as optimal treatment for this condition.

CC002

Neuromielite ottica in età pediatrica: il laboratorio può essere utile?

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A 6-years-old female was admitted following occasional finding of bilateral papilledema with haemorrhage signs during ophthalmologic consultation for unspecific visual impairment during school time and everyday activities. She was previously healthy and reported no additional symptoms. Neurologic examination only revealed torpid photomotor reflex and slow eye-blink reflex. No other abnormal signs nor alterations in routine laboratory workup were found. Brain CT scan was negative. Magnetic resonance angiography (MRA) showed: tiny subcortical white matter T2-hyperintense lesion, FLAIR-hyperintensity of both optic nerves with mild swelling of left one, swelled cervical spinal cord with 2 hyperintense lesions; no post contrast enhancement. Neuromyelitis optica spectrum disorder (NMOSD) was then suspected and serum and CSF were collected for OCB, AQP4-Ab and MOG-Ab testing. OCB were found in CSF and MOG-Ab positivity was found in both serum and CSF; titre in serum was 1:320 and in CSF 1:40. Visual acuity (VA) at onset (T0) was 1/100 in both eyes.High dose IV methylprednisolone (MP) for 5 consecutive days was then administered; afterwards (T1) serum was again tested for MOG-Ab, resulting in persisting positivity with titre 1:160; VA was 5/10 in right eye (RE) and 2/10 in left eye (LE). No maintenance therapy was established. Follow-up MRI showed tiny white matter lesions, no alterations of optic nerves and persisting cervical lesions. After 3 weeks (T2, almost one month from onset) clinical worsening was noted, with VA 5/10 in RE and 1/20 in LE. MOG-Ab tested positive at titre 1:320. A second cycle of high dose MP was administered for 5 days, followed by oral prednisone. At the end of IV steroids (T3) VA was 5/10 in RE and 1,25/10 in LE and MOG-Ab titre was 1:160. One week later (T4) VA was 6,4/10 in RE and 5/10 in LE; MOG-Ab titre persisted at 1:160. At two-months follow-up (T5) VA was 6,3/10 in RE and 5/10 in LE, and MOG-serostatus remained positive at 1:160. 2g/kg IVIg was then administered for 5 days during steroid tapering. Mycophenolate mofetil was initiated thereafter. At one-year (T6) follow-up VA was 10/10 in both eyes and MOG-Ab negative.The need of MOG-Ab laboratory follow-up is still debated, nevertheless it might be useful in predicting clinical relapse.

CC003
Una trombofilia da indagare accuratamente

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Direct Oral Anticoagulants (DOACs) affect laboratory coagulation tests. Activated Carbon (AC) has been described to selectively remove DOACs interference on coagulation assays without affecting assays results.We present a case concerning a 45 year old woman coming from a spoke hospital to our laboratory for a thrombophilia screening: Lupus Anticoagulant (LA), Protein C, Proteins S, Factor VIII, resistance to Activated Protein C (APCr). We ignore pharmacological therapy and clinical history of the patient.We always perform Thrombin Time and Prothrombin Time (PT) before LA screening tests. The patient showed a Thrombin Time ratio > 11.00 (normal values <1.20) and a PT ratio = 1.45 (normal values <1.21). These findings are compatible with Dabigtran therapy. We perform dilute Thrombin Time in this patient and we find a dTT = 222 ng/ml. Thrombophilia assays requested showed: LA false positivity (SCT screening ratio = 1.34, SCT mix ratio = 1.05, SCT Confirm ratio = 1.17, DRVVT screening ratio = 1.85, DRVVT mix ratio = 1.25, DRVVT confirm ratio = 1.32) normal factor VIII (VIII= 87%) normal Chromogenic Protein C (PC = 122%), normal Free Protein S (FreePS = 101%), high normal Coagulative Protein S (PSC=195%) and normal APCr ratio (APCr ratio = 3.58). In order to resolve DOACs interference on coagulation tests we spike citrated plasma with 20 mg/ml of AC. Sample was than gently mixed for five minutes, than centrifuged at 2000 g for 5 minutes and the supernatant was transferred to a new tube. We measure dTT and TT on AC treated plasma and we found a dTT <10 ng/ml and a TT ratio = 1.02. Than we perform again all coagulative assays and we found: normal LA (SCT screen ratio = 1.02 and DRVVT ratio = 1.08), high abnormal factor VIII levels (VIII = 215%), normal PC (119%), normal Free PS (99%), normal PSC (102%) and reduced normal APCr ratio (APCr ratio = 2.45). As described in the literature AC selectively resolved DOACs interference in all coagulative assays performed in our patient.

CC004
Monitoraggio della risposta al trattamento con plasma iperimmune in un paziente affetto da COVID-19

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Introduzione: SARS-CoV2 è un nuovo Coronavirus in grado di causare una malattia respiratoria acuta (COVID19) con decorso a volte grave, la cui diffusione ha reso necessaria una modalità diagnostica veloce ed accurata quale la RT-PCR. Le offerte terapeutiche per i pazienti sono in corso di studio, il trattamento con plasma iperimmune sembra fornire risultati promettenti. Abbiamo monitorato un'infezione con decorso severo da SARS-CoV2 in RT-PCR di un paziente trattato con plasma iperimmune.
Materiali e Metodi: L'RNA virale è estratto da tampone naso-faringeo raccolto in UTM Copan con MagNA Pure 24 System (ROCHE). La ricerca del virus è realizzata con sonde specifiche per i tratti dei geni E e RdRP dell'RNA di SARS-CoV-2, su piattaforma LightCycler z480 (ROCHE). Per il gene E sono considerati positivi gli amplificati con Cross-point (Cp) <36, per il gene RdRP con Cp <39.
Risultati: La RT-PCR per SARS-CoV-2 in un paziente di 50 anni ospedalizzato con severa insufficienza respiratoria e necessità di ventilazione assistita ha evidenziato un Cp di 27,99 per il gene E, il test ripetuto a 24h un Cp di 32,53 mentre per RdRP di 36,15. In accordo con gli esami strumentali, si è posta diagnosi di infezione COVID19. A 7 giorni la stessa ricerca mostra un Cp per E di 32,01 ed un Cp per RdRP di 31,07, le condizioni cliniche sono peggiorate con trasferimento in Rianimazione e seguente trattamento prima con Idrossiclorochina e Lopinavir-Ritonavir, poi con Tocilizumab. Al giorno 9 il Cp per E è di 30,08 ed il Cp per RdRP è di 31,02 con quadro ancora critico. Al giorno 15 il Cp di E è di 37,5 ed il Cp di RdRP>40. Per il persistere della grave insufficienza respiratoria, si è decisa l'infusione in 3 somministrazioni di plasma iperimmune (giorno 20). Al giorno 23 il Cp di E è di 38,62 ed il Cp di RdRP>40; al giorno 30 il Cp di E è di 37 ed il Cp di RdRP di 38,5. Dal giorno 40 in poi la ricerca del virus è stata sempre "undetectable", contestualmente al graduale recupero delle condizioni cliniche del paziente.
Conclusioni: La RT-PCR ha mostrato un decorso parallelo alla clinica, evidenziando una riduzione rapida dell'andamento dell'infezione virale subito dopo la somministrazione di plasma iperimmune, confermandosi pertanto un valido strumento per il monitoraggio di SARS-CoV2.

CC005

Un soggetto con pancitopenia e febbre persistente

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A 75-years-old female with pancytopenia (WBC:2700/μL, Hb:74gr/L, PLT:63000/μL), for about 10 days has unresponsive fever to antibiotic therapy and persistent cough. She has celiac disease, HCV+ chronic liver disease, COPD, hepatosplenomegaly and lymphadenopathy. Laboratory findings show elevated ferritin (1049 ng/mL) and triglycerides (231 mg/dl) and normal fibrinogen. Hepatorenal, thyroid and autoimmune function, tumor markers are normal; infectious and culture tests are negative. The fever persists, profuse night sweats appear, pancytopenia has a rapid downhill course (WBC:430/μL, Hb:67gr/L, PLT:5000/μL), ferritin (1800ng/mL) and triglycerides (370mg/dL) increase further. Bone marrow aspiration and biopsy are performed. Morphological examination shows megakaryocytic and erythroid hypoplasia and medium-large size pathological cells (38%) with moderately condensed chromatin, sometimes with "hand-mirror" cell morphology and numerous figures of hemophagocytosis. Atypical T lymphocytes CD3+CD5-CD7-CD2++CD8 +CD56- are present on the flow cytometric examination. The hemophagocytic syndrome (HLH) in lymphoproliferative disease is suspected and confirmed by biopsy. Despite high-dose corticosteroids administration there is a clinical worsening with progressive multi-organ involvement until the death. HLH is a rare and life-threatening hematologic disease caused by excessive activation of immune system resulting in a systemic hyperinflammation with tissue destruction and multiorgan failure. The classification of HLH by Histiocyte Society distinguishes the primary or genetic and secondary forms which can be triggered by various conditions as infections, autoimmune diseases and malignancy (44% lymphomas). It is extremely rare for a patient to survive when secondary HLH is triggered by a malignancy. Diagnosis of HLH is defined by finding five of the eight criteria: fever, splenomegaly, cytopenias, hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, low or absent NK cells activity, elevated soluble CD25 and hemophagocytosis. HLH diagnosis is very challenging and integration of clinical-anamnestic, instrumental and laboratory information are essential for to guide an prompt diagnosis and therapeutic strategy.

CC006

L'importanza della morfologia ematologica in una bambina Etiope affetta da anemia severa

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Severe malnutrition anaemias with haemoglobin (Hb) values lower than 40 g/L, even if thought not compatible with life, are not so uncommon in Ethiopia. Here a severe anaemia due to vitamin B-12 deficiency and/or folic acid is reported. A 16 month-old girl from a rural village was hospitalized in Adwa hospital. On physical examination her general appearance was acutely sick looking, lethargic and with severe pallor, muscle hypotonia and shortness of breath. The pulse rate was 120 beats/minute, respiratory rate 24 breaths/minute and O2 saturation 90%. The abdomen was slightly distended, the liver palpable, no fluid collection and no splenomegaly.The anthropometry measurements (weight for age and mid upper arm circumference) according to WHO growth standards, were both indicative of severe acute malnutrition. Her blood count, using Sysmex XN550 instrument, showed: WBC 16.5 109/L, RBC 0.69 1012/L, Hb 21 g/L, the mean corpuscular haemoglobin 30.9 g/dL, a mean cell volume 98.6 fL, PLT count 55 109/L.Creatinine and Urea, measured to exclude the Hemolytic-Ureamic Syndrome, were both normal. The visual inspection of the centrifuged sample did not show any sign of haemolysis or jaundice.The flags reported by the XN550, the RBC/PLT distributions, the lack of the RDW determination, indicate the presence of morphologic anomalies. In this condition, the lymphocyte count is not reliable being overestimated, and the morphological evaluation on the blood smear is strongly recommended.Her blood film showed remarkable anisopoikilocytosis with macrocytes, fragmented cells, tear drops and bizarre shapes. Hypersegmented neutrophils and some erythroblasts were also seen. Considering the local impossibility of detecting hemoglobinopathies, a congenital haemolytic anaemia due to a membrane abnormality was not excluded even though the number of polychromatophilic RBCs does not support this hypothesis. Moreover, consanguineous marriage is strictly forbidden in Ethiopia.The morphological alterations, the absence of haemolysis and the clinical data confirmed the suspicion of deficiency anaemia arising from severe malnutrition. This clinical case confirms once again the appropriateness of the microscopic evaluation for detecting morphologic anomalies otherwise not recognizable.

CC007
Marcata iper-eosinofilia in un paziente cardio-trapiantato

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L'emocromo di un paziente maschio di circa 70 anni cardiotrapiantato, eseguito con strumentazione XN9000 (Sysmex), evidenzia una marcata eosinofilia: leucociti 11,29x109/L, eosinofili 5,48x109/L (48,5%), neutrofili 4,31x109/L (38,2%), linfociti 0,94x109/L (8,3%), monociti 0,52x109/L (4,6%), basofili 0,04x109/L (0,4%). Il middleware di settore (Dasit Management System) allarma il campione e lo indirizza alla revisione microscopica, in quanto è presente una regola per cui, in caso di eosinofilia superiore a 2,5x109/L, il campione viene bloccato e viene eseguito uno striscio di sangue periferico.
Alla revisione microscopica non si conferma la eosinofilia e la formula leucocitaria viene modificata in: eosinofili 0,5%, neutrofili 85,9%, linfociti 6,3%, monociti 4,7%, basofili 0,5%, mielociti 1,6%, metamielociti 0,5%.
Analizzando meglio i campioni di tale paziente nel tempo emerge che ad aprile 2020, per qualche settimana, e successivamente a luglio, al citogramma WDF compare uno sdoppiamento della nuvola dei neutrofili e che la nuvola più a dx è a volte classificata come costituita da neutrofili e a volte da eosinofili. Tale sdoppiamento non si osserva nei periodi in cui il paziente si presenta leucopenico. Al riesame degli strisci periferici si osserva inoltre la presenza di neutrofili ipersegmentati (fino a otto lobi) e di altri neutrofili normo o iposegmentati.
La nostra interpretazione è che i due cluster di neutrofili nel citogramma WDF corrispondano ai neutrofili normosegmentati e a quelli ipersegmentati e che lo strumento interpreti talvolta erroneamente il cluster a maggiore complessità cellulare come eosinofili.
Al momento non ci è nota la causa di tale doppia popolazione e della sua fluttuazione: è stata esclusa la presenza di malaria, possibile causa di pseudoeosinofilia, non ci sono stati cambiamenti nella tipologia di farmaci somministrati e non si registrano cambiamenti di reagenti nei periodi indicati.
Da questo caso emerge l'importanza della revisione sia microscopica che dei grafici dei campioni fermati da regole di validazione opportunamente implementate nei software dedicati.

CC008
Informazioni inaspettate da un esame emocromocitometrico

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Si riporta il caso di un uomo di 56 anni, giunto al pronto soccorso del Policlinico di Tor Vergata-Roma per un episodio di vertigini posizionali. All'esame obiettivo nulla da rilevare. A seguito della consulenza otorinolaringoiatrica si eseguiva manovra liberatoria secondo Epley per nistagmo. Gli esami ematochimici evidenziano un valore di MCV di 78.5 fl e RDW-CV di 16.7% con valori di LDH, Bilirubina Tot. E Diretta nella norma. L'emocromo è stato eseguito con l'analizzatore ematologico XN 9000 (Sysmex Co) e ha dato i seguenti allarmi morfologici: "WBC Abnormal Scattergram" ed "Abnormal Lymphocytes". Alla revisione microscopica, si evidenziano un discreto numero di emazie contratte e di emazie a bersaglio, ma nessuna alterazione a carico dei leucociti. Un'attenta analisi dei citogrammi di formula leucocitaria, del canale WDF, ha messo in evidenza una diminuzione dei valori dell'intensità di fluorescenza di Neutrofili [NE-SFL 31.6 ch (44.1-54.1)], di Linfociti [LY-Y 41.9 ch (65.9-80.3)] e di Monociti [MO-Y 58.6 ch (101.5-126.7)]. In letteratura, è riportato che la presenza di varianti emoglobiniche possa determinare una minore disponibilità del marcatore fluorescente per la colorazione degli acidi nucleici, utilizzato dagli analizzatori Sysmex XN(XE). Pertanto, è stata eseguita l'analisi delle emoglobine mediante cromatografia ad alta risoluzione (HLC-723 G11 TOSOH). Il tracciato cromatografico ha effettivamente rilevato la presenza di una variante emoglobinica, in zona D/E, con un tempo di eluizione compreso tra 2.72-2.73 minuti ad una concentrazione di 24.5% (HbA: 58.8%, HbA2: 3.5% HbF: 0.1%). L'analisi molecolare, in corso presso un laboratorio specializzato, permetterà di identificare la variante emoglobinica. Questo caso clinico dimostra come l'utilizzo della tecnologia Sysmex XN permette di rilevare alcune varianti emoglobiniche in assenza di riscontri clinici associabili, utilizzando parametri strumentali disponibili in Laboratorio con l'analisi dell'emocromo. L'identificazione dei portatori di varianti emoglobiniche, spesso asintomatici, è molto importante per le complicazioni che potrebbero essere indotte da terapie farmacologiche, infezioni virali ed esposizione a sostanze tossiche.

CJ01

INFOSPHERE: THE INFLUENCE OF DISINFORMATION

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The Infosphere is the totality of the information domain (1); it is increasingly characterized by the saturation of attention span and the fragmentation of the media, which act in a pervasive manner, determining the phenomenon of information overload.
These transformations contribute to the shift from the economy of attention to that of polarized emotions.
In this new system emerges the difficulty of a critical and experiential faculty in distinguishing the real from the false, which is obstructed by an unprecedented acceleration of mental time, which emphasizes the traits of hyperactivity, transience and uncertainty, which are typical of postmodern age and the complexity of our society (2).
In 2016 the Oxford English Dictionary added the word post-truth to its dictionary, a neologism that refers to a society where objective facts are less influential in shaping public opinion than information that moves emotions or reinforces personal beliefs.
The collapse of the authority and hierarchies, of the credibility and reliability of classic sources, the process of self-composition of information schedules by the user, as well as the new relationship that exists between digital individuals, who are configured in simple users, producers and distributors of contents, leads to the systemic collapse of intermediation and the consequent definition of a key phenomenon for understanding the infosphere: the disintermediation.
In the global of Infosphere, the cognitive, perceptual and mnemonic transformations imposed by technologies deactivated the universalism of reason, reduced sensitivity and destroyed the foundations of ethical behavior.
This "information disorder" exploits polarized opinions, emotional sharing, cognitive effects deriving from information overload, cognitive biases and echo chambers, fake news and disinformation that change the exposure of the conscious mind to information that becomes so rapid, so short, that critical processing is being disabled.
The primary information infrastructure in the Infosphere is the infomercial arena (the intertwining of the English words information and commercial) which uses mythopoiesis as an elementary dynamic of construction of reality: myths, commonplaces, fake news, conspiracy theories, propaganda, recurring narrative structures determine modes of behavior.
The COVID-19 epidemic has quickly unleashed an infodemic (from the English infodemic, in turn composed of the s. Information and epidemic) not only through online platforms such as Facebook, YouTube, or messaging services, but, also through traditional media, such as TV, but, above all, on the markets, flooding them with an avalanche of misleading announcements 24 hours a day, fake news, narratives on conspiracy theories and much more.
In "*Communicating risk in public health emergencies"(3) in 2017, WHO declared: "Risk communication is a vital element for public health. During public health emergencies, people need to know what health risks they face, the nature and extent of the event, and what actions they can take to protect their health and life."*
The problem of "fake news pollution" and the war of (dis) information - which , by now, has reached and flooded the field of scientific dissemination, and which proliferates in the alternative media, but remains in the professional media - affects, is increasingly weighting on the cognitive, perceptive and mnemonic sphere of humanity.
The use of the scientific method constitutes a valid remedy to the fallacy of idiosyncratically oriented perceptions, given that prejudices and misperceptions are more frequent when working on ambiguous and often discordant materials.
It is methodologically incorrect to formulate ad hoc hypotheses to save shaky theories, and that the "facts" themselves are hypothetical observation statements, because the researcher's mind is not a clean slate and therefore approaches problems based on his "pre-understanding" of reality.
Our society needs people who ask themselves about contemporaneity without filters and without limits, but through principles and scientific method for the production of knowledge and for the understanding of a reality where "nothing is as it seems".

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CJ02

MISINFORMATION, FAKE NEWS: THE HEALTH THREATS

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The search for health-related information has been one of the main reasons for access to the Internet since the 1990s. However, in parallel with the benefits of the wide dissemination, concerns about the risks of incorrect or incorrectly interpreted/applied information soon emerged, considering its impact on lifestyles and decisions regarding diagnostic and therapeutic procedures. With the advent of blogs and forums, where users have begun to introduce information themselves and, more recently, with the explosion of social platforms, the circulation of incredible amounts of information has reached uncontrollable levels. This speed clearly also applies to

misinformation (false/misleading information) and disinformation (consciously deceptive/manipulative information). The viral spread of fake news may be followed by changes, often risky, in habits and choices, sometimes so harmful as to cause death, such as inappropriate use of drugs/remedies or presumed such, changes in diet or psychosocial behavior (violence, racism) and the reduction in vaccination coverage. As a consequence the world has faced several outbreaks of vaccine-preventable diseases (30% global measles increase in 2018) and WHO included Vaccine hesitancy in the top 10 threats to global health(1). The tsunami of information, accurate and not, circulating about the Covid-19 pandemic (infodemic)(2) is the mirror of the difficulty to find reliable resources and has become a major threat to public health. Between February and April 2020 in Iran there were more than 5000 cases, with hundreds of deaths from methanol poisoning, due to the spread of its healing power against the Coronavirus on social media. Sometimes even politicians in institutional positions can more or less knowingly convey unscientifically validated information and cause irreparable harm, such as the death of a man in the USA who had ingested a product containing chloroquine following declarations by President Trump about the possible usefulness of hydroxychloroquine for the Coronavirus(3).
International institutions and organizations have been focusing for years on the issue of dis/misinformation, also for its political implications, trying different countermeasures. It is now evident that a multidisciplinary and holistic approach is needed at social, political, sanitary and scientific levels. In fact, despite the great advances in the detection capabilities of fake news that have been achieved also through the application of artificial intelligence systems, a complete monitoring of the information circulating - not only on the network, but also in traditional media (radio/TV) - is not sufficient, nor is it technically and ethically feasible. Therefore, the challenge we face is to strengthen communication and information with quality standards, protecting the right of speech and opinion, and to create a resilient society that is able to cooperate in the recognition of misinformation, as made explicit by the hashtag #TakeCareBeforeYouShare of the UNESCO awareness campaign launched on World Social Media Day (30 June 2020), which calls for fact-checking and verification of sources before sharing on the emotional wave(4).

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CJ03

YOUNG SCIENTISTS AND SOCIAL NETWORK: A GLOBAL COMMUNITY

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Social networks offer formal and informal ways to facilitate the communication between individuals.
The use of such networks in health has its threats that have to stay under control when conducted between unprofessional people in order to avoid desinformation, unreliable sources or fake news. On the other hand, when used in professional people, they are an invaluable tool for health workforce to exchange information, knowledge or experience. In this context, Young Scientists from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) are networking together in the IFCC-Task Force-Young Scientist. Thanks to social network, IFCC-TF-YS perform different social, professional and educational activities, including webinar or mentorship programme. In order to improve connectivity and communication among young professionals, IFCC-TF-YS also launched Lab-surfing (www.lab-surfing.com), an international community to connect young laboratory professionals from all around the globe.
Recently, young laboratory medicine professionals had and continue to have a central contribution during the current health crisis. With their professional commitment, they are at the forefront during COVID-19 pandemic, adding value to patient care and well-being, as well as to protection of the population. In this context, the need of improving visibility and promoting involvement of laboratory medicine professionals at an international level was identified. In this common and global objective, young scientists from IFCC-TF-YS participated in making a video underlying their role during the crisis. This initiative, as well as the edition of the video, was conducted by a union of young medical biologists from France SJBM, with the support of the national representative society SFBC and IFCC-TF-YS.
With the participation of many countries, and more than 140 000 views, the video permitted to promote contribution of lab workforce during the pandemic, link young scientists from all over the world, and promote international cooperation. With the persistence of the pandemic, this project has several perspectives, including creation of a larger-scale video, in order to involve more countries.

52° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

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

PO001
La refertazione del liquido pleurico

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Molte patologie, più o meno comuni, possono causare la raccolta di un versamento in cavità pleurica. In base al sospetto clinico, l'analisi del liquido pleurico dovrebbe procedere seguendo uno schema logico in grado di orientare verso le probabili cause del versamento (trasudato: scompenso cardiaco congestizio, cirrosi, sindrome nefrosica, embolia polmonare; essudato: cancro, polmonite, trauma, tubercolosi, artrite reumatoide e LES).Il liquido aspirato dalla cavità pleurica deve essere raccolto immediatamente ed analizzato per il dosaggio di LDH e proteine (criteri di Light). Il confronto dei livelli sierici di queste sostanze rispetto a quelli dosati contemporaneamente nel liquido pleurico consente di effettuare la prima grande distinzione fra essudati e trasudati. Se il liquido è un trasudato di solito non sono necessari altri test, mentre se risulta essere un essudato la diagnosi differenziale richiede ulteriori analisi. Per identificare gli essudati si applica un criterio aggiuntivo a elevata specificità: la misurazione del gradiente di concentrazione dell'albumina fra siero e liquido pleurico. In quasi tutti i pazienti con versamento trasudatizio questo gradiente supera 1,2 mg/dl. Il data base, da noi creato in Access, prevede la refertazione dell'aspetto, del colore, del numero di cellule, ma anche di esami quali glucosio ed amilasi. In un essudato si evidenziano i rapporti liquido pleurico/siero dei seguenti parametri: proteine (> 0,5), LDH (> 0,6), albumina (¤ 1,2) ed amilasi (>1,0). Ulteriori esami di approfondimento sono costituiti da marcatori tumorali, amilasi pancreatica e trigliceridi (>110 mg/dL: chilotorace).La nostra proposta informatica elimina esami obsoleti quali pH e peso specifico e può essere applicata anche ad altri liquidi biologici (es., liquido peritoneale) per la differenziazione di un trasudato da un essudato.

Cangiano G, Latte A, Di Maina E et Al. Informatizzazione di laboratorio del rischio nefrolitiasico. Biochimica clinica, 2012, vol. 36, 6:602

PO002
Implementazione del sistema di codifica LOINC per le prestazioni del laboratorio analisi

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Introduzione Per favorire lo scambio di informazioni in ambito sanitario a livello nazionale e, in un prossimo futuro, in ambito internazionale, si è reso necessario standardizzare i sistemi di classificazione delle prestazioni. Il Tavolo di lavoro permanente per la Sanità Elettronica, a cui afferiscono le diverse regioni, ha definito gli standard tecnici per la creazione del Documento di Referto secondo lo standard CDA2 HL7. Tali standard prevedono l'utilizzo esclusivo di ICD9-CM per la codifica delle diagnosi e di LOINC (Logical Observation Identifiers Names and Codes) per la codifica dei dati di laboratorio(1).
Scopo L'utilizzo di un sistema univoco di codifica delle prestazioni di laboratorio permette la comprensione delle informazioni sanitarie da parte di specialisti su tutto il territorio; LOINC infatti, si pone l'obiettivo di creare identificatori universali impiegati in informatica sanitaria permettendo lo scambio di dati clinici di laboratorio tra ambienti informatici eterogenei.
Materiali e metodi Nel 1994 il Regenstrief Institute for Health Care ha pubblicato il sistema di codifica LOINC e ha sviluppato un programma (RELMA) per accedere al database LOINC affinchè ciascun laboratorio potesse mappare i propri codici di laboratorio nei corrispondenti codici LOINC. I codici LOINC sono costituiti da 6 parti: analita, tipo di campione, proprietá, aspetto temporale, scala e metodo in modo da individuare in maniera univoca ed universale una singola e specifica prestazione di laboratorio con le sue specifiche caratteristiche(1).
Risultati e conclusioni L'introduzione della codifica LOINC delle prestazioni di laboratorio permette di ottenere informazioni riconosciute universalmente con l'obiettivo di allineare l'Italia agli standard internazionali. La definizione del metodo inoltre, limita la ripetizione di esami e favorisce l'interpretazione del dato contribuendo così ad una migliore gestione del paziente.

(1) C. J. McDonald et al. LOINC, a Universal Standard for Identifying Laboratory Observation: a 5-year update, 2003 Clinical Chemistry, p.624-633

PO003

Fatal evolution of an Anaplastic Lymphoma Kinase, ALK-

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U.O.C. Ematologia

A 55 years old male refers to Hematology for an evaluation about marked asthenia and numerous lymph nodes with lymphomatous pattern detecting echography. Therefore, the patient undergoes excisional biopsy of lateral cervical lymph node that establishes the diagnosis of Anaplastic Large Cells Lymphoma, negative Anaplastic Lymphoma Kinase (ALCL,ALK-). The blood count is normal. Bone marrow aspiration and biopsy are performed. Observation of bone marrow smears shows medium size to large anaplastic cells (25%) which have nuclei with irregular contours sometimes convoluted with two or more prominent nucleoli, inhomogeneous basophilic cytoplasm and frequently nuclear and cytoplasmic vacuoles. These cells show increased forward and side scatter and atypic CD45++CD3-CD5-CD4+CD25+CD30+ immunophenotype detected by flow cytometric analysis, suggestive of disease localization. The patient undergoes a therapeutic program that provides chemotherapy and hospitalization for autotransplant. At the time of admission patient's blood count performed with ADVIA2120i shows anemia (Hb:80gr/L), thrombocytopenia (PLT:41x109/L), mild leukocytosis (WBC:12,74x109/L) and presence of LUC (4%) which can be seen as a heterogeneous population on the perox cytogram upward extending indicating large size cells. The smears show medium and large pathological cells with a fine chromatin pattern, scant basophilic cytoplasm, nuclear and cytoplasmic vacuoles; these cells have the same immunophenotype founded in the bone marrow. Patient's clinical conditions rapidly worsen until his death. ALCL are a T-cell lymphomas classified in ALK+ and ALK- according to ALK protein expression. Two entity share morphological and immunophenothipical features as strong CD30 expression and frequent loss of T-cell markers, frequently of CD3 and CD5 while CD4 is often expressed. Patients with ALK# ALCL are older than ALK+ and appear to have a poorer outcome. Bone marrow involvement is uncommon and leukemic phase is extremely rare and the most of those cases reported are ALCL ALK+ in childrens. Although uncommon, diagnosis of leukemic phase ALCL must be considered for its fatality.

Swerdlow SH, Campo E, Pileri SA, et al. (2016). The 2016 revision of the World HealthOrganization classification of lymphoid neoplasm. Blood. 127:2375-90

PO004

Valutazione della Proteina di Bence Jones in elettroforesi capillare zonale/immunosottrazione

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È stata valutata l'applicabilità nella nostra routine di laboratorio della determinazione qualitativa e quantitativa della proteina di Bence Jones (PBJ) mediante elettroforesi capillare zonale (uCZE) e immunosottrazione (uISE). Tali tecniche vengono largamente impiegate per l'elettroforesi delle sieroproteine e la tipizzazione delle componenti monoclonali sieriche nei laboratori ad elevata produttività analitica, offrendo vantaggi di automazione e riproducibilità e garantendo al tempo stesso una risoluzione paragonabile all'elettroforesi ad alta risoluzione in gel d'agarosio. L'immunofissazione urinaria (uIFE), ad oggi gold-standard per la ricerca della PBJ, risulta spesso poco accurata nella sua quantificazione, soprattutto a causa della non facile delimitazione della banda al tracciato di riferimento urinario. Su urine del mattino di 24 pazienti affetti da gammopatie monoclonali in follow-up, sono state eseguite: proteinuria e creatinuria (Architect, Abbott); uCZE e uISE (Capillarys 2, Sebia) con pretrattamento dei campioni (dialisi e concentrazione) di circa 2 ore; uIFE (Hydrasys, Sebia). La quantificazione della PBJ in uCZE è stata effettuata rapportando il valore della PBJ (mg/L) alla creatinuria (g/L) mentre in uIFE è stata eseguita la valutazione della entità della banda (lieve, moderata, marcata). In 24/24 campioni uISE e uIFE hanno mostrato risultati concordanti per l'identificazione della PBJ (18 PBJ k, 6 PBJ lambda), in 9/24 si è osservato un numero maggiore di picchi PBJ all'uISE rispetto alle bande evidenziate all'uIFE. In 13/24 campioni con entrambe le tecniche è stata evidenziata una immunoglobulina completa, in 1/24 solo all'uIFE.

I risultati ottenuti in uISE e uIFE sono concordanti, ma è necessario ampliare la popolazione in studio. La uCZE/uISE ha mostrato un più elevato potere risolutivo rispetto all'uIFE, consentendo una migliore delimitazione dei picchi PBJ e quindi una più accurata quantificazione, dal momento che per l'uIFE i più comuni metodi in commercio si avvalgono di una elettroforesi non ad alta risoluzione. Come sottolineato in precedenti studi, si auspica una ottimizzazione della procedura che riduca il tempo di pretrattamento dei campioni, fattore limitante ad impatto sfavorevole sull'attività globale del laboratorio proteine.

PO005

Comparison of two PEG precipitation methods for routine detection of macroprolactin

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Prolactin (PRL) is an anterior pituitary hormone present in the serum of healthy subjects both in monomeric (23KDa, 85% of total PRL) and as macroprolactin (MacroPRL, 50-150KDa).MacroPRL is biologically inactive and may cause an increase in measured serum prolactin concentration without corresponding activity. Hyperprolactinemia is a condition in which a subject has higher-than-normal levels of the monomeric hormone in the blood and the presence of MacroPRL may interfere with the correct diagnosis of hyperprolactinemia.The aim of our study is to validate the polyethylene glycol (PEG) precipitation test in our laboratory to introduce it as a routine screening for hyperprolactinaemic patients.To do this, we compared two protocols for PEG precipitation of MacroPRL, which differ in speed and time of centrifugation.An adequate number of serum samples (n=75) were divided into 3 groups: low, medium and high concentration (respectively PRL <700, 700÷1500 and >1500mIU/L).To remove the MacroPRL, the serum was mixed with an equal volume of a 25% w/v solution of PEG-6000 dissolved in phosphate buffered saline (PBS, pH 7.4), incubated for 10 min at RT and then centrifuged at 2800 x g for 30min (Procedure 1-P1) or 14000 x g for 5min (Procedure 2-P2).PRL was measured in the supernatant with the Siemens PRL assay on a Dimension Vista® System. Samples from each group were pooled to test the linearity and the precision of both methods. To evaluate linearity, 2-fold serial dilutions of each pool were tested with the two methods (P1 R2=0.996; P2 R2=0.994).To assess the precision, the PRL of each pool was measured 3 times/ day for 3 days. The intra-assay Coefficient of Variation (CV) was 2.2% and 1.6% respectively, while the inter-assay CV was 2.3% for P1 and 2.4% for P2. Finally, to evaluate which of the two protocols guarantee a better recovery of monomeric PRL, 40 samples were tested with different PRL values (702.7-2095.7 mIU/L):P2 allowed a higher recovery of monomeric PRL than P1 (T-student test:P< 0.0001).In conclusion, the comparison of the two procedures showed similar performance in terms of linearity and precision.However, procedure 2 allowed a better discrimination between macroprolactinemia and hyperprolactinemia.Further studies are necessary to confirm our conclusion.

PO006

Comparison of High Sensitive and Conventional Cardiac Troponin I assays on the Siemens Dimension Vista®

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Cardiac Troponin is a useful marker for patients with myocardial injury, playing a pivotal role in the diagnostic criteria for acute myocardial infarction (AMI). In the last years, high sensitivity cardiac troponin assays are spreading worldwide, replacing the conventional tests. The aim of this study is to compare the analytical and clinical performance of high sensitivity troponin I (Siemens Dimension Vista® TNIH) assay with the cardiac troponin I assay (Siemens Dimension Vista® CTNI). We enrolled 193 patients (110 males and 83 females, age range: 15-93 years), presenting to the emergency department with acute chest pain. TNIH and CTNI were measured in litium-heparin plasma samples on Dimension Vista® 1500 System analyser (Siemens Healthineers, Erlangen, Germany). The analytical measurement range was 3-25000 ng/l and 15-40000 ng/l for TNIH and CTNI, respectively. All statistical tests were performed by MedCalc Statistical Software (Ostend, Belgium) and the statistical significance was set at p value <0.05.TNIH and CTNI concentrations were assessable in 182 (94%) and 63 (33%) patients, respectively. Comparing the two assays, Spearman's rank correlation coefficient was 0.964 (p<0.001) and Cusum test indicated no significant deviation from linearity (Passing-Bablok regression y= -6.31+1.16x). Bland-Altman plot showed good agreement between the two assays (mean bias =12.6 ng/l). The clinical concordance rate, determined considering the upper reference limit of the assays (CTNI URL = 40 ng/l; TNIH URL = 78.5 ng/l for males and 53.8 ng/l for females), was also satisfactory (overall rate = 94%, Cohen's Kappa=0.80). Of the 10 patients with discordant results, 6 were finally diagnosed with cardiovascular disease other than AMI (i.e. blood hypertensive crisis, congestive heart failure, atrial fibrillation, cardiac valvulopathies), while the remaining 4 patients suffered from respiratory disease. The study confirmed the higher analytical sensitivity of Dimension Vista® TNIH assay, compared to Dimension Vista® CTNI assay, as declared by the manufacturer. In addition, the clinical performances of TNIH assay was very satisfactory, demonstrating a high ability to diagnose AMI.Undoubtedly, the results supported the introduction of TNIH assay in diagnostic laboratories.

PO007

IgE monoclonal gammopathy: the clinical relevance to perform the immunofixation using IgE antisera

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Immunoglobulin E monoclonal gammopathy is a rare kind of plasma-cell disorder, associated to an aggressive clinical course and a short survival in the case of multiple myeloma. Laboratory plays a crucial role in the identification of this pathology in order to start a correct treatment. Herein, we describe two cases of IgE monoclonal gammopathy of undetermined significance (MGUS) without any common biological and clinical symptoms, identified at the San Gerardo Hospital, Monza (Italy), from 2005 to 2019. The aim of this study is to highlight the key clinical impact of serum immunofixation using anti-IgE and anti-IgD antisera when an initial monoclonal component is fixed by light chains antisera only.Reviewing the published cases from 1967 to 2018, although uncommon, IgE monoclonal gammopathy presents clinical features similar to the other plasma cell dyscrasias. Bone lesions, anemia, renal failure, hypercalcemia, and the presence of Bence-Jones protein are commonly noted However, given the rarity of this disease, knowledge is limited to the small amount of cases reported in literature. In our two patients, hypercalcemia was not observed, a mild anemia was only present in the second case, and renal failure was not noted since serum creatinine values were normal. Even though IgE gammopathy seems to be more aggressive in clinical course compared to other monoclonal gammopathies, our second case suggests that a prolonged survival is possible, as previously described. The numbers of subjects affected by IgE monoclonal gammopathy is very low and different variants are present; therefore, it is important to be cautious in drawing general conclusions about this pathology. Moreover, the quantification of serum IgE could be underestimated due to the prozone effect using the antigen-antibody based methods currently available, as previously reported. However, serum protein electrophoresis provides a reasonable quantitative value for the monoclonal immunoglobulin present in serum.In conclusion, laboratory plays a fundamental role in diagnosis and we suggest that for all gammopathies presenting free light chains without a heavy chain counterpart to undergo a screening for the presence of IgD and IgE proteins.

PO008

Formyl peptide receptor 1 signalling in acute inflammation and neural differentiation induced by traumatic brain injury

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Traumatic brain injury (TBI) is a shocking disease frequently followed by behavioral disabilities including risk of cerebral atrophy and dementia. N-formylpeptide receptor 1 (FPR1) is expressed cells and neurons in the central nervous system. It is involved in inflammatory processes and during the differentiation process in the neural stem cells. We investigate the effect of the absence of Fpr1 gene expression in mice subjected to TBI from the early stage of acute inflammation to neurogenesis and systematic behavioral testing 4 weeks after injury. C57BL/6 animals and Fpr1 KO mice were subjected to TBI and sacrificed 24h or 4 weeks after injury. Twenty-four hours after injury TBI Fpr1 KO mice showed reduced histological impairment, tissue damage and acute inflammation (MAPK activation, NF-B signaling induction, NRLP3 inflammasome pathway activation and oxidative stress increase). Conversely, four weeks after TBI Fpr1 KO mice showed reduced surviving proliferated cells in the Dentate Gyrus compared to the WT group. Behavioral analysis confirmed this trend. Moreover, TBI Fpr1 KO animals displayed reduced neural differentiation (evaluated by beta-III tubulin expression) while up-regulation of astrocytes differentiation (evaluated by GFAP expression). Collectively, our study reported that immediately after TBI Fpr1 increased acute inflammation, while after four weeks Fpr1 promoted neurogenesis.

PO009

Biochemical and Pharmacological Studies on Anacardium occidentale L. cashew nuts on an experimental model of gut ischemia/reperfusion injury

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Background: Several antioxidant and pharmacological properties of cashew nuts (from Anacardium occidentale L.) have recently been described. It is a medicinal plant with important therapeutic effects. This study aimed to verify the effect of an oral administration of cashew nuts in a rat model of ischemia/reperfusion (I/R) of the gut. Methods: Adult male rats were subjected to intestinal I/R injury by clamping the superior mesenteric artery for 30 minutes and then allowing animals to 1 h of reperfusion. Results: Rats subjected to I/R of the gut showed a significant increase in lipid peroxidation, tissue myeloperoxidase activity, protein carbonyl content, reactive oxygen species generation and decreased antioxidant enzyme activities. Increased immunoreactivity to nitrotyrosine, poly ADP ribose polymerase (PARP), P-selectin, intercellular adhesion molecule -1 (ICAM)-1 was observed in the ileum of rats subjected to I/R. Administration of cashew nuts (100 mg/kg) significantly reduced the mortality rate, the fall in arterial blood pressure, oxidative stress and restored the antioxidant enzyme activities. Cashew nuts treatments reduced cytokines plasma levels, nitrotyrosine and PARP expression as well as adhesion molecules expressions. Conclusions: Our study demonstrates that cashew nuts administration exerts antioxidant and pharmacological protective effects in superior mesenteric artery occlusion–reperfusion shock and other cardiovascular diseases.

PO010

Anti-inflammatory and anti-oxidant activity of Hidrox® in rotenone-induced Parkinson's disease in mice

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In developed countries, the extension of human life is increasingly accompanied by a progressive increase in neurodegenerative diseases, most of which do not yet have effective therapy but only symptomatic treatments. In recent years, plant polyphenols have aroused considerable interest in the scientific community. The mechanisms currently hypothesized for the pathogenesis of Parkinson's disease (PD) are neuroinflammation, oxidative stress and apoptosis. Hydroxytyrosol (HT), the main component of Hidrox® (HD), has been shown to have some of the highest free radical evacuation and anti-inflammatory activities. Here we wanted to study the role of HD on the neurobiological and behavioral alterations induced by rotenone. A study was conducted in which mice received HD (10 mg/kg, i.p.) concomitantly with rotenone (5 mg/kg, o.s.) for 28 days. Locomotor activity, catalepsy, histological damage, dopamine transporter (DAT) content, tyrosine hydroxylase (TH) and accumulation of ¤-synuclein were initially evaluated. Subsequently, apoptosis, neuroinflammation, the role of the inflammasome and finally oxidative stress. Taken together, the results obtained highlight HD's ability to reduce the loss of dopaminergic neurons and the damage associated with it by counteracting the three main mechanisms of PD pathogenesis. Therefore, HD represents a promising nutraceutical choice against PD. Moreover, it shows an excellent safety profile and is subject to fewer regulations than traditional pharmaceuticals to improve brain health of patients.

PO011

Alterazioni morfologiche linfocitarie dello striscio di sangue periferico in paziente affetto da Felty's syndrome.

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Introduzione
La Felty's syndrome (FS) è distinta da una triade di sintomi: artrite reumatoide (AR), splenomegalia e neutropenia. Circa l'1-3% dei pazienti con AR ha una forma complicata da FS1. Questo quadro predispone a infezioni gravi, fino a shock settico e allo sviluppo di leucemia linfocitica granulare a grandi cellule T (leucemia GLG a cellule T)1,2.
Descrizione del caso e metodi
L'8/1/2020 un paziente di 75 anni si presenta al Day-Hospital geriatrico dell'ospedale di Desio con polmonite infettiva su BPCO, severa anemia (HB 5,6 g/dl) e neutropenia (560/mm3); viene ricoverato in cure subacute. In anamnesi ha Felty's syndrome, BPCO, insufficienza renale cronica, diabete tipo 2; una pregressa TC torace e addome mostrava alterazioni linfonodali e splenomegalia, come da malattia linfoproliferativa. Il 13/1/2020 all'emocromo (Sysmex DI-60 e SP-10) si rilevano numerosi linfociti atipici con nucleo plurilobato e vacuoli (definiti flower cell). Questa particolare morfologia può essere indicativa per infezione da virus HTLV3, per cui il paziente viene sottoposto a test sierologico, risultato negativo. Si suggerisce la tipizzazione delle popolazioni linfocitarie che evidenzia linfopenia selettiva CD4 e B con un aumento della popolazione T CD3+. Il 14/1 il paziente sviluppa un quadro di sepsi, che lo porta al decesso.
Discussione
L'osservazione allo striscio di numerosi linfociti plurilobati con morfologia "flower cell" deve, in assenza di anamnesi nota, far sospettare inizialmente una infezione da HTLV e conseguente processo linfoproliferativo tipo leucemia a cellule T dell'adulto (ATLL)3. In questo caso il dirigente di laboratorio può consigliare il dosaggio di anticorpi anti-HTLV. Se queste risultano negative o se si è a conoscenza della diagnosi di AR o FS, il sospetto può ricadere su leucemia GLG a cellule T, fortemente associata a queste patologie1,2 e che raramente può presentarsi con nuclei a morfologia polilobata.4,5
Conclusioni
La valutazione delle anomalie morfologiche dei linfociti all'emocromo è connessa ad una buona conoscenza del quadro clinico ed anamnestico dei pazienti, per indirizzare il clinico a ulteriori approfondimenti laboratoristici a supporto della diagnosi.
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PO012

Alterazioni morfologiche delle principali linee cellulari nei pazienti COVID-19

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INTRODUZIONE
Le alterazioni ematologiche più comuni riscontrate nei pazienti COVID-19 sono linfocitopenia, neutrofilia e lieve trombocitopenia1. L'infezione determina alterazioni morfologiche delle cellule ematiche in un gruppo di pazienti COVID-19.2
Lo scopo di questa analisi è descrivere le anomalie morfologiche osservate allo striscio di sangue periferico e valutare una possibile correlazione tra PCR e tali anomalie.
MATERIALI E METODI
I campioni sono stati analizzati tra marzo e maggio 2020 presso l'U.O.C Laboratorio Analisi dell'Ospedale di Desio con l'utilizzo dello strumento Sysmex SP-10 e DI-60.
RISULTATI
255 sono stati i pazienti COVID-19 ma solo di 78 è presente lo striscio periferico. 56/78 mostrano anomalie morfologiche delle principali linee cellulari. Le più comuni sono a carico di neutrofili (n=23) e trombociti (n=21); in entrambi è stata osservata la presenza di vacuoli multipli e di varie dimensioni nel citoplasma. 5 pazienti presentano neutrofili con citosol ipergranulato e cromatina condensata. In 24 pazienti sono stati riscontrati linfociti di piccole dimensioni con scarso citosol basofilo e protrusioni (blebs), alterazioni non osservati in lavori precedenti. L'8% dei pazienti presenta cellule immature vacuolate come promielociti e mielociti.La nostra analisi ha rilevato che i pazienti che presentano anomalie morfologiche hanno livelli di PCR significativamente più alti rispetto ai pazienti senza anomalie(80.89 vs 43.15mg/L;$P<0.05$).
DISCUSSIONE
La presenza di linfociti con blebs è riportata in casi di leucemia pro-linfocitica a cellule T.3 Nessuno dei pazienti con tali alterazioni presenta neoplasie ematologiche. Le protrusioni della membrana possono indicare apoptosi o essere un meccanismo di motilità dei linfociti verso i tessuti coinvolti.4 Valutando i livelli elevati di PCR si può ipotizzare una possibile correlazione tra l'infezione virale e le diverse anomalie riscontrate.
CONCLUSIONE
L'infezione da SARS-COV2,su alcuni pazienti Covid-19, provoca alterazioni morfologiche su striscio di sangue periferico come i blebs linfocitari,neutrofili vacuolati e ipergranulati. Inoltre i livelli di PCR su pazienti con tali anomalie sono significativamente più alti. BIBLIOGRAFIA
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PO013

VALUTAZIONE "LABINSIGHT WORKFLOW ANALYSIS" SULLA GESTIONE DI CONTROLLI DI QUALITÀ INTERNI SULLE PIATTAFORME ATELLICA SOLUTION NEL LABORATORIO SMEL2 DELL'ASST PAPA GIOVANNI XXIII DI BERGAMO

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In febbraio 2020, personale certificato Green Belt Lean Six-Sigma (Bio-Rad Laboratories), ha analizzato per due giorni il flusso di lavoro relativo alla gestione dei CQI sui 7 moduli Siemens AtellicaTM Solution (4 Chimica, 3 Immunometria) nell'Area Corelab del nostro Laboratorio, certificato UNI EN ISO 9001:2015, con metodologia Lean e Six Sigma, al fine di individuare aree di miglioramento in una visione del Controllo di Qualità Orientata al Paziente. Sono stati considerati: capacità produttiva dei sistemi (n ° campioni e test eseguiti), interventi manuali e rischiosi (etichettatura, aliquotazione, caricamento) e produzione di "Waste" (rifiuti, attese). Sono stati analizzati: modalità di arrivo campioni ed esecuzione CQI, manualità nell'uso dei CQI e validazione degli esiti. L'osservazione, rivolta al processo e non agli operatori, dimostra come sia possibile: contenere la spesa; semplificare i CQI in uso senza alterarne la calendarizzazione, legata all'avvio della routine/urgenza ed alle manutenzioni delle macchine (pre-valutazione: 10 CQI, di cui 6 InteliQ in tubo primario pronti all'uso e 4 da aliquotare, corrispondenti a 36 tubi e 22 aliquote; post-valutazione: 8 CQI, di cui 7 InteliQ e 1 da aliquotare, corrispondenti a 49 tubi e 6 aliquote, dove l'aumento del 36% dei tubi è compensato dal calo del 73% di aliquote); ridurre le azioni manuali, richiedenti 150''/die (pre: 294, pari a 12h25'; post: 134, 54% in meno, pari a 5h58'); standardizzare l'uso dei CQI automatizzandolo; implementare il software "Mission ControlTM" e valutare RMI (Risk Management Index), Indicatore che quantifica il rischio del test in base a statistiche di esami richiesti, statistiche e frequenze di CQI e probabilità di danno, suggerendo frequenza e regole di Westgard (W) idonee al livello di rischio accettato al fine di ridurlo. Nella simulazione con dati di 3 mesi, RMI<0,001 per hsTnI con regola W-1,3s non cambia anche portandola a 1,4s. Ciò permette di ottimizzare le risorse sfruttando il rischio quantificato (RMI), in modo da ridurre l'esecuzione dei CQI per test con RMI basso e aumentarla per quelli con RMI>1. Tale valutazione, quindi, aiuta a razionalizzare efficacemente i CQI, consentendo l'implementazione della qualità delle prestazioni assicurando la centralità del Paziente.

PO014

Performance evaluation of Mindray 1200i: HBV, HIV Ab/Ag assays.

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Background: Enzyme immunoassays are currently the most widely used screening immunoassays for hepatitis B virus identification and for human immunodeficiency virus HIV 1-2. Chemiluminescence has obtained great attention for its high sensitivity, good specificity and simple equipment. Fourth generation assays have recently been proposed as new HIV screening tests. Mindray CL-1200i HIV is a fourth-generation HIV test that is able to simultaneously detect the p24 antigen and anti-HIV antibodies 1-2. The aim of this study is to compare Abbott system, used routinely, with the new Mindray 1200i platform for the quantitative determination of HBsAg in human serum, its Anti-HBs antibody, for the qualitative determination of other HBeAg antigen and its anti-HBe and Anti-HBc antibody and human immunodeficiency virus (HIV 1/2 Ag/Ab). Methods: All samples were compared on both CL-1200i and Abbott Architect. Analytical precision, linearity, limit of detection and carry over were assessed. Results: The results showed a good comparability between Mindray CL-1200i and Abbott. The agreement between the results ranged from 99% to 100% and the discrepancy rate from 0% to 1%. Among 541 samples tested by the two methods, discrepant results were obtained with 2 (1%). There were no false negative results, thus achieving 100% sensitivity of each test. No interference or cross-reactivity was observed with known interfering substances and virologic markers. Bland-Altman and Passing-Bablock plots were calculated to evaluate the agreement between the updated values with the two assays. It has been shown that the carry over effect is not present. Conclusions: The results were overall accurate, showing low variability and minimun discrepancy compared to Abbot system. This study represents the first rigorous comparison between Abbott and Mindray 1200i. We observed a good correlation and a high agreement between HBV and HIV assays with the two automated systems, CL-1200i shows high sensitivity and specificity and is suitable for early HIV screening. It can detect minimal HBsAg levels and all HIV1-2 and HIV p24 antigen positive samples. In conclusion the clinical efficacy of the new highly sensitive analyzer makes it useful for routine screening for HBV and HIV detection.

PO015

Drug of abuse presence in the hair of children under the age of 2: how to report the results

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Introduction and aim
The presence of drugs of abuse in the hair of children under the age of 2 has different origins: in utero exposition, breast feeding, passive smoke, or contact with contaminated objects.[1] In 2019, the laboratory of Desio Hospital was asked to test the hair of a 9-month-old child for cocaine, benzoylecgonine (BZE) and cannabinoids (THC-COOH). The child and the mother were living in a recovery community and the woman was suspected of abusing drugs. Being this test uncommon in children of young age, current cut-offs and reporting mode were further investigated.
Materials, methods and results
Written consent from the parents was obtained. The hair analysis with liquid chromatography-mass spectrometry detected cocaine = 0.39 ng/mg and BZE = 0.15 ng/mg, whereas THC-COOH was not.
Discussion
Hair analysis in children under the age of 2 is an important clinic and forensic diagnostic tool. Children's hair is structurally different compared to adults and this determines the impossibility to distinguish the way and time of drug assumption.[1] The guidelines on hair testing highlight the caution needed in the interpretation of results in pediatric age and the difficulty in distinguishing whether the exposition happened in utero or after birth.[2] A different cut-off for the pediatric population compared to adults is missing, as in the regional council decisions of Lombardy, Italy.[3]According to the Lombardy and international cut-off values, the tests would have been considered negative. But, being advised from the Italian Observatory for Smoke, Alcohol and Drugs of Abuse (OssFAD), the drug concentrations were reported with a conclusive comment underlining the compatibility of the child's results with passive exposure to cocaine.
Conclusion
It is recommended for hair test reports in children to indicate the numerical result and not to adopt the current cut-offs, because it indicates a direct or indirect exposure to drugs of abuse.
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PO016

Low Serum Testosterone: A Comparison of Certified Immunoassay and LC-MS/MS in male adults

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BACKGROUND
The usefulness of testosterone replacement therapy at testosterone levels between 2.3 to 3.5 ng/mL(grey zone), remains controversial. Testosterone immunoassays are plagued by high variability, especially in the lower concentrations. The CDC Hormone Standardization Program has approached this problem by certifying those testosterone measurement procedures that have a mean bias of ±6.4% when compared to the CDC reference method. Few immunoassays achieved certification, especially at low levels of testosterone1,2 , whereas most of the LC-MS/MS approaches met the CDC criteria providing higher accuracy in total testosterone (TT) measurement.The aim of the present study was to compare low TT concentrations determined by a CDC certified immunoassay with LC-MS/MS.
METHODS
Serum testosterone from 19 adult males (33-89 y) with levels lower than 3.5 ng/mL, was measured by immunoassay. In order to investigate if testosterone–deficiency diagnosis was affected by the method of measurement, the same samples were further analyzed by LC-MS/MS.
RESULTS
All serum samples measured by certified immunoassay, presented a TT level below the reference range obtained by LC-MS/MS3, confirming the diagnosis of testosterone deficiency.Linear regression analyses comparing each immunoassay result with the corresponding LC-MS/MS, showed a slope of 0.47,indicating an overestimation by immunoassay at low concentrations. The concordance correlation coefficient between TT in LC-MS/MS and TT immunoassay was 0.968.
DISCUSSION
Due to the difference related to the specificity and sensitivity of TT methodologies, there is variability in thresholds used to diagnose testosterone deficiency. All the patients evaluated whose TT values fall in the "grey zone" were confirmed as having TT levels below the LC-MS/MS reference range.3
CONCLUSIONS
In our evaluation, both certified immunoassay and LC-MS/MS accurately diagnosed testosterone deficiency in adult males.

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PO017

GESTIONE DEL RISCHIO: fase analitica degli esami eseguiti in regime di urgenza

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La qualità è sicuramente un punto cardine di qualunque azienda, sia che produca beni o che eroghi servizi, quindi, anche per le aziende sanitarie, la qualità è fondamentale. Esistono diverse norme da seguire per poter garantire beni e prestazioni di qualità ai propri clienti, le norme ISO 9000 ne costituiscono un esempio importante. La ISO 9001:2015 focalizza l'attenzione sul risk based thinking, richiedendo all'organizzazione di comprendere il proprio contesto e di determinare i rischi, come base per la pianificazione. La gestione del rischio diviene così il fulcro centrale per qualsiasi tipo di organizzazione: il rischio va esaminato in ogni realtà dell'azienda in un'ottica sistemica. Per le aziende ospedaliere risulta centrale il rischio clinico. Il laboratorio analisi del presidio ospedaliero di Borgomanero dell'ASL NO da sempre gestisce il proprio rischio clinico con specifiche procedure e protocolli. Una porzione fondamentale del rischio clinico per un laboratorio è rappresentata dal rischio analitico presente nell'utilizzo delle strumentazioni di Laboratorio. Per tenere sotto controllo questo rischio il Laboratorio si affida ad un software commercializzato da Bio-Rad Laboratories s.r.l. Attraverso il programma Mission:Control™ viene stimato il rischio relativo ai principali esami eseguiti in regime di urgenza: si tratta di oltre 50 analiti eseguiti su 3 strumenti diversi. Il software si articola in due moduli Risk Calculator™ e QC Designer™, nel primo viene calcolato il rischio attuale, nel secondo viene proposta una strategia da applicare ai controlli di qualità secondo il rischio accettabile secondo gli standard di Laboratorio. Principale misura di questo programma per la stima del rischio è l'E(Nuf), l'errore nei pazienti già refertati. Risk Calculator™ fornisce un'indicazione sugli E(Nuf) attuali sulla base dell'inserimento dei seguenti parametri ottenuti per ciascun analita: la media e la deviazione standard del controllo di qualità, le regole di Westgard applicate al controllo di qualità, l'errore totale accettabile, il numero di test e il numero di controlli di qualità effettuati giornalmente. Emerge, per i test considerati, una situazione abbastanza rassicurante e vengono elaborate nuove strategie di controllo qualità ad hoc da applicare a ciascun analita attraverso il modulo QC Designer™. Questo modulo propone una nuova strategia di controllo di qualità per ogni analita nel rispetto dell'indicazione del rischio accettabile fornita (numero minimo di E(Nuf) possibili consentito dal software) mediante l'inserimento dei seguenti parametri: il numero di provette processate sull'analizzatore che esegue il test, le regole di Westgard da considerare per le nuove strategie di controllo di qualità, il numero di sedute di controllo di qualità giornaliere che si desiderano effettuare e le informazioni ottenute da Risk Calculator™.Nel report prodotto da QC Designer™ ogni test presenta una gestione del rischio personalizzata attraverso una strategia di controllo caratteristica con delle regole di Westgard e un numero di controlli specifici. Questo consente in molti casi un minor numero di controlli giornalieri e quindi un minor spreco di risorse, sia in termini di reagenti e controlli che in termini di risorse umane.

PO018

MONITORAGGIO DEL TRASPORTO DEI CAMPIONI BIOLOGICI. ESPERIENZA DELL'ISTITUTO MULTIMEDICA DI MILANO

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Dal 2018 il Servizio di Medicina di Laboratorio del Gruppo MultiMedica si è affidato a una ditta specializzata nella logistica e nel trasporto dei materiali biologici. Il personale dedicato al servizio, che ha ricevuto una specifica e adeguata formazione, riceve il programma giornaliero da eseguire direttamente per via telematica sugli smartphone in dotazione, con i quali tramite apposita applicazione si può procedere al carico e scarico informatico del materiale tramite lettura dei barcodes posizionati su ogni singolo collo. Software di tracking online consentono di monitorare costantemente le missioni di ogni singolo mezzo, rilevando non solo il percorso seguito ma anche tutti i parametri utili all'individuazione immediata di potenziali Non Conformità (deviazione dal percorso, monitoraggio della temperatura di trasporto, brusche accelerazioni e decelerazioni....), e quindi alla loro immediata gestione e risoluzione. Specifici processi ampiamente collaudati per la gestione delle emergenze consentono di avere la necessaria continuità di servizio in ogni momento. Il software di tracking permette di adempiere a tutte le funzioni necessarie al corretto svolgimento del servizio. Il sistema è in grado di eseguire, per effetto di algoritmi di ricerca operativa, un'ottimizzazione in tempo reale dei tragitti con conseguente contenimento dei costi e delle emissioni inquinanti. Tutte le operazioni di carico e scarico sono accompagnate dall'annotazione delle movimentazioni dei contenitori su appositi log di sistema. Il tracking degli spostamenti consente quindi di avere un costante controllo dell'associazione tra il materiale trasportato, il mezzo (con controllo in tempo reale della temperatura del vano di carico) e il singolo operatore. Una reportistica dedicata riassume i dati salienti delle missioni effettuate e le eventuali anomalie riscontrate. Il personale è qualiicato, propriamente formato e in possesso di specifico patentino ADR. Per la conservazione e il trasporto del materiale si seguono le normative in vigore, impiegando contenitori con triplo imballaggio con contenitori terziari isotermici, che consentono il trasporto a temperatura controllata e in sicurezza, e contenitori secondari a tenuta stagna, sterilizzabili e dotati di adeguate soluzioni anti-sversamento.

PO019

CREAZIONE DI UN MODULO ELETTRONICO PER LA REGISTRAZIONE DELLE NON CONFORMITA' NEL LABORATORIO SMEL2 DELL'ASST PAPA GIOVANNI XXIII DI BERGAMO

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Dal 20/08/2019 il Laboratorio, certificato UNI EN ISO 9001:2015, ha cambiato la modalità di registrazione delle Non Conformità (NC) sostituendo il file excel dedicato, che prevedeva l'inserimento di molto testo, con un foglio di calcolo "csv" usando il pacchetto open source dell'applicazione "Drive - Moduli Google", che può contenere ogni tipo di dato (numeri, testi, ecc). Nel Modulo si registrano: matricola operatore, data, barcode campione, provenienza, settore, tipologia di richiesta (routine/urgenza) e di NC (Campione non pervenuto, vuoto, insufficiente, provetta errata, non correttamente identificato, pervenuto in modo non idoneo, coagulato, emolizzato, errato rapporto sangue/anticoagulante, diluito, contaminato), trattamento (refertazione con nota, annullamento esiti, modifica richiesta, comunicazione telefonica, ristampa etichetta, recupero campione, richiesto nuovo). Lo stesso Modulo registra le NC anche per Strumenti (malfunzionamenti meccanici, idraulici, pressori, d'avvio, motori, di software, d'analisi; trattamento: risoluzione in autonomia/ tramite assistenza telefonica, richiesta d'intervento tecnico) e Segreteria (errori di anagrafica/provenienza/ dati/refertazione, cancellazione esami e/o richiesta; trattamento: cancellazione e/o modifica di esami e/ o richiesta, richiesta dati mancanti, riallineamento anagrafica e/o richiesta ad una esistente, risoluzione telefonica). L'unico svantaggio del Modulo è la dipendenza dall'operatore a registrare le NC, a fronte di molteplici vantaggi: velocità e facilità di registrazione, alta personalizzazione, elaborazione statistica automatica ed efficace (corredata da un file excel completo dei dati), sia delle NC sia dei relativi trattamenti (entrambi selezionabili da un menù a scelta multipla), cui far seguire l'Azione Correttiva appropriata, accesso da tutti i pc tramite un link sul desktop. L'aumento e il riscontro di nuove registrazioni (N=127 nel 2018, contro N=2011 in 20/08/2019 – 31/12/2019) testimonia il successo del Modulo, dovuto all'essere user-friendly e flessibile: permette, quindi, di monitorare attivamente alcuni Indicatori adottati non su base autoreferenziale bensì scelti fra quelli proposti dall'IFCC Working Group "Laboratory Errors and Patient Safety", a guida della performance del Laboratorio.

PO020

SERUM 25-HYDROXYVITAMIN D MEASUREMENT: COMPARATIVE EVALUATION OF THREE AUTOMATED IMMUNOASSAYS

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Aim The different analytical methods for the measurement of serum 25-hydroxyvitamin D (25OHD) are not yet fully harmonized and no consensus exists on a threshold of 25OHD defining a deficiency status. In this study we compared the results from the assays of 25OHD concentrations performed with three different methods to evaluate 1) the presence of potential biases and 2) how much this biases can influence the assignment of patients to the specific 25OHD deficiency/sufficiency categories. Methods LIAISON 25OH Vitamin D TOTAL DiaSorin, ELECSYS VITAMIN D TOTAL II Roche and LUMIPULSE G 25OH VITAMIN D Fujirebio were used. Comparability of methods across the assays was established performing the Passing Bablok regression and the Bland Altman analysis to prove whether the differences found were less than the preliminarily established maximum acceptable bias. Results. Precision and accuracy were verified and the maximum acceptable bias was calculated as 12.4%. We compared the 25OHD results obtained by Liaison with those obtained by Elecsys and then Lumipulse, respectively. Moreover, we performed the same analysis also to results obtained by Elecsys versus Lumipulse. All the Passing Bablok regressions exhibited the presence of a proportional and constant systematic error. The Bland Altman analysis between Liaison and Elecsys showed a bias=-22.5% and between Liaison and Lumipulse a bias=16.2%. Finally, the Bland Altman analysis between Elecsys and Lumipulse revealed a bias=38.2%. All three mean biases were well above the maximum acceptable bias (12.4%) so the 25OHD concentrations measured were not comparable. To evaluate whether the three methods had the same ability to classify patients into different categories of vitamin D levels, we categorized the results obtained by each method in reference classes. Lumipulse categorized most patients into the class with the lowest 25OHD concentrations (>20 ng/mL) whereas Elecsys categorizes the least patients. Conclusions All the compared immunoassays have shown excellent accuracy and reliability in measuring 25OHD levels. Nevertheless, we showed that the assays are not interchangeable due to the lack of comparability of the results as well as to the disagreement in the sub classification of hormone deficiency or sufficiency

PO021

Barbiturici, benzodiazepine e antidepressivi triciclici: verifica delle performance analitiche su sistema Cobas 8000-c 702 (Roche).

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Con l'introduzione della nuova strumentazione Cobas 8000-c702 (Roche) presso il Laboratorio Generale dell'AOU Careggi di Firenze, si è resa necessaria la verifica delle metodiche per la determinazione semiquantitativa su siero di barbiturici (BARBI), benzodiazepine (BENZO) e antidepressivi triciclici (ADT) sul nuovo sistema analitico, come previsto dalle linee guida nazionali e internazionali. Per le prove di verifica, sono stati utilizzati reagenti e calibratori DRI Serum Tox (Thermo Scientific) e come materiale di controllo MAS Tox Control (Biorad). Le performance dichiarate dal produttore per BARBI, BENZO e ADT, espresse in termini di CV% intra-assay, sono rispettivamente per il livello L1 (basso) 8.1, 1.1 e 1.8, e per il livello L2 (alto) 7.9, 0.6 e 0.7; in termini di CV% inter-assay rispettivamente per L1 10.4, 3.7 e 3.5 e per L2 6.8, 2.9 e 1.5. Le prestazioni analitiche dei metodi sono state verificate sia in termini di imprecisione intra-assay (10 ripetizioni) e inter-assay (10 ripetizioni in 10 giorni) utilizzando 3 campioni biologici (C1, C2, C3), sia in termini di accuratezza, espressa come bias % rispetto al valore atteso, utilizzando 2 calibratori (STD) per ciascun analita (10 ripetizioni in 10 giorni). Il limite di accettabilità scelto dal laboratorio per CV% intra-assay, inter-assay e accuratezza è <10%. BARBI: Precisione metodo: CV% intra-assay C1 = 7.9, C2 = 2.6, C3 = 2.1; CV % inter-assay C1 = 21.8, C2 = 16.1, C3 = 10. Accuratezza: bias% STD 500 ng/mL = 6.5, STD 3000 ng/mL = 2.3. BENZO: Precisione metodo: CV% intra-assay C1 = 1.4, C2 = 1.7, C3 = 4.5; CV% inter-assay C1 = 7.4, C2 = 3.0, C3 = 8.2. Accuratezza: bias% STD 50 ng/mL = -4.6 , STD 200 ng/mL = -9.9. ADT: Precisione metodo: CV% intra-assay C1 = 6.6, C2 = 4.1, C3 = 4.3; CV% inter-assay C1 = 22.4, C2 = 29.3, C3 = 19.4. Accuratezza: bias% STD 300 ng/mL = 1.9, STD 500 ng/mL = - 22.6. I risultati ottenuti evidenziano valori di imprecisione intra-assay in linea con quanto dichiarato dall'azienda produttrice e nei limiti di accettabilità scelti, mentre i valori di imprecisione ottenuti nelle prove inter-assay evidenziano un notevole scostamento per BARBI e ADT. Il bias è accettabile per tutte le concentrazioni dei vari analiti ad eccezione dello STD 500 ng/mL ADT.

PO022

Samples Management and Data Protection through SARS-Cov2 infection

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Abstract: Privacy and security of data are emerged as fundamental parts of routine clinical and laboratory practice. The adoption of electronic medical record, the connectivity of information systems and instruments to Internet have increased the risk for violation of patients privacy. The European Union General Data Protection Regulation 2016/679 (GDPR 2016/679) defines how personal information must be used by adopting measures that guarantee high level of security. Accordingly, transparency about the generation and managing of data in research became crucial. The most important ethical issue document is represented by the informed consent, which can be signed by the donor to authorize the collection, storage and use of the samples and associated data for specific research purposes. In May 2020, a screening for novel coronavirus SARS-CoV2 infection in all the patients and employees at the Santa Lucia Foundation, in Rome has been conducted, together with the voluntary opportunity for all the participants to donate biological materials for future researches for COVID-19 disease.

Materials: All participants were subjected to naso-oropharyngeal swab test for viral SARS-CoV2 RNA detection, and blood sampling, for Anti-SARS-CoV-2 antibodies assessment, in electrochemiluminescence immunoassay “ECLIA” (Roche). In addition, those who gave voluntary consent for donation to the Biobank, carried out an adjunctive tube for serum sample. According to the GDPR, the informed consent form is divided into two parts: the first for the donation of samples to the biobank and the second for personal data treatment authorization, including i. authorization to personal data processing, ii. genetic data processing, iii. transfer of biological samples and data to third parties in or iv. outside Europe (Research Institute, University, other biobanks), and v. consent to access to the electronic medical record.

Results: A total of 742 patients and personnel participated at the screening for COVID-19: 468 gave the consent for donation of biological sample to the Biobank, while 43 denied consent. Among donors, 22 (4,7%) refused consent to the transfer/communication of biological samples/associated data to third parties in Europe while 35 (7,5%) denied the transfer/communication outside Europe. Regarding the consent for the genetic data processing, only 5 participants (1,1%) refused authorization.

Conclusions: The fear of an uncontrolled dissemination of personal data, especially on internet or media, is one of the main reasons that preclude participation in research studies. Donation of biological materials and data for biobanking expose patients and donors to risk of threatening of their privacy, but represent a great need for future of translational medicine. Informed consent defines the authorizations for the processing of patient data and the possibility to withdraw at any time. Finally, transparency and security are the pillars for the patients healthcare.

PO023

Determinazione dell'enzima adenosina deaminasi (ADA) nel liquido pleurico su piattaforma Roche Cobas 8000.

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La determinazione quantitativa dell'adenosina deaminasi (ADA) su liquido pleurico è utilizzata come ausilio nella diagnosi di tubercolosi. In seguito al cambio di piattaforma analitica da Dimension Vista 1500 (Siemens) a Cobas 8000–c702 (Roche), si è resa necessaria la verifica delle performance del metodo sulla nuova strumentazione. Sono stati utilizzati Adenosine Deaminase Assay Kit, Calibrator e Control Set (Diazyme Laboratories). Le performance dichiarate dal produttore sono: coefficiente di variazione (CV) % inter-assay <5% e intra-assay <2%. Per la verifica delle performance del metodo sono state valutate: - precisione intra-assay: media (M), deviazione standard (ds) e CV% di 3 campioni di pazienti conservati a -20°C ripetuti 10 volte nella stessa seduta analitica; - precisione inter-assay: M, ds e CV% di 7 campioni a diverse concentrazioni (range 2.38-55.05 U/L) eseguiti in triplo nel corso di 5 distinte sedute analitiche; - accuratezza: il calibratore dell'azienda produttrice è stato analizzato come campione incognito (n=10; valore atteso: 49.1 U/L). Lo spostamento rispetto al metodo precedente è stato valutato confrontando i risultati di 3 campioni umani conservati a -20°C. Precisione intra-assay: per tutti i campioni analizzati è stato ottenuto un CV<5% (C1: M ±ds = 2.1±0.07 U/L, CV%=3.4; C2: 5.46± 0.10 U/L, CV %=1,42; C3: 25.45±0.33, CV% =1.30). Precisione inter-assay: i CV% ottenuti sono risultati <10% per 6 campioni e 12.1% per un campione (M±ds =6.8±0.82 U/L). Accuratezza: si è ottenuta una media dei replicati di 50.5 U/L, con un bias di circa il 3%. Il confronto Dimension Vista e Cobas 8000 ha mostrato i seguenti risultati: campione 1: 22.8 U/L e 24.9 U/L rispettivamente, campione 2: 6.4 U/L e 6.9 U/L, campione 3: 19.9 U/L e 22.1 U/L. L'errore % è risultato quindi essere rispettivamente: 8.9, 7.8, e 10.4. I valori di imprecisione intra ed inter-assay ottenuti sono più alti di quelli dichiarati dall'azienda produttrice ma in linea con i limiti di accettabilità prefissati (10%). Anche se l'assenza di cicli di VEQ forniti da provider accreditati rende complessa una valutazione sull'accuratezza del metodo, i dati ottenuti sono comunque ritenuti sufficienti per l'utilizzo del metodo su Cobas 8000 senza modifiche significative rispetto alla metodica già in uso.

PO024

Thyroid Hormone Assays on Mindray CL-1200i: comparison with other immunoassays platforms.

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Background: Thyroid hormones with their metabolic activity have an essential function for most cellular metabolic processes.The improvements in the analytical sensitivity characteristics of the laboratory tests for thyroid hormones of TSH and of the antibody proteins involved in the pathogenesis of thyroid diseases have changed the diagnostic and therapeutic strategies. Most of the dosages used in thyroid diagnostics are based on immunoassay by using automated platforms performing many analytical advantages, precision, rapid measurements and good sensitivity. The aim of this study is to evaluate and verify the analytical performances of the new Chemiluminescence Immunoassay (CLIA) System CL-1200i from Mindray with serum of TSH, FT4, FT3, Tg, TPOAb and TgAb, for the diagnosis of thyroid disorder, compared with other immunoassays platforms. Methods: We assessed the accuracy, linearity, comparison of the tests, limit of detection, cross reactivity, analytical interferences of 6 analytes (TSH, FT4, FT3, Tg, TPOAb and TgAb) on serum samples according to the guidelines of the CLSI EP15-A3 protocols. The imprecision was expressed as coefficient of variation (CV %). Thyroid analytes were assayed on Mindray CL-1200i and compared with other analytical systems: Beckman Coulter for Tg, Anti-Tg and Anti-TPO and Abbott for FT3, FT4, TSH.Results: The agreement between the results ranged from 99% to 100%. Satisfactory results were obtained in the within run and between run precision studies. The linearity range was acceptable. The clinical samples, respectively with the highest values and those with the lowest values, were tested in triplicate, the carry over effect was not observed. Bland-Altman and Passing-Bablock plots were calculated to evaluate the agreement between the updated values with the two assays.Conclusions: The results obtained in the performance comparison showed a low variability with correlation coefficients of (r)> 0.975 for all the analytes, therefore the systems are comparable to each other. CL-1200i showed high sensitivity and specificity. In this study the Mindray CL-1200i demonstrated that has excellent analytical performance and good reliability, the assays of thyroid panel tests are accurate and are suitable for use in the routine clinical laboratory.

PO025

Stima del lockdown nella rilevazione delle Componenti Monoclonali in Elettroforesi siero proteica

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Durante i due mesi lockdown, in seguito alla pandemia da SARS Cov-2, numerose attività ospedaliere hanno subito un calo rilevante di richieste. Anche il lavoro del Laboratorio Analisi Cliniche ha registrato una riduzione considerevole del numero di prestazioni per gli esami di routine. Infatti il lavoro è stato limitato ai pazienti interni e alle urgenze afferenti al Pronto Soccorso. Questa comunicazione ha lo scopo di riportare l'andamento del lavoro nel settore Proteine, esso ha il compito principale di rilevare la presenza di Componenti Monoclonali (CM) nel siero mediante Elettroforesi siero-proteica (ELP) e successiva tipizzazione con immunofissazone sierica. Il calo delle richieste ha portato ad una diminuzione del numero di CM rilevate. Da Gennaio a Maggio del 2017 sono state eseguite 23573 ELP , nello stesso periodo del 2018 le ELP sono state 27144, ugualmente nel 2019 le ELP sono state 26662, invece nello stesso periodo del 2020 le ELP sono state 20845, un calo percentuale del 21.86 % rispetto al 2019. Si riportano ora i dati delle CM trovate dal 2017 al 2020 sempre i primi 5 mesi: 679, 676, 892, 546, un calo di 346 CM pari al 38.79 % rispetto al 2019. La prevalenze delle CM sul numero totale di ELP eseguite è del 2.83% durante gli ultimi tre anni, tale prevalenza concorda con i dati in letteratura; di queste all'incirca il 46% sono di nuovo riscontro, mentre il 54% rappresentano monitoraggi di CM già note. Quindi delle 346 CM mancanti si può stimare che 159 sono proprio le CM non rilevate, mentre la stima delle CM in monitoraggio è di 187 CM. Dai dati per i 5 mesi e per anni dal 2017 al 2019, si evince un progressivo incremento di richieste di EPS. Al contrario nei primi 5 mesi del 2020, a seguito della riduzione del carico di lavoro della routine, si è riscontrato una diminuzione delle richieste di EPS nei mesi del lockdown marzo ed aprile, mentre già a Maggio si è avuto una ripresa dell'attività. Si auspica che l'incremento di lavoro successivo possa tradursi in un recupero delle diagnosi mancate e che il ritardo diagnostico non comporti conseguenze dal punto di vista clinico.

PO026

Hemoglobin Görwihl a Variant with Impaired Glycation

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The Hemoglobin A1c (HbA1c) concentration is the most suitable marker for long-term for diabetic care. The methods used to measure glycated hemoglobin (Hb) may be affected by pathophysiologic and pharmacologic interferences, such as mutant variants of Hb that cause erroneous results on the determination of HbA1c. This may lead to inconsistent HbA1c values for certain patients and indicate a need to search for Hb variants. We present the case of a 42-year-old male diabetic patient who has a hemoglobin variant which results in an error in the determination of HbA1c.He went to the Department of Laboratory Medicine of the University Hospital of "Tor Vergata" to perform routine blood tests; laboratory results showed that the fasting glucose value was 97 mg/dl and the percentage of HbA1c were 5.8% (40 mmol/mol).The chromatogram performs with Tosoh G11 HPLC Analyzer showed the presence of an additional peak and the sample was analyzed also by capillary electrophoresis through the use of Capillarys 2 Flex Piercing. Also in this case, the electropherogram showed the presence of an atypical hemoglobin. The sample was sent to the Tosoh Bioscience molecular genetic laboratory to carry out genetic analyzes to understand what type of variant is present. DNA analysis allowed the C-G point mutation in codon 5 of the gene that codes for β-globin, which replaces the amino acid proline with alanine (CCT-GCT; βPro-Ala).These studies allowed the identification of Hb Görwihl a variant identical to that found in a German patient with an exceptionally low HbA1c value of 1.5%. Hb Görwihl is not associated with clinical symptoms or hematologic abnormalities but it demonstrates impaired glycation of the ◘ βchains.

PO027

ASSESSMENT OF THE LABORATORY CLINICAL BIOCHEMISTRY AND COAGULATION REQUESTS DURING THE SARS-CoV-2 PANDEMIC IN THE SMEL2 LABORATORY OF THE PAPA GIOVANNI XXIII HOSPITAL IN BERGAMO

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Laboratory Medicine has provided a fundamental contribution and it has assumed an essential role during the pandemic caused by Coronavirus SARS-CoV-2. We have compared the Laboratory Clinical Biochemistry and Coagulation requests (both Siemens) among 22/2/2020 - 3/05/2020 with the same period in 2019, both extracted by our LIS Concerto (Dedalus): it is clear how the pandemic has deeply modified the Laboratory requests. In fact, in 2020 those requests have been 26% less than 2019 (N = 777.281, versus N = 1.055.861 in 2019), against a slight increase in Urgent internal requests (6% more than in 2019). Laboratory Biochemical Chemistry requests decreased by 27,3% compared to 2019, while those of Coagulation increased by 10,4%. Moreover, this comparison shows that Laboratory Biochemical Chemistry requests have not changed in their typology but in their frequency, because the “ranking” of the most requested tests in 2019 in pandemic becomes: Glucose -45%, Creatinine -27,2%, Potassium -18,4%, Sodium -17,2%, ALT -20,9%, AST -20,1%, Urea -21,4%, LDH +15%, PCR -6%, Bilirubin -16,1%, Calcium -26,9%. Also noteworthy are the increase in Procalcitonin requests (+70,4%) and the introduction of Interleukin-6 test (N = 2.921 requests). The frequency of Laboratory Coagulation requests has also changed compared to 2019: PT -4,9%, aPTT +2,8%, D-Dimer +906%, Antithrombin +18%, Fibrinogen +12,4%. The closure of the withdrawal centers for outpatients (it’s about 60% fewer requests) produced the drop in requests found in the pandemic, while the almost total conversion of the wards in areas exclusively dedicated to assist Covid-patients, is the origin of the increased Urgent requests. The increase in Laboratory Coagulation requests reflects the importance that the D-Dimer test has assumed in the pandemic compared to those of Clinical Biochemistry, whose basic performance, probably, have been partly replaced by the increase in the use of blood gas analyzers (+36%, Siemens) and glucometers (+49%, Roche). The pandemic has demonstrated the Laboratory's maturity in facing a new vital challenge, supported by its ability to remodel itself according to the new emerging needs, combining Quality, Competence and Diagnostic Efficiency to better answer to the new diagnosis and treatment needs.

PO028

Una stima dei carichi di lavoro e del fabbisogno di personale per la professione del Tecnico Sanitario di Laboratorio Biomedico

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Scopo La disponibilità di un sistema di misura del carico di lavoro in un laboratorio clinico consente di raggiungere alcuni obiettivi: analisi e valutazione delle attività in corso; revisione delle attività precedenti; programmazione delle attività; confronto dell'attività tra laboratori. Scopo del presente lavoro è proporre un modello preliminare di analisi dei carichi di lavoro per i tecnici sanitari di laboratorio biomedico (TSLB).
Materiali e metodi I sistemi per il calcolo del carico di lavoro si differenziano per la più o meno approfondita rilevazione delle diverse fasi operative. La misura dei carichi di lavoro in un laboratorio clinico va effettuata in maniera rigorosa e oggettiva, facendo riferimento in primis alla numerosità delle prestazioni erogate. Tale misura deve inoltre tener conto di altri elementi essenziali quali: orari di apertura del servizio, tipologia di esami eseguiti, attività complementari all'erogazione della prestazione (controlli di qualità, manutenzione e controllo delle apparecchiature); attività non direttamente riconducibili alla prestazione, ma fondamentali per raggiungere determinati standard qualitativi (riunioni, training del personale, reportistica).
Risultati Per la definizione del calcolo del fabbisogno di TSLB si è partiti dall’analisi dei seguenti fattori: volume di attività; orario di arrivo dei campioni; orario di apertura del servizio; tempo impiegato per svolgere le diverse attività. I dati sono stati elaborati per effettuare il calcolo delle ore uomo/die necessarie per svolgere un determinato volume di attività. Il calcolo del fabbisogno di personale si ottiene dividendo le ore uomo/annue necessarie per le ore/uomo annue lavorate da un TSLB.
Conclusioni L'analisi dei carichi di lavoro è un importante strumento di gestione organizzativa, particolarmente utile in aziende dove la risorsa umana rappresenta il maggior capitale in termini di professionalità. Tale lavoro si propone di suggerire uno strumento per calcolare quanto più fedelmente possibile il fabbisogno di personale tecnico sanitario necessario per svolgere determinati volumi di attività. E’ fondamentale che il sistema adottato si accompagni a verifiche di qualità e all’avvio di procedure di consenso tra i diversi professionisti della medicina di laboratorio.

PO029

Laboratory findings of COVID-19 patients admitted to Desio Hospital: a retrospective study

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Background

Italy was one of the hardest hit European countries by the novel coronavirus SARS-CoV-2 epidemic.1,2The aim of the study was to evaluate the clinical and laboratory characteristics of some cases with coronavirus disease 2019 (COVID-19).

Methods

A retrospective study was conducted on 175 confirmed COVID-19 cases, admitted during March(follow-up through April 30th, 2020) 2020 to Desio Hospital, and were classified into non-survivors (n=62) and survivors (n=113), according to mortality outcome.

Results

The median age was 71 years (IQR 58-80). 70% were males and 23% were admitted to ICU. The most frequent symptoms were dyspnea, fever, and cough. CBC showed leukocytosis in 18% and leukopenia in 14%. Lymphocytopenia (55%) and thrombocytopenia (20%) were also observed.Of the 62 non-survivors, 61 were classified as severe ($p<0.001$).3 The non-survivors were older (80 vs 62 yrs; $p<0.01$), had more comorbidities (93% vs 73%; $p<0.01$) such as cardiovascular diseases and diabetes. Their SpO2 (86% vs. 92%; $p<0.01$) and PaO2/FIO2 (307 vs 206; $p<0.001$) were lower. Compared to survivors, the non-survivors had a significantly higher neutrophil count, a higher N/L ratio and lower hemoglobin values ($p<0.01$). Furthermore, leukocytosis (25.8% vs 13.3%; $p<0.05$) and lymphocytopenia (79% vs 43%; $p<0.01$) were more frequent. Indices of liver damage (AST), renal dysfunction (creatinine and urea), inflammation (CRP and PCT), and coagulation function (aPTT, INR, and D-Dimer), plus creatinine kinase (CK) and hs-troponin T were higher in non-survivors ($p<0.05$). DiscussionAmong non-survivors, leukocytosis was more frequent than leucopenia. Neutrophil count, N/L ratio, and lymphopenia were greater in accordance with other studies.4,5 The increased N/L ratio and CRP might be related to cytokine storms, characterized by massive recruitment of neutrophils in the lung interstitium and reduced lymphocyte control.3,6

Conclusions

Multiple factors such as older age, comorbidities, higher blood leukocyte count, neutrophil count, PCR, D-dimer, AST, creatinine, LDH, CK and hs-troponin T, and lower lymphocyte count were related to non-survivors ($p<0.05$).

1.Deng Y, 2020
2.Gao Y, 2020
3.Zhou Y, 2020
4.Guan WJ, 2020
5.Huang C, 2020
6.Chen N, 2020

PO030

Determinazione di specifici anticorpi IgG anti SARS-CoV-2. Due metodi in chemiluminescenza a confronto.

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Introduzione: La determinazione di specifici anticorpi (Abs) IgG anti SARS-Cov-2 è indicata per l'identificazione di soggetti che sono venuti a contatto con il virus e per il monitoraggio della popolazione. In commercio sono disponibili molteplici test. In questo studio abbiamo valutato le performance analitiche e confrontato la determinazione degli Abs IgG anti SARS-Cov-2 utilizzando due metodi in chemiluminescenza in automazione.

Metodi: La valutazione è stata condotta a Maggio 2020. Sono stati raccolti i sieri di soggetti pervenuti consecutivamente presso il laboratorio di Baggiovara (Modena) e processati su iFlash1800 (YHLO BiotechCo.,Ltd., Shenzhen, China) e ARCHITECT ci2800 (Abbott Laboratories, Ireland). Entrambi gli strumenti utilizzano microparticelle paramagnetiche rivestite di antigene SARS-CoV-2 alle quali si legano gli Abs IgG presenti nel campione. Sono considerati positivi valori ▫10,0 AU/ml in iFlash 1800 e ▫1.4 Index in Architect. La correlazione per risultati qualitativi è stata valutata mediante il test ▫. Il test di McNemar è stato applicato alla tabella di frequenza 2x2 per verificare l'esistenza di differenze tra i risultati ottenuti dai due strumenti analitici. Inoltre, abbiamo valutato la ripetibilità, l'imprecisione e la concordanza dei risultati. I risultati sono stati considerati statisticamente significativi con p ▫0,05.

Risultati: Gli anticorpi IgG anti Cov-2 sono stati determinati su 208 campioni. Di questi, 137 (65.8%) erano IgG positivi con Iflash 1800, e 114 (60.3%) con Architect. 113 campioni (54.3%) erano positivi con entrambi gli strumenti. La correlazione tra i due strumenti è risultata significativa (▫=0.77; 95%CI 0.71-0.82, $p<0.0001$). Per 24 campioni (11.5%) non c'è stata concordanza tra i due strumenti, ma questo risultato non sembra impattare sull'applicabilità dei due metodi (McNemar test: diff -11% da -15.5 a -6.6). Complessivamente, la ripetibilità (<9%), e l'imprecisione intermedia (<8% per iFLash 1800 e <1% per Architect) sono state valutate ottimali.

Conclusioni: Il nostro studio ha mostrato una buona prestazione analitica per le IgG SARS-CoV-2 per entrambi gli strumenti considerati, suggerendo che le IgG potrebbero essere utili per studi epidemiologici e per il monitoraggio di soggetti immunizzati.

PO031

The prevalence of antibodies to SARS-CoV-2 in healthcare workers of Modena. The screening on more than 7000 subjects.

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Introduction:Healthcare workers (HCWs) are considered at high risk of SARS-CoV-2 infection, and during outbreak more than 30 thousand of them were infected. From April, in Emilia-Romagna Region, the screening for all HCWs has started through serological tests. In this retrospective study, we evaluated the prevalence of IgG and IgM antibodies (Abs) anti SARS-CoV-2 in HCWs affiliated to AUSL of Modena.
Methods:We analysed data on asymptomatic HCWs, to assess the immunological status for the regional screening, that allows Abs to be detected every two weeks. In case of positivity to serological test, SARS-CoV-2 RNA detection in nasopharyngeal/oropharingeal swab takes place by RT-PCR.
Results: From 30 March to 31 May 2020, 7711 HCWs were screened using commercial immunocromatography and chemiluminescent tests to detect specific Abs IgG and IgM to SARS-CoV-2. 7361 (95.4%) were IgG and IgM negative while 350(5%) were IgG and/or IgM positive. In particular, 72 (0.9%) were IgG+ and IgM+, 233 (3%) were IgG+ and IgM-, 45 (0.6%) were IgG- and IgM+. At follow up, 4.6% of analyzed subjects were positive, of these 4.2% was IgG+ and only the 0.4% was IgM+. The RT-PCR performed on positive HCWs revealed that 7% of them was positive for viral RNA.
Conclusions: Serological tests could bridge the gap between the disease and symptoms onset, allowing to detect both active and past infection when used as screening. Results of serological tests were influenced by several factors: in asymptomatic people it is difficult to establish the moment of contact, so it is plausible that the serological tests were negative because the body hasn't had the time to develop the antibodies. Conversely, detection of a very low percentage of IgM in conjunction with high IgG levels could be due to a remote contact with the virus, but also due to a scarce sensibility of tests. Furthermore, it is more likely that the asymptomatic people develop a little or no immune reaction because they didn't developed symptoms. Although the prevalence of SARS-CoV-2 infection is low among HCWs of Modena, Abs detection could be useful to detect subjects who developed an immune response, and to understand the epidemiology of the infection.

PO032

The accuracy and clinical validity of Next Generation Sequencing in the genetic testing of two orthopaedic rare diseases

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Background:Next Generation Sequencing(NGS) technology has become essential also for the diagnosis of inherited orthopaedic rare diseases.We used a Health Technology Assessment approach to evaluate the accuracy, clinical validity and the budget impact of a protocol with NGS-Ion Torrent™ equipment in substitution to one with Sanger sequencing in two musculoskeletal rare diseases: Osteogenesis Imperfecta(OI) and Hereditary Multiple Exostoses(HME).
Methods:First, we screened literature searching for guidelines, systematic reviews, and clinical trials. Second, we performed a mutational analysis for individuals with clinical diagnosis or suspect of OI or HME. We analyzed EXT1 and EXT2 genes for HME, and genes related to both dominant(Col1A1,Col1A2 IFITM5) and recessive forms (CRTAP,LEPRE1,PPIB,SERPINF1,WNT1,TMEM38B,BMP1, of OI. We compared results obtained with NGS to Sanger sequencing. Finally, we performed a Budget Impact analysis applying the typical Activity Based Costing methods in order to identify the cost drivers associated with each type of sequencing analysed.
Results:The literature search identified only 2 studies reporting a good accuracy of NGS to detect disease-causing variants. In our primary study, in 200 OI patients, we identified with NGS, 150 different mutations in as many patients. 64(43%) patients had mutation in COL1A1 gene, 68(45%) patients in COL1A2, with both NGS and Sanger. The remaining patients had mutations in the genes associated to OI. Likewise, for HME,we observed genetic anomalies in 184/200 HME patients.We identified 91(49%) different mutations for EXT1 gene, and 26 for EXT2 with both NGS and Sanger sequencing. Two mutations were identified only with NGS protocol. No false positive or negative results were observed. Introducing NGS protocol produces a per-patient saving of ▫715 for OI and ▫85 for HME.Introducing NGS protocol produces a saving of ▫ 37.565 for OI and ▫ 4.440 for HME on the budget.
Conclusions:Our is the first study provides data of the application of NGS in a large cohort of patients with rare orthopedics disease, highlighting its good accuracy and reliability. Adoption of NGS by public health laboratories

reduces time and costs to diagnosis and it offers the budget holder room for saving.

PO033

Pilot "External Quality Assessment" to evaluate Inter-laboratory agreement for free light chains measurement in cerebrospinal fluid, serum and for K Index evaluation

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Introduction

The kappa index, calculated by dividing the cerebrospinal (CSF)/serum kappa free light chain (KFLC) ratio by the CSF/serum albumin ratio, is gaining increasing interest as an indirect marker of intrethecal activation of the humoral immune response. However, the lack of consistent data on inter-laboratory agreement in CSF and serum KFLC measurements is one of the factors that hamper the use of kappa index in routine practice.ObjectiveTo assess inter-laboratory agreement in CSF and serum KFLC measurements and kappa index values.

Methods
Fifteen paired CSF and serum samples were analyzed in all participating laboratories (nr=8). Four centers used Binding Site instruments and assays, 3 centers used Siemens instruments and assays, and one center used a Siemens instrument and a Binding Site assay. Absolute individual agreement between laboratories was calculated using a two-way mixed effects intraclass correlation coefficient (ICC). Cohen's kappa coefficient was used to measure inter-laboratory agreement on positive (¤5.8) kappa index values.

Results
Within Binding Site laboratories, ICC for KFLC measurements was 0.96 (95%CI: 0.9-0.98) for CSF, 0.93 (95%CI: 0.63-0.98) for serum and 0.97 (95%CI: 0.94-0.99) for kappa index values.Within Siemens laboratories, ICC for KFLC measurements was 0.99 (95%CI: 0.97-100) for CSF, 0.93 (95%CI: 0.48-0.98) for serum and 0.95 (95%CI: 0.89-0.98) for kappa index values.ICC calculated for all laboratories was 0.93 (95%CI: 0.87-0.97) for CSF KFLC, 0.81 (95%CI: 0.53-0.93) for serum KFLC and 0.65 (95%CI: 0.43-0.84) for kappa index. Cohen's kappa coefficient for a positive kappa index was 0.89 across Binding Site laboratories, 0.70 across Siemens laboratories, and 0.77 across all laboratories.

Conclusion
There was an excellent agreement in CSF KFLC measurements and in kappa index values within laboratories using the same instrument and assay (Binding Site or Siemens), while serum KFLC measurements were less concordant. Agreement across all laboratories was decreased when including the laboratory using a Siemens instrument coupled with a Binding Site assay in the analyses. Concordance for a positive kappa index was substantial across all laboratories and within Siemens laboratories, and very good within Binding Site laboratories.

PO034
Cloni linfocitari in due casi di malattia mieloproliferative JAK2+.

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La mutazione JAK2V617F è stata riscontrata in un'elevata percentuale di casi di malattie mieloproliferative (SMP) BCR/ABL1- mentre è poco comune nei LNH. Alcuni studi hanno dimostrato un aumentato rischio di sviluppo di malattia linfoproliferativa (MLP) in pazienti con SMP BCR/ABL1-, significativamente più elevato nei maschi ed in presenza della mutazione JAK2V617F. Riportiamo i casi di due pazienti di sesso maschile con SMP JAK2+ ed evidenza di un clone linfocitario nel sangue periferico. Il primo paziente di 72 anni, viene inviato a consulenza ematologica per anemia (Hb:113gr/L) ipocromica e microcitica, leucocitosi (WBC:1594/µL) con linfocitosi (Linfociti:5560/µL) e trombocitosi (PLT: 1405/µL). Il paziente presenta un ulcera sanguinante ad una gamba, verosimilmente causa dell'anemia. Viene richiesta la ricerca della mutazione JAK2 e vengono effettuati uno striscio di sangue periferico e una tipizzazione linfocitaria. Il secondo paziente di 84 anni, già in terapia farmacologica per SMP JAK2+ diagnosticata presso altra struttura, presenta anemia (Hb:98gr/L) ipocromica e normo-macrocitica, leucocitosi (WBC:34000/µL) senza linfocitosi (Linfociti:1390/µL) e trombocitosi (PLT: 604/µL). Viene eseguita una valutazione morfologica e successivamente lo studio dell'immunofenotipo linfocitario. Allo striscio di entrambi i pazienti sono presenti linfociti di piccole e medie dimensioni con cromatina mediamente addensata. Nel primo caso, l'analisi citometrica evidenzia una quota di linfociti B (3336/µL) con immunofenotipo CD19+CD20+ +CD22+CD5-CD10-FMC7+CD11c+KAPPA; è presente la mutazione JAK2V617F. Nel secondo caso è presente una quota di linfociti B (523/µL) con immunofenotipo CD19+CD20++CD22+CD5-CD10-FMC7+CD11c+LAMBDA. La coesistenza di una SMP e di una SLP è molto rara e spesso è difficile stabilire se le due condizioni abbiano origine dallo stesso clone o da cloni differenti. Da un punto di vista clinico ci può essere una sovrapposizione di sintomi come l'anemia o la splenomegalia e la scelta terapeutica può essere difficile non essendoci in letteratura molti dati a riguardo. Lo studio citometrico della clonalità dei linfociti B nelle SMP JAK2+ può essere utile per poter individuare i pazienti a rischio di sviluppare una MLP e migliorarne la gestione clinica.

PO035

Comparison of electrochemiluminescence and ELISA methods in the detection of IL-6

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Interleukin-6 (IL-6) is a pleiotropic cytokine that is released under a wide range of conditions. IL-6 plays a well-known role in the chronic low-grade inflammation characteristic of obesity, diabetes and cardiovascular disease, as well as the acute immunological crises of infection and sepsis. IL-6 concentrations are typically quite low, in the range of 0.2–7.8 pg/ml but can exceed levels of 1600 pg/mL in sepsis.Several studies have shown IL-6 to be an independent prognostic biomarker where high IL-6 levels were associated with a poorer outcome and the cutoff point of serum IL-6 level was investigated as an indicator of treatment strategy. Therefore, we compared the quantitative analysis of IL-6 through two different analytical methods. The performance of two IL-6 detection methods was compared: Elecsys ECL IL-6 (Roche) and IL-6 Human Instant ELISA™ Kit (Invitrogen, ThermoFisher Scientific). Each assay was performed according to the manufacturer instructions. Sixty-eight serum samples from subjects with viral infection were analyzed. IL-6 measures with ECL and ELISA methods significantly correlated (Pearson Correlation Coefficients $r=0.77$ $p<0.0001$), but absolute concentrations differed among assays. While ELISA kit was able to detect the very low levels of IL-6 typically found in healthy individuals, but failed to measure 9/68 (13%) samples with very high concentrations (>200 pg/ml), ECL was able to capture higher values (only 2/68 samples require dilution and reassay). All serum samples were divided into subgroups based on IL6 levels detected with ELISA and the data were again compared by inserting a multiplication factor. Its ranges from 2 up to 5 and it was dependent on the IL-6 values: it was higher for low levels of IL-6 (0.1-7 pg/ml) and decreases with increasing cytokine (100-200 pg/ml). In particular, values lower than 45 pg/ml with ELISA, were 4 times higher with ECL IL-6. This study establishes the validity of IL-6 measurement by ECL and ELISA immunoassays. However, there are some discrepancies in quantification related to different analytical sensitivities and procedures. This make it necessary to choose a single method to evaluate sample in longitudinal manner or at least to know the corrections to be adopted to switch from one method to another.

PO036

Appropriateness of cryoglobulin test request: a retrospective assessment over 5 years

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INTRODUCTION

Cryoglobulinemia is a rare pathology which is difficult to diagnose both clinically and in the laboratory. To improve analytical accuracy, some authors have suggested the repetition of sample examinations, especially with persistent clinical suspicion, to avoid false-negative results. On the other hand, clinical recommendations suggest that positive CRG analysis must be confirmed in a second test at an interval of >12 weeks to allow classification of cryoglobulinemic syndrome. The aim of the work is to verify adherence to the recommendations by clinicians.

METHODS

We study CRG analyses over a 5-year period (2015 - 2019) at the Department of Laboratory Medicine, Modena (Italy): 6,716 samples for 4,963 subjects (mean age 59 ± 18 years; 60% female). The data were extracted from Laboratory Information System.

RESULTS

Initial negative testing was not confirmed in 10% (n=44) of those retested (n=455, 11%), In our study, of the 354 subjects with positive CRG initial testing, positivity was not confirmed in 17%, independently of the time of retesting. We documented at least two tests only in 16% (n=809) of all tested subjects (11% of negative and 39% of positive CGR). Estimates suggest that in up to 13% (n= 105) initial reported outcomes are not confirmed.

CONCLUSION

Although results from a single serum sample should not be considered sufficient for cryoglobulinemia diagnosis or disease exclusion, we report poor adherence to these recommendations, suggesting that clinicians avoid recommended confirmatory testing, especially if initial results are negative ($p<0.001$).The results show that 10% of initial CRG negative outcomes could be positive, suggesting a high rate of potential misdiagnoses especially as confirmatory retesting in this study was only requested for 1 in 10 CRG negative subjects. The low estimates for confirmatory CRG testing call for intervention from laboratories. The authors suggest the inclusion of a note when CRG is found for the first time, such as: “To confirm cryoglobulinemia, it is recommended to repeat testing after 12 weeks”. In case of a first negative

CRG test, the note could be: “In the presence of strong clinical suspicion of cryoglobulinemia, it is recommended to repeat the analysis to exclude pre-analytical problems”.

PO037

Inaspettata componente monoclonale in una bambina epatotrapiantata di 15 mesi

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La gammopatia monoclonale è raramente riscontrata in età pediatrica. La malattia linfoproliferativa post-trapianto (PTLD) comprende un gruppo eterogeneo di malattie che si manifestano in corso di terapia immunosoppressiva post-trapianto. La prevalenza nella popolazione generale di 1/3.800 varia in base all'organo trapiantato e all'agente immunosoppressivo utilizzato; è più elevata nella popolazione pediatrica, probabilmente perché non precedentemente esposta al virus di Epstein Barr (EBV), correlato alla patogenesi. Poichè i sintomi iniziali sono aspecifici, la PTLD dovrebbe essere considerata in tutti i trapiantati.

Paziente di 7 mesi sottoposta ad epatotrapianto (OLT) per cirrosi scompensata da atresia biliare, già operata senza successo a 41 giorni di portoenteroanastomosi secondo Kasai, sviluppa componente monoclonale (CM) all’età di 15 mesi. Il decorso post-trapianto è privo di complicanze, ad eccezione di un’infezione da Herpesvirus umano 6. Inizia terapia immunosoppressiva con Basiliximab, Metilprednisolone, Tacrolimus e prosegue esclusivamente con Tacrolimus a dosaggi decrescenti. La funzionalità del graft epatico, i parametri auxologici e lo sviluppo psicomotorio si mantengono nella norma. A 8 mesi dall’OLT viene ricoverata per infezione respiratoria acuta; AST, ALT, ALP, GGT, PT, Alb nella norma; LDH 458 U/L (192-321); Bil tot 0.17 mg/dL (0.2-1.2). All’elettroforesi sieroproteica è presente CM IgG k, 8.3 g/L. Risulta negativa la ricerca del DNA-EBV e del DNA-Citomegalovirus. Si riscontra positività per anticorpi anti-microsomi epatici di tipo 1, in assenza di epatite autoimmune all’esame istologico su biopsia epatica e di segni di rigetto. Nei tre mesi successivi le condizioni cliniche rimangono stabili con assenza di linfoadenomegalie e splenomegalia. La posologia del Tacrolimus viene ridotta per riscontro di livelli ematici elevati rispetto all’epoca post-trapianto; per acidosi metabolica da nefropatia iatrogena da Tacrolimus viene somministrato sodio bicarbonato per os.

La CM, spia di disordini linfoproliferativi osservata in soggetti adulti sottoposti a OLT, nel caso descritto EBV negativo potrebbe rappresentare un marcatore precoce di PTLD. Tale ipotesi deve essere confermata attraverso uno stretto monitoraggio clinico-laboratoristico-strumentale.

PO038

The biobank of the ASST "Papa Giovanni XXIII" of Bergamo: role and opportunity in the pandemic context Covid-19

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AIM

The aim of this work is to describe the role of the ASST Papa Giovanni XXIII Research Biobank (ASST-PG23) of Bergamo in the Covid19 pandemic context. Lombardy was the most affected Italian Region and the province of Bergamo has registered 14177 cases (June 23, 2020). For this reason, the ASST-PG23 can plays an important role in sample collection and analytic research.

MATERIALS AND METHODS

The ASST-PG23, established in 2014 and UNI EN ISO 9001: 2015 certified, is a non-profit Service Unit for the collection, preparation and storage of biological material. The ASST-PG23 covers the lack of a centralized and reliable system for the storage of biological samples and associated epidemiological clinical data. The ASST-PG23 contributes to the research by providing a large quantity of samples indispensable for the results to be statistically valid.After the Pandemic COVID-19 infectious pathology, the Biobank and Database project for Clinical and Basic Research in SARS-COV-2 infections was developed with the aim of preserving clinical samples and data for the development of retrospective studies and future prospect studies to be submitted to the Ethical Competence Committee. The ASST-PG23 is equipped with laboratory instrumentations, rooms with electronic controlled access, 27 freezers (-20 ° C / -70 ° C) with remote control, 10 liquid nitrogen containers and finally the software for the management of samples and data “Freezerworks” which guarantees anonymization and traceability complete the equipment.

RESULTS

The ASST-PG23 increased its activities by carrying out the biobanking of 10288 aliquots belonging to 4719 patients discharged or hospitalized by the ASST-PG23 First Aid or in post-discharge follow-up, and healthcare personnel under surveillance for the infection, as at June 26, 2020.In particular, 1256 respiratory samples (nasopharyngeal swab and broncho-alveolar lavage) and 8812 blood aliquots (plasma, serum, whole blood) were collected, including the positive serum for neutralizing IgG anti SARS-COV-2 deriving from the surveillance of healthcare personnel and anyone who has been in contact with them as defined by the Lombardy Region.ASST-PG23 samples have currently been used to start about 20 research projects, representing one of the most important assets available to the national and international scientific community. From May 2020, the ASST-PG23 is part of the Italian Node of the European Research Infrastructure of Biobanks and BioMolecular Resources (BBMRI-ERIC).

CONCLUSIONS

ASST-PG23 represents an important bioresource for diagnosis and research. Initially the samples were collected spontaneously and unevenly within our hospital. With the institution of the ASST-PG23 the samples were organized according to national and international standards. After reaching this primary objective, the ASST-PG23 during the pandemic period, continued with the implementation of the COVID-19 sample collection. This implementation is considered as a precious resource for collaborative studies, to develop knowledge scientific useful to produce new accurate and fast diagnosis systems as well as effective vaccines and antiviral drugs.

PO039

DIAGNOSTICA DELLE INFEZIONI VIRALI DELLE DONNE IN GRAVIDANZA : ESPERIENZA IN UN SINGOLO CENTRO

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BACKGROUND E OBIETTIVI: Le infezioni virali durante la gravidanza possono essere trasmesse dalla madre al feto in maniera verticale. Gli agenti infettivi più frequentemente coinvolti nella trasmissione materno fetale sono: Citomegalovirus, virus della Rosolia, Toxoplasma gondii. Lo scopo del lavoro è stato quello di evidenziare la presenza di anticorpi IgM / IgG diretti contro tali virus nelle donne in gravidanza residenti sull'isola d'Ischia, valutando il rischio di infezione congenita nei neonati. MATERIALI E METODI: Da Gennaio 2017 a novembre 2019 sono state arruolate 210 donne in gravidanza, nelle quali è stata testata la presenza di anticorpi per Toxoplasmosi, Rosolia e Citomegalovirus, adoperando la metodica Vidas. Tale tecnica si avvale del principio ELFA che prevede l'associazione del metodo ELISA con rivelazione finale in fluorescenza. RISULTATI: Dall'analisi dei risultati, si evince che il 76,50 % delle donne in gravidanza non è protetto dalla Toxoplasmosi, mentre il 21,08 % risulta immunizzato. Si è, inoltre, osservato un caso di infezione contratta durante la gravidanza e trasmessa al feto. In aggiunta l'80 % delle donne gravide ha contratto l'infezione da citomegalovirus e rosolia. I risultati ottenuti sono in linea con i dati epidemiologici nazionali. CONCLUSIONE: Alla luce di quanto osservato, appare chiara l'importanza dello screening anticorpale, nonché l'eventuale diagnosi precoce delle infezioni da Toxoplasma, Citomegalovirus e Rosolia durante la gravidanza. E' proprio grazie ad una tempestiva diagnosi che si può ridurre il rischio dell'infezione al feto o allorché ciò non dovesse essere possibile, si può comunque intervenire precocemente con opportune cure farmacologiche rivolte alla madre, con l'obiettivo di migliorare il decorso della malattia e offrire al nascituro maggiori probabilità di sopravvivenza e guarigione.

PO040

ESPERIENZA DELLA MEDICINA DI LABORATORIO DEL P.O. "SAN PAOLO" NELLA PANDEMIA SARS-COV2

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L'attuale pandemia provocata dal ceppo di b-Coronavirus SARS CoV-2 ha reso necessario misure volte ad identificare tempestivamente i casi positivi, sintomatici ed asintomatici, per attivare idonee misure di contenimento alla propagazione del virus. L'analisi della presenza dell'RNA virale mediante Real-time PCR, da tampone rino-faringeo e/o da campioni delle basse vie respiratorie, è la procedura considerata più attendibile per porre diagnosi di infezione di SARS CoV-2. L'esigenza di analizzare rapidamente e con costi ridotti ampie fasce di popolazione, ha richiesto l'introduzione anche di test sierologici. L'UOC di Medicina di Laboratorio del P.O. "San Paolo" dell'ASL Napoli1 Centro, individuata quale unico laboratorio di riferimento per l'area metropolitana di Napoli, dal 16 marzo al 30 giugno 2020, ha esaminato circa 11000 campioni mediante Real-time PCR (kit AllplexTM 2019-nCoV Seegene e kit Roche Tib Molbiol). L'RNA virale è stato estratto con sistemi automatizzati basati sull'utilizzo di biglie magnetiche. Sono stati, inoltre, eseguiti 1520 test sierologici con metodica in chemiluminescenza (Maglumi 2019-nCoV IgG/IgM) e 270 test rapidi in immunocromatografia (Techno Genetics). Dei campioni analizzati, mediante Real-time PCR, il 6,5% è risultato positivo alla presenza dell'RNA virale, di questi il 56% era di sesso maschile con una prevalenza nella fascia di età compresa tra i 50 e 60 anni. Nella fase iniziale dell'infezione, in circa il 30% dei soggetti in cui è stata rilevata la presenza virale, si è osservata una positività ai tre geni target E, N e RdrP. Tali soggetti durante la fase di follow-up hanno mostrato la persistenza dell'infezione per più settimane, evidenziata, di contro, dalla sola positività dell'amplificazione del gene N. I test sierologici eseguiti come screening del personale sanitario e dei pazienti asintomatici hanno evidenziato una positività alle IgG e /o alle IgM nel 2,6% dei casi, di questi solo il 12,5% è risultato essere positivo anche al tampone. Il test sierologico è una tecnologia ancora non pienamente validata, inficiata dalla possibile cross-reattività con altri coronavirus, a causa di tale limitazione l'analisi Real-time PCR rappresenta il test di riferimento sia in fase di screening che di follow-up della popolazione.

PO041

Cryofibrinogemia: laboratory procedure and clinical effects

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Introduction: Cryofibrinogenemia is a rare cryopathy, identified in 1955 by Korst and Kratochvil. The clinical picture presents itself as a typical vasculitis and more severe course of a cryoglobulinemia, it is an occlusive vascular disorder of the small vessels that causes non-healing skin ulcers compared to the precipitation of cryofibrinogen. Less known and less investigated than cryoglobulinemia is to be considered whenever suggestive clinical manifestations and research of negative cryoglobulinemia are present.The lack of shared diagnostic criteria determines the cryofibrinogenemia still remains an under-diagnosed pathology but with significant morbidity and an increase in mortality.Methods: In laboratory practice, the search for cryofibrinogenemia must be associated with the search for cryoglobulinemia to perform the differential diagnosis between the two pathologies.In the laboratory one blood tube with anticoagulant (EDTA) and one without are received, both are stored at 37 ° C until the plasma / serum is separated which is then kept at 4 ° C for 7 days to observe the presence of any cryoprecipitate in the case both cryofibrinogenenmia will form in the plasma tube alone. In the event that the precipitate is present in both tubes, it is necessary to investigate the presence of cryoglobulin.In the presence of cryoprecipitate, the necessary washes and hot resuspension are carried out to carry out typing on agarose gel, in turn hot, with antisera: anti-fibrinogen (Agilent, USA), IgG, IgM, IgA, Kappa and Lambda (SEBIA, Florence). The presence of precipitate against the antifibrinogen antiserum confirms the presence of cryofibrinogemia.Conclusions: The laboratory should equip itself to carry out the study of "cryoproteins", is proteins that can precipitate at low temperatures.Since the preliminary phase is fundamental for the success of the examination, it is necessary that the dedicated sampling centers organize themselves to maintain the heat chain until the separation of the serum / plasma, while the subsequent identification can be performed in a single laboratory. reference by territorial area.

PO042

Clinical case: IgA lambda or heavy chain disease, the role of the laboratory is crucial

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Introduction: Electrophoresis is the method for separating serum proteins and detecting the presence of monoclonal components (CM). When an abnormal peak is detected, an agarose immunofixation (IFE) with specific antibodies against the heavy chains α, γ, μ and against the light chains κ-λ is performed to typify the immunoglobulin (Ig) consisting of an associated heavy chain to a specific light chain.It can happen, however, that the light chain, especially if lambda, is not always easily detectable, due to particular steric conformations of the molecule, thus being able to lead to an incorrect diagnosis of heavy chain disease.Clinical case: a 52-year-old patient performs an electrophoresis and immunofixation at the Corelab Laboratory (AOU-AUSL Modena) during the periodic check for IgG Lambda multiple myeloma.An immunotyping in zonal capillary electrophoresis (Capillarys Tera, SEBIA, Florence) was performed which showed no particular anomalies in the tracing and an immunofixation (IFE) on agarose gel (IF 2/4 SEBIA, Florence) which revealed the presence of a heavy chain IgA not associated with any light chain. We then proceeded to incubate 10 L of serum with 100 ▫L of total anti-kappa antiserum and further 10 ▫L with 100 ▫L of total Lambda for 24 hours. After centrifugation at 10,000 rpm on the supernatant a 3-track IFE with anti-IgA antisera, total anti-kappa and total anti-lambda is performed. The precipitation of IgA remained visible in the serum incubated with anti kappa at IFE, while in the incubated with total Lambda no immunoprecipitate was detected, indicating that all immunoglobulin had been complexed and eliminated from the reaction environment. The patient therefore presented with a CM IgA-Lambda in the serum.Conclusions: In patients with MM being treated, a change in the immunoglobulin produced by the plasma cell clone may occur, which can make it less greedy towards the antisera used in laboratory practice. Ig epitopes could in fact be altered or inaccessible, in particular with reference to lambda-type light chains. This could lead to an incorrect diagnosis of heavy chain disease.The continuous contact with the clinician and above all the experience of the laboratory technician can lead to an accurate identification of a CM essential to formulate a correct clinical diagnosis and to direct towards an appropriate treatment of the patient.

PO043
IMPIEGO DI PIU' SISTEMI AUTOMATICI PER LA RICERCA ED IDENTIFICAZIONE DI ALLOANTICORPI

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Introduzione:I tests di screening anticorpali rappresentano un punto critico per l'evasione delle richieste trasfusionali,per la refertazione dei donatori e per la gestione di donne gravide e in età fertile.L'identificazione dell'anticorpo irregolare clinicamente significativo (RAIA) può essere difficoltosa e richiedere,in alcuni casi,un confronto metodologico e l'impiego di ulteriori supporti,oltre ad una comprovata esperienza dell'operatore.Scopo dello studio è stato quello di valutare l'utilità di diverse metodologie automatizzate,nonché l'utilizzo di pannelli eritrocitari enzimatici di supporto alle medesime,al fine di giungere ad una corretta diagnosi e giusta terapia.
Metodi:I campioni risultati positivi al RAIA, sono stati sottoposti ad identificazione anticorpale mediante l'impiego del pannello eritrocitario a 11 cellule (ID-DiaPanel Biorad).Di questi,i campioni con positività aspecifiche sono stati testati con un ulteriore pannello papainizzato a 11 cellule(ID-DiaPanel P Biorad).Infine tutti i campioni che hanno presentato delle reattività non determinabili sono stati testati con il pannello eritrocitario a 11 cellule(ResolvePanel A SystemOrtho).
Risultati:Su un totale di 41.000 RAIA effettuati nel 2019,247 sono risultati positivi(36 donatori di sangue e 211 pazienti/donne gravide).Dei 247 campioni positivi testati,182 (73%)sono stati identificati con il pannello eritrocitario Biorad,42(17%)con il pannello papainizzato e 23(10%)con quello dell' Ortho.Con il pannello papainizzato si è riuscito ad identificare anticorpi anti D- c- E-K- Jka, mentre con il sistema Ortho sono state identificati gli anticorpi anti-M e anti-Cw.
Conclusione:Il sistema Biorad ha presentato una buona performance metodologica,permettendo l'identificazione del 90% dei campioni analizzati.Tuttavia tale metodica ha mostrato difficoltà nell'identificazione degli anticorpi freddi, meglio rilevati con il sistema Ortho.Inoltre l'impiego routinario di un pannello pretrattato con papaina, che intensifica reattività mascherate o a basso titolo,è risultato di grande supporto.Questo implica che disporre di più sistemi è un grosso vantaggio per l'identificazione di anticorpi parimenti non determinabili con un solo sistema, riuscendo ad ottenere un risultato completo e una giusta refertazione.

PO044
GESTIONE DEL LABORIATORIO DI IMMUNOEMATOLOGIA PIASTRINICA PRESSO IL SIT DELL'ASL CASERTA

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Introduzione:Gli anticorpi antipiastrine possono avere un'origine alloimmune o autoimmune.IL SIT dell'ASL Caserta dall'anno 2019 ha implementato metodiche di diagnosi sierologica e molecolare per la trombocitopenia alloimmune ed autoimmune istituendo un laboratorio di immunoematologia piastrinica. Scopo del lavoro è stato quello di investigare le possibili cause per una risoluzione efficace ed efficiente della patologia.
Metodi:Da gennaio 2019 sono giunti al SIT,30 casi di trombocitopenia.Su tali pazienti è stata effettuata l'anamnesi,esame emocromocitometrico(Sismex),ricerca degli anticorpi antipiastrine con metodica capture P(Immucor).
Risultati:16 neonati a termine con 100.000/mmc piastrine,positivi al test per la ricerca degli autoanticorpi antipiastrine e le rispettive madri positive al test per la ricerca degli alloanticorpi antipiastrina.Una paziente donna di 30 anni con anamnesi negativa per patologie oncoematologiche,sotto terapia farmacologica con tetracicline, si presenta alla nostra attenzione con 87000/mmc piastrine,risulta positiva alla ricerca di autoanticorpi antipiastrine e negativa alla ricerca degli alloanticorpi antipiastrine.10 pazienti uomini di età media 60 anni,con anamnesi negativa per patologie ematologiche ed oncologiche si presentano alla nostra attenzione con 80000/mmc piastrine risultano positivi alla ricerca degli autoanticorpi antipiastrine.3 pazienti uomini di 65 anni con anamnesi positiva per ipercolesterolemia familiare resistente a terapia farmacologica sottoposto ad LDL Aferesi,si presentano con una conta di 80000/mmc piastrine risultano positivi alla ricerca degli autoanticopi antipiastrine.
Conclusioni:I dati mettono in luce che la maggior parte delle trombocitopenie riscontrate sono determinate dalla trombocitopenia autoimmune neonatale fetale,nel caso della paziente donna la trombocitopenia è stata provocata dalle tetracicline e dopo sospensione del farmaco è stato osservato un incremento della conta piastrinica fino alla ripristino della normalità.I pazienti uomini con trombocitopenia sono ad eziologia idiopatica in essenza di alterazione di natura batteriologica e segni di splenomegalia.Concludendo l'efficienza e l'efficacia di metodi diagnostici sono fondamentali per il clinico per la risoluzione della patologia.

PO045

Controllo interno come indicatore di qualità nella gestione trasfusionale di pazienti con alloimmunizzazione eritrocitaria multipla

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INTRODUZIONE: Il corretto approccio ai test pre-trasfusionali di pazienti con alloimmunizzazione eritrocitaria multipla consente di procedere all'assegnazione di unità di emazie concentrate filtrate ai fini della completa sicurezza trasfusionale.
MATERIALI E METODI: Da Gennaio a Maggio 2020, sono pervenute, presso il trasfusionale, 15 richieste di emazie per pazienti con alloimmunizzazione eritrocitaria multipla. Come descritto nel Manuale del Buon Uso del Sangue, è stato eseguito il protocollo di Type&Screen (gruppo ABO, Rh, Kell -schedine Bio-Rad-- e Test di Coombs Indiretto (TCI) -ID-DiaCell I-II-III Bio-Rad-). Alla positività del TCI, è seguita l'identificazione anticorpale (ID-DiaPanel a 11 cellule Bio-Rad-) e nei casi di immunizzazione multipla è stata eseguita la metodica di adsorbimento, incubando il siero del paziente con emazie omozigoti per l'antigene corrispondente ad ogni anticorpo identificato. In tal periodo, è stato aggiunto un controllo interno a conferma della corretta identificazione anticorpale, cimentando il plasma del paziente con unità di emazie esprimenti l'antigene per cui si vuole verificare la presenza dell'anticorpo identificato. Infine, si è provveduto all'assegnazione di unità di emazie ad ogni paziente eseguendo prove di compatibilità completa.
RISULTATI: Implementando con un controllo interno di sicurezza interlaboratoristica il protocollo applicato presso il SIT ASL CE, è stata confermata la corretta identificazione anticorpale per tutti i 15 casi esaminati. Inoltre, è stata valutata la frequenza degli alloanticorpi riscontrati ed essa riflette il grado di maggiore immunogenicità degli antigeni eritrocitari: D>K>c>E>C>e.
CONCLUSIONE E DISCUSSIONE: Premesso che presso il SIT ASL CE sono rispettate le procedure standardizzate per lo screening di anticorpi irregolari clinicamente significativi, la metodologia applicata nel suddetto arco temporale ha permesso di eseguire un ulteriore controllo interno che avvalora la corretta gestione dei casi di alloimmunizzazione eritrocitaria multipla.

PO046

The quest for indicators of profound hematologic response in AL amyloidosis: compelte response remains the optimal goal of therapy

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Background: Complete response (CR), defined as negative serum & urine immunofixation plus a normal FLC-ratio (FLCR), is considered the optimal goal of therapy in AL amyloidosis. Recently, different groups proposed a modification of this criterion based on different cutoffs of FLC. Objective: We compared the outcome of patients who obtained a profound FLC reduction (not CR) with those who reached CR and we checked whether very low FLC levels identify a better outcome among CR patients.Methods: Our dataset was searched for newly-diagnosed patients who reached at least one the following endpoints 6 months after treatment: 1) CR or, in patients who do not qualify for CR, 2) normalization of involved-FLC (N-iFLC), dFLC <10 mg/L (dFLC10) and normalization of FLCR (N-FLCR). We calculated overall survival (OS) from the time of response assessment. Results: 434 patients were included: CR (161), N-iFLC (114), dFLC10 (144) and N-FLCR (220). No differences were seen between the CR and all the others in the main baseline variables. The reasons for not qualifying for CR in the N-iFLC group was abnormal FLCR only in 4 (3%), positive s&u-IFE only in 81 (72%), and both in 29 (25%). The median follow-up of living patients was 60 months. CR patients enjoyed a longer OS (median not reached) compared to patients who reached any of the other endpoints. In particular, median survival N-iFLC was 91 months (P=0.033), in dFLC<10 85 months (P<0.001) and in N-FLCR 79 months (P<0.001). We then evaluated whether achieving a normal iFLC or a dFLC10 was associated with a better survival among CR patients. There was no significant difference in OS and TNTD between subgroups of patients in CR according to iFLC or dFLC response. In addition, no differences were seen in cardiac (51 vs. 47%, P=0.34) and renal (51 vs. 47%, P=0.34) responses amongst patients in CR with or without the additional factors.Conclusion: CR is associated with best survival in AL amyloidosis and should be the goal of therapy if tolerability and patient frailty allow. Higher sensitivity tools to identify residual clonal disease (mass-spectrometry on serum and urine, next generation sequencing and next generation flow cytometry on bone marrow) are needed to detect patients in CR at higher risk of relapse.

PO047

The Role of Eosinophilic Cationic Protein as a New Biomarker in SARS-CoV-2 positive patients with different severity of illness

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Background: Severe acute respiratory syndrome coronavirus (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), with a clinical outcome ranging from mild to severe, including death. To date, it is unclear why some patients develop severe symptoms. The routine laboratory tests and host immunity in COVID-19 patients with different severity of illness were compared after patient admission and also after discharge from the hospital after some time for follow up. Eosinophilic Cationic Protein (ECP) has various biological activities, including antibacterial, antiviral, antiparasitic and neurotoxic functions, and it contributes to the regulation of fibroblast activity. ECP, also induces airway mucus secretion and interacts with the coagulation and complement systems. ECP has been developed as a marker for eosinophilic disease and quantified in biological fluids including serum, bronchoalveolar lavage and nasal secretions. It is found in diseases such as allergic asthma and allergic rhinitis but also occasionally in other diseases. Methods: We evaluated 59 positive patients who underwent a nasopharyngeal swab PCR analysis for SARS-CoV-2, monitoring them through ECP and many routine laboratory tests such as ferritin, lactate dehydrogenase, 25-hydroxyvitamin D (25(OH)D),C-reactive protein (CRP),IL-6, during the period from 20 March to 25 May 2020. ImmunoCAP FEIA Fluorometric Enzyme Immunocapture Assay (ThermoFisher Scientific) measures the level of ECP in serum. Result: A total of 59 COVID 19-positive patients (64 + or - 10 years, male 74%) were classified as having mild(HB) (n = 33), severe (S) (n = 19) and extremely severe (ES) (n = 7) illness. ECP levels were significantly higher in" ES"[64,1 microg/L (18-117), p<0.001]; "S" showed [21.8 microg/L (5.5-46.3), p=0.016] and "HB" with level [16,5 microg/L (2,3-30,8), p=0.021]compared to Control Group [9.7 microg/L (6,1-13,4)].Interestingly, 14 patients monitored after discharge from hospital show altered ECP values (X=24,6 microg/L) correlated with poor oxygen saturation. Conclusions: We believe that the serial determination of ECP levels is useful for monitoring most patients with COVID 19 but above all to analyze the patients in the follow up to report a possible dysfunction of the respiratory tract

PO048

L'IMPORTANZA DEL MONITORAGGIO TERAPEUTICO DELL'ISAVUCONAZOLO, AL FINE DI MIGLIORARE LA TERAPIA FARMACOLOGICA: UN CASO CLINICO

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L'isavuconazolo (ISA) è un antimicotico appartenente alla famiglia dei triazoli, approvato recentemente sia dalla FDA che dall'EMA per il trattamento di Aspergillosi Invasive (AI) e mucormicosi. Il vantaggio terapeutico dell'ISA risiede nella minore frequenza e gravità degli effetti avversi rispetto alle comuni profilassi (in particolare all'uso del voriconazolo), minori interazioni farmacologiche e nella possibilità di essere somministrato sia per via intravenosa che orale, rendendolo idoneo per terapie a lungo termine. Il presente lavoro descrive il caso clinico di un uomo di 37 anni sottoposto a trapianto bilaterale di polmone a seguito di complicazioni causate da Fibrosi Cistica (FC). La terapia immunosoppressiva consisteva nella somministrazione di tacrolimus, micofenolato e prednisone. Nei mesi successivi al trapianto è stato possibile osservare un progressivo peggioramento respiratorio riconducibile alla presenza di Aspergillus flavus. Inizialmente il paziente è stato trattato con amfotericina b e voriconazolo successivamente sostituiti con ISA, al fine di limitare le interazioni farmacologiche con il tacrolimus. Il paziente è stato sottoposto a prelievi di sangue venoso 2 volte a settimana per un arco di 35 giorni, oltre il quale è stato nuovamente ricoverato per un peggioramento delle immagini radiologiche e dei test di funzionalità respiratoria. Grazie alla misura della concentrazione dell'ISA con metodo LC-MS (cromatografia liquida con spettrometria di massa) effettuato sui campioni prelevati precedentemente è stato possibile evidenziare che il fallimento della terapia era dovuto alle concentrazioni plasmatiche del farmaco inferiori all'intervallo terapeutico. L'assenza di raccomandazioni o linee guida sul monitoraggio terapeutico dell'ISA, la variabilità intraindividuale e la poca conoscenza del comportamento farmacologico nelle diverse condizioni cliniche, rende la misura della sua concentrazione plasmatica uno strumento utile per migliorare la gestione farmacologica dei pazienti, cosi da permettere il raggiungimento di concentrazioni efficaci ed aumentare così la probabilità del successo terapeutico.

PO049

COVID 19: LA SICUREZZA TRASFUSIONALE AI TEMPI DELL' EPIDEMIA DA CORONAVIRUS

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INTRODUZIONE: Alla fine del 2019, nella città cinese di Wuhan, sono stati diagnosticati i primi casi di polmonite interstiziale atipica. L'agente patogeno è stato identificato come un beta-coronovirus denominato SARS_CoV-2 (Severe Acute Respiratory Syndrome Coronavirus2). Sebbene i coronavirus generalmente infettano il tratto respiratorio superiore o inferiore, è comune la diffusione virale nel plasma o nel siero. Scopo di questo lavoro è la descrizione delle strategie adottate dal nostro Servizio Trasfusionale al fine di garantire la sicurezza trasfusionale soprattutto nei pazienti con un sistema immunologico compromesso.
MATERIALI E METODI: Il conteggio dei globuli bianchi residui (r-WBC) è stato eseguito nei concentrati piastrinici (CP) e nei campioni di emazie concentrate filtrate tramite una lettura in fluorescenza con il KIT Adam r-WBC (ADAM, Macopharma). L'inattivazione dei patogeni nei CP è stata eseguita con Amatosalen e raggi UVA (Intercept TM, CerusCoropration).
RISULATI: Nel periodo di epidemia da covid 19 i pazienti oncoematologici sono stati trasfusi con CP inattivati e con un conteggio medio dei r-WBC di 0.04x106. Per tali pazienti abbiamo selezionato unità di emazie concentrate filtrate con un conteggio di r-WBC inferiore o uguale a 0.02 x106 in modo da evitare la trasmissione del virus attraverso i WBC. Qualora i pazienti richiedevano trasfusione di plasma, è stato consegnato plasma sottoposto ad inattivazione virale con trattamento solvente/ detergente. Tutto il plasma fresco congelato di tipo B e plasma da aferesi non inattivato è stato distribuito alle industrie per la produzione di plasma derivati.
CONCLUSIONI:Poiché l'RNA del covid-19 può essere rilevato nel plasma o nei linfociti, il nostro Servizio Trasfusionale ha esso in atto tutte le possibili strategie per rendere sicura la trasfusione degli emocomponenti. Altre strategie adottate sono state: 1) misurare la temperatura corporea prima della donazione di sangue;(2) ulteriori domande nel questionario sullo screening dei donatori in merito al fatto che il donatore o i parenti abbiano i sintomi correlati oppure abbiano soggiornato in aree a rischio entro 28 giorni;(3) richiamare tutti i donatori e chiedere le loro condizioni fisiche attuali dopo la donazione.

PO050

Riduzione del rischio di alloimmunizzazione in regime d'urgenza trasfusionale: tipizzazione estesa mediante gelcards

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Introduzione: L'alloimmunizzazione eritrocitaria se non viene diagnosticata in modo corretto e gestita in maniera appropriata può causare severe reazioni emolitiche mediate da anticorpi diretti verso gli antigeni dei sistemi gruppo ematici. Per ridurre al minimo le alloimmunizzazioni, il protocollo adottato dal nostro SIT prevede la trasfusione di emazie concentrate quanto più compatibili (better cross match) soprattutto in pazienti politrasfusi come quelli oncoematologici, talassemici e pazienti di giovane età. Scopo del nostro lavoro è stato quello di valutare l'indice di immunizzazione di tali pazienti trasfusi in regime d'urgenza dopo tipizzazione sierologica estesa con gelcards Biorad.
Materiali e Metodi:Nel periodo tra giugno e dicembre 2019, sono pervenute 2500 richieste in regime d'urgenza per pazienti oncoematologici e per pazienti di giovane età (18-40 anni). Per ogni richiesta oltre al gruppo ABO diretto e indiretto, test di coombs indiretto e fenotipo Rh, è stata eseguita la determinazione degli antigeni del sistema Kell, Duffy, Kidd, MNS, tramite la metodica in microcolonna che prevede l'utilizzo di schedine contenenti anticorpi monoclonali contro l'antigene posti nella matrice del gel e un controllo negativo.
Risultati:il 97,7% dei pazienti è risultato Kp(a- b+), il 2,0 % Kp(a+ b+), 0,3% Kp(a+ b-). Il 50% dei pazienti presentava il fenotipo MN,il 28% MM e il 22% NN. Per quanto riguarda il sistema s il fenotipo più diffuso è ss nel 45% dei pazienti, nel 44% è Ss e nell'11% dei pazienti è SS. Il 50% dei pazienti presentava il fenotipo Jk(a+ b+), il 26% Jk(a+b-) e il 24% Jk(a- b+). Il 49% dei pazienti presentava il fenotipo Fy (a+ b+), 34% Fy (a-b+), 17%Fy (a+ b-). Di questi pazienti solo il 2% si è immunizzato ma per antigeni differenti da quelli testati.
Discussioni e Conclusioni:Dall'analisi dei dati è emerso che la frequenza dei fenotipi riscontrati è in linea con quelli evidenziati da Mollison (Mollison et al. Blood Transfusion in Clinical Medicine,1997). L'utilizzo di tali schedine permette lo studio dell'assetto antigenico dei pazienti in cui il rischio di immunizzazione è più elevato. L'esecuzione di tale test risulta comunque rapida (circa 10 minuti) e poco dispendiosa rispetto ai tempi e ai costi della biologia molecolare.

PO051

Persistence of minimal residual disease as detected by next-generation flow cytometry hinders organ response in immunoglobulin light chain (AL) amyloidosis.

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Immunoglobulin light chain (AL) amyloidosis is caused by a small B cell clone producing light chains that form amyloid deposits and cause organ dysfunction. Chemotherapy against the underlying B cell clone aims suppressing the production of the toxic light chain and restore organ function. However, even complete hematologic response (CR), defined as negative serum and urine immunofixation and normalized serum free light chain (FLC)-ratio, does not always translate into organ response.

Next-generation flow (NGF) cytometry on bone marrow aspirates is used to detect minimal residual disease (MRD) in multiple myeloma. In the present study, we evaluated MRD by NGF (Euroflow panel) in 92 AL amyloidosis patients in CR.

Fifty-four percent of analyzed AL amyloidosis patients in CR had persistent MRD in the bone marrow as assessed by NGF (median 0.03% abnormal plasma-cells). There were no differences in baseline clinical variables in patients with or without detectable MRD at NGF. Undetectable-MRD at NGF was associated with higher rates of renal (92% vs 57%, p=0.005) and cardiac response (95% vs 71%, p=0.046), as assessed based on changes of renal and cardiac biomarkers, respectively. Hematologic progression was more frequent in MRD-positive patients (0 vs 25% at 1-year, P=0.001).

Altogether, NGF on bone marrow aspirates can detect MRD in approximately half the AL amyloidosis patients in CR, and persistent MRD can explain persistent organ dysfunction. Thus, this study supports testing MRD in CR patients, especially if not accompanied by organ response. In case MRD persists, further treatment could be considered, carefully balancing residual organ damage, patient frailty, and possible toxicity.

PO052

The role of serological test in combination with RT-PCR to support diagnosis of COVID-19. Preliminary data from the RO.S.A.D.A Study.

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Diagnostic tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, named also COVID-19) infection are always more widespread and constantly evolving. The WHO provides the reverse-transcription polymerase chain reaction (RT-PCR) as laboratory testing strategy recommendation for COVID-19 performed in upper and lower respiratory specimens.SARS-CoV-2 infection can also be detected indirectly through serological testing; however, systematic data on the diagnostic performance and the predictive value of these tests are still lacking. The aim of this study was to evaluate the role of serological tests and their predicitive values, in combination with RT-PCR, to support diagnosis of COVID-19. We conducted a retrospective study on 637 patients admitted to the Emergency Department and Internal Medicine Ward of S.Giuseppe Hospital (Empoli,Italy) for suspected COVID-19. All sera were first tested by rapid test for qualitative detection of anti-SARS-CoV-2 IgG/IgM. Sera positive for IgG and/or IgM were subsequently tested for the quantitative detection using a chemiluminescent method. Of the 637 patients, 59 had a COVID-19 diagnosis; 104 were positive for anti-SARS-CoV-2 IgG/IgM antibodies by a qualitative test and 57 for the viral RNA by RT-PCR, with a concordance rate between the two tests of 24% (25/104). Moreover, 32 out of the 57 patients with positive RT-PCR were negative for anti-SARS-CoV-2 IgG/IgM antibodies by qualitative test with a concordance rate of 56.1% and, on the contrary, two COVID-19 patients were negative for RT-PCR but positive for anti-SARS-CoV-2 antibodies. Double positive tests (qualitative and quantitative) over the total number of positive results ranged from 27% to 37%, according to the single isotype or combination of antibodies used. The best odd ratio (OR) for associations of anti-SARS-CoV-2 positive tests with COVID-19 was reached by combining 3 or 4 tests (OR:12.9-22.7). Since our results showed a weaker association between SARS-CoV-2 infection and the positive single qualitative test (31-37%), compared to the double positive qualitative test or the double, triple, and quadruple qualitative and quantitative tests (63-90%), the significance of the qualitative test should be evaluated according to the isotype detected and to its combination with the quantitative test

PO053

Monocyte Distribution Width (MDW) as a new parameter useful in COVID-19 patients in emergency setting

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INTRODUCTION AND AIM: clinical laboratory plays a role in diagnosis (viral RNA research, antibody response) and evaluation of infection severity, progression and response to treatments of COVID-19 patients. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) defined a panel of tests1 recommended for clinical evaluation including blood cell count, coagulation and biochemical tests. MDW (Monocyte Distribution Width) is a new parameter describing the standard deviation of monocytes' volume. MDW is included in the differential white blood cell count (CBC-Diff) and has been shown to be an early indicator of sepsis (Crouser2). The aim of the study is to verify the contribution of MDW in the diagnostic and prognostic pathway of patients with suspected SARS-CoV-2 infection in Emergency Department (ED) setting.
MATERIALS AND METHODS: the retrospective study included 189 consecutive patients entering the ED of Baggiovara Civil Hospital (Modena) tested for SARS-CoV-2 RNA using oro-nasopharyngeal swab. A complete and differential white blood cell count (CBC-Diff) including MDW (K2 EDTA, DxH 900 Beckman Coulter Inc) has been performed for all patients.
RESULTS: 123 patients were negative for viral RNA, and 66 were positive. Among the latter 25 were discharged and 41 hospitalized. New MDW parameter showed an AUC of 0.83 (sensitivity 84.9 and specificity 75.6) with a cut off 20.1 between negative and positive. Moreover different median values have been observed in negative (18.26), positive discharged (21.60) and positive hospitalized (23.29) patients.
DISCUSSION and CONCLUSIONS: MDW, included in CBC-diff, is a parameter quickly available for the clinician without additional request. The observed differences are associated with different severity of the COVID-19 clinical history indicating a potential valuable prognostic role. MDW could be inserted in a diagnostic algorithm as a support for clinical evaluation.
BIBLIOGRAPHY: 1IFCC Information Guide on COVID-19; 2Crouser et al. “Improved Early Detection of Sepsis in the ED With a Novel Monocyte Distribution Width Biomarker” Chest 2017 Sep;152(3):518-526

PO054

MDW and IFCC laboratory parameters in COVID patients in Emergency setting

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INTRODUCTION AND AIM: IFCC defined a panel of tests1 including blood cell count, coagulation (D-Dimer and Prothrombin Time-PT) and biochemical tests (albumin, LDH, GOT, GPT, bilirubin, urea, creatinine, troponin, PCT, CRP, ferritin, IL 6), recommended for clinical evaluation of COVID patients. MDW (Monocyte Distribution Width), a new parameter describing the standard deviation of the mean volume of monocytes, included in the differential white blood cell count (CBC-Diff), has been shown to be an early indicator of sepsis (Crouser2 2017). The aim of the study is to verify the contribution of IFCC parameters and MDW in the diagnostic and prognostic pathway of patients with suspected SARS-CoV-2 infection in Emergency Department (ED) setting.
MATERIALS AND METHODS: the study retrospectively included 189 patients entering the ED of Baggiovara Civil Hospital (Modena) tested for SARS-CoV-2 RNA on oro-nasopharyngeal swab samples. A complete and differential white blood cell count (CBC-Diff) including MDW has been performed for all the patients (K2 EDTA DxH 900 Beckman Coulter Inc); a venous blood sample for clinical biochemistry tests (AU680 and DxI Beckman Coulter Inc.) and sodium citrate for coagulation tests (ACL TOP-IL) were collected, according to IFCC panel1.
RESULTS: 123 patients were negative for viral RNA, and 66 were positive. Among the latter, 25 were discharged and 41 hospitalized. The performance for each test was evaluated through AUC ROC: aPTT, GPT, lymphocyte, CRP had high specificity; PT, urea, PCT, D-Dimer had high sensitivity. LDH (AUC 0.80, sensitivity 76.3, specificity 72, cut off 795 U/L) and MDW (AUC 0.83, sensitivity 84.9, specificity 75.6, cut off 20.1) showed high sensitivity and specificity.
DISCUSSION AND CONCLUSIONS: combination of multiple tests is required for diagnostic classification of COVID patients. Among parameters tested, LDH and MDW perform better showing both high sensitivity and high specificity. MDW has features to be inserted in a diagnostic algorithm for COVID patients without additional request.
BIBLIOGRAPHY: 1IFCC Information Guide on COVID-19; Crouser et al. “Improved Early Detection of Sepsis in the ED With a Novel Monocyte Distribution Width Biomarker” Chest 2017 Sep;152(3):518-526

PO055

An unexpected tricky case of plasma cell leukemia

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INTRODUCTION: plasma cell leukemia (PCL) is a rare and aggressive form of monoclonal gammopathy (MG), defined by at least 20% circulating plasma cells and a total plasma cell count in peripheral blood of at least 2 × 109/l. PCL can be divided into primary PCL (PCL) and secondary PCL (sPCL) following previously diagnosed multiple myeloma (MM).
CLINICAL CASE: an unexpected tricky case of PCL with septic shock at onset
A 65-year-old woman, with silent clinical history, entered the ED in a state of septic shock. She had increased pro-inflammation indices (CRP 30.3 mg/dl, procalcitonin 45.7 ng/ml) and severe multi-organ failure signs (creatinine 6.68 mg/dl, eGFR 6 ml/min, total bilirubin 1.37 mg/ml, amylase 600 U/L, lipase 135 U/L, troponin 414 ng/L), D-Dimer >20000 ng/ml-FEU. Blood cell count (DxH 900 Beckman Coulter, Inc.) reported normochromic anemia (Hb 9.5 g/dl) and normal leukocyte and platelet counts. The instrumental flag "blasts" prompted peripheral blood microscopic review which revealed atypical plasma cells (35% WBC – 2.13 × 109/l). Total body CT showed no organ alterations or adenomegalies. Laboratory specialist communicated suspected PCL to ED physician and was executed a specific scanner in dorsal spine that showed multiple bone lytic lesions; serum calcium resulted 12.9 mg/dl. Laboratory test during recovery showed hypogammaglobulinemia, lambda free gamma monoclonal light chains in serum and urinary immunofixation, high free lambda chains (12426 mg/dl) and free kappa/lambda ratio <0.01. Peripheral blood and bone marrow flow cytometric analysis identified a plasma cell population with abnormal immunophenotype (CD138 +, low CD 38 expression, CD19-, CD56 ++, CD45 +/- heterogeneous). Moreover blood culture was positive for E. coli, sensitive to all tested antibiotics.
CONCLUSION AND DISCUSSION: despite the absence of important alteration of the blood count, the "blast" instrumental alarm led to microscopic review with evidence of a PCL. The timely connection between laboratory specialist and ED physician allowed to rapid identification of a rare and serious condition with an adequate care setting.
REFERENCE: Gundesen MT et al; Plasma Cell Leukemia: Definition, Presentation, and Treatment. Curr Oncol Rep. 2019; 21(1): 8. - doi: 10.1007/s11912-019-0754-x

PO056

L'IMPORTANTE CONTRIBUTO DEI DONATORI DI SANGUE AI TEMPI DEL COVID-19

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Introduzione:Stante l'emergenza epidemiologica legata al COVID-19, numerose regioni hanno segnalato una riduzione delle presentazioni dei donatori nelle sedi di raccolta pubbliche (intra-ospedaliere) e associative (sul territorio).Scopo del nostro studio è documentare come il SIT Aziendale ASL Caserta ha gestito l' emergenza della pandemia nel reclutamento dei donatori di sangue.
Materiali e Metodi: dal 9/03/2020 al 4/05/2020 sono state reclutati 3900 donatori di cui 2514 uomini e 1.386 donne. Le donazioni effettuate sono state:57 (maschi) plasmaferesi,1 (maschio) piastrinoaferesi,2456 (maschi) prelievo di sangue intero, 22 (donne)plasmaferesi, 1364 (donne) prelievo di sangue intero.Al fine di evitare contagi sono state adottate diverse misure preventive: i potenziali donatori sono stati invitati a presentarsi presso i servizi trasfusionali o i punti di raccolta solamente dopo aver prenotato la donazione.Al momento della prenotazione, è stato effettuato un veloce pre-triage telefonico per valutare lo stato di salute (contatti stretti con soggetti covid-19 nelle ultime 3 settimane,presenza di febbre,tosse diarrea e altri sintomi associati al covid-19). Al fine di evitare assembramenti il tempo che intercorreva tra una donazione e l'altra è stato di 30 minuti. Prima di accedere agli ambienti preposti al dono è stata misurata la temperatura corporea di tutti. All'interno dei locali vengono utilizzati gel igienizzante e mascherine.
Risultati:In collaborazione con l'Università Vanvitelli e UOC Malattie Infettive è stato eseguito su tutti i donatori arruolati il dosaggio delle IgG e IgM per il Covid-19. Sono risultati positivi al dosaggio delle IgM 5 donatori asintomatici. I donatori risultati positivi alla ricerca delle IgM sono stati sottoposti al tampone faringeo riscontrando un esito negativo.
Discussioni e Conclusioni: Nonostante la forte emergenza epidemiologica che stiamo fronteggiando, la solidarietà manifestata dalla popolazione italiana ha permesso presso il nostro servizio di registrare un forte incremento delle donazioni di sangue. Una risposta straordinaria non solo per ripristinare le scorte ma anche assicurare la compensazione interregionale qualora si registrassero nuove carenze nel paese alle prese con le misure restrittive anti-coronavirus.

PO057

IMPLEMENTAZIONE DI UNA NUOVA PROCEDURA INFORMATICA PER LA GESTIONE DELLE RICHIESTE DOMICILIARI

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INTRODUZIONE:L'emotrasfusione domiciliare puo'essere considerata una valida alternativa all'ospedalizzazione per quei pazienti(oncologici,ematologici etc) che per varie problematiche legate alle patologie di cui sono portatori non hanno la possibilita'di accedere al centro trasfusionale. L'appropriatezza della terapia trasfusionale inizia al momento della prescrizione da parte del medico che ha in cura il paziente,il quale deve da un lato far riferimento alle linee guida trasfusionali del COBUS e dell'altro verificare che non ci siano strategie terapeutiche alternative alla trasfusione. Lo scopo del nostro studio è stato quello di valutare l'appropriatezza prescrittiva delle richieste per la trasfusione domiciliare.
MATERIALI E METODI: Sono state valutate le non conformità relative alle richieste domiciliari pervenute presso il Sit ASL Caserta nell'anno 2019. Di queste richieste sono stati valutati: dati anagrafici del paziente,il consenso informato e informativa alla trasfusione di sangue,il grado di urgenza,la patologia e il valore di Hb e/ o conteggio delle piastrine.
RISULTATI E DISCUSSIONI: Nell' anno 2019 sono giunte 300 richieste di terapie domiciliari ,di queste 98 (30%) sono risultate non conformi ai parametri valutati: dati anagrafici del paziente (2.8%),consenso e informativa alla trasfusione(10%), grado d'urgenza(5%), patologia e motivo della richiesta (3.5%),valore di Hb e/o conteggio plt(8.7%).
Conclusioni: Dall' analisi dei dati è emerso che la percentuale più alta di non conformita è per la mancanza di consenso informato ed informativa e per la mancanza del valore di Hb e/o valore di plt. Visto le numerose richieste inappropriate ,il nostro SIT ha introdotto in seguito ad una serie di audit interni avviati dagli operatori del servizio trasfusionale,una procedura informatica che prevede l'invio online di moduli di emotrasfusione semplicemente inviando una e-mail alla nostra posta elettronica aziendale indicando nell'oggetto la dicitura "domiciliare". Questo tipo di organizzazione ha portato numerosi vantaggi: tempi piu'rapidi nell'accettazione della richiesta,minore stress da parte dei familiari che portano la richiesta,e una maggiore collaborazione con i medici di base.

PO058

CAMBIAMENTI ORGANIZZATIVI DURANTE L'EMERGENZA COVID E VERIFICA DELLA QUALITA' ANALITICA: DESCRIZIONE DI UN ESEMPIO PRESSO IL LABORATORIO DEGLI SPEDALI CIVILI DI BRESCIA

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INTRODUZIONE. La pandemia Covid-19 ha costretto i Servizi di Medicina di Laboratorio ad affrontare repentini cambiamenti organizzativi, mettendo a rischio il mantenimento di un'adeguata qualità analitica. In questo frangente, sono risultati utili i documenti messi a disposizione da SIBioC, che, nel nostro caso, hanno permesso al Laboratorio dell'ASST Spedali Civili di Brescia di affrontare due trasferimenti dell'esame Procalcitonina in tempi rapidi e ravvicinati, dapprima dal Laboratorio di Microbiologia e Virologia (sfruttando la presenza della stessa strumentazione in entrambe le sedi: Diasorin Liaison XL; Liaison BRAHMS PCT), e successivamente presso l'area in cui vengono eseguiti gli esami in emergenza (su Roche Cobas e801; Elecsys BRAHMS PCT). Le prove di verifica di quest'ultimo trasferimento, qui descritte, sono state condotte utilizzando un foglio elettronico approntato secondo il protocollo messo a disposizione dal GdS "Statistica" (Biochim clin 2016;40:129-42).
METODI. Le prove effettuate sono state: 1) Comparazione dei due sistemi analitici mediante regressione di Passing-Bablok su 44 campioni umani 2) Verifica delle prestazioni analitiche della metodica su Cobas e801, applicando, su due tipologie di campioni (1 pool di plasma umano e 2 materiali di controllo commerciali), lo schema 5X5.
RISULTATI. Usando i campioni raccomandati (plasma in litio eparina per Cobas e in sodio citrato per Liaison) le prove di comparazione hanno dimostrato una buona concordanza fra i due sistemi analitici (slope=0.93; 95% CI 0.78÷1.01; intercetta=-0.01; 95% CI -0.07÷0.02), permettendo la trasferibilità dei livelli decisionali. I confronti effettuati con lo schema 5X5 sono risultati all'interno dei limiti di accettabilità (imprecisione rispetto alle specifiche dichiarate dalla ditta produttrice, inesattezza rispetto al valore assegnato ai materiali di controllo, errore totale rispetto al traguardo analitico scelto per il monitoraggio delle prestazioni). Il valore Sigma ottenuto con i 2 campioni di controllo era pari a 7.4 e 7.0.
CONCLUSIONI. La messa a disposizione da parte dei GdS SIBioC di protocolli operativi e di strumenti di facile utilizzo consente di verificare il rispetto della qualità delle prestazioni analitiche anche in condizioni di emergenza organizzativa.

PO059

Droplet Digital PCR for the detection of BRCA1 Large Genomic Rearrangements: application to the germline and somatic test in High Grade Serous Ovarian Cancer

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Background. With the introduction of Olaparib as target therapy for patients affected by High Grade Serous Ovarian Cancer (HGSOC) who are carriers of both germline and somatic BRCA mutations, the genetic test performed on tumor tissues became relevant like the best-know germline test. In this context, the evaluation of Large Genomic Rearrangements (LGRs) represents a challenge due to the characteristics of tumor-derived DNA. Here we describe a droplet digital PCR (ddPCR) assays for the evaluation of target BRCA1 LGRs on blood, formalin-fixed, paraffin-embedded (FFPE) and Fresh Frozen Tissue (FFT) samples.

Methods. ddPCR technology was used for LGRs analysis of BRCA1 exon 2, 20 and 21 selected as the most common LRGs accounted in our cohort of patients. We analyzed DNA derived from blood and FFT/FFPE samples in previously genotyped samples (n=15) and in a validation group of unknown samples (n=50). A reference range of copy number (CN) was calculated in order to support the classification of BRCA CN status.

Results. ddPCR assays allowed the identification of LGRs in BRCA1 exon 2, 20 and 21 in both blood and tissue samples. We were able to correctly classify CN alteration firstly through the interpretation of ddPCR fluorescence plots and also with the evaluation of reference range of CN.

Conclusions. This study shows the utility of ddPCR to accurately assess LGRs in blood samples as well as in archival FFPE and FFT specimens. The introduction of ddPCR in a comprehensive workflow, encompassing both germline and somatic test, represents an improvement in the BRCA1/2 gene testing pipeline. Moreover, ddPCR is confirmed to be a method able to solve CN differences in complex and heterogeneous DNA mixture. This approach can overcome challenges related to BRCA genetic test, especially on FFPE tumor analysis. Finally, ddPCR represents an attractive alternative option to the established standard methods, currently used in clinical routine.

PO060

TENPROProstate: an innovative biosensor for personalized treatment of prostate cancer

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After skin cancer, prostate cancer (PC) is the most common cancer among men. The gold standard for PC diagnosis is based on the PSA (prostate-specific antigen) test. Basing on this preliminary screening, the physician makes a decision on whether to proceed with biopsy to confirm cancer and evaluate its aggressiveness. Nevertheless, the specificity of the PSA test is suboptimal and, as a result, about 75% of men who undergo a prostate biopsy because they have elevated PSA levels do not have cancer. Moreover, more than 50% of new diagnosed PC are indolent cancer. Overdiagnosis leads to unnecessary overtreatment of PC with undesirable side effects.In the last decade, a lot of studies were focused on the identification of biomarker panels able to overcome the limits of PSA, increasing diagnostic specificity and improving the ability to detect clinically significant tumors. In particular, several evidences were available indicating that the combined use of some molecules increases the diagnostic specificity of PSA. The simultaneous determination of several markers increases the costs borne by the patient and the National Health System. On this basis, there is an urgent need to identify an innovative technology that would allow the determination of a panel of biomarkers at a low cost. This requirement finds a valid solution in plasmon resonance biosensors, which are fast, efficient and highly analytical sensitive systems. We developed a biochip that evaluates the circulating levels of about 10 molecules significantly associated with PC aggressiveness. This test allows to obtain a risk index that the patient is suffering from an aggressive PC starting from a single blood sample. The definition of the level of aggressiveness of the disease in the initial phases of the diagnosis allows to reduce the over-treatment in patients with organ-confined PC, addressing them to radical prostatectomy only when the tumor classification indicates a pathology with a high risk of progression. Collectively, TENPROProstate will: 1) reduce the risk of overdiagnosis and overtreatment ; 2) improves life expectancy and quality of life; 3) reduce health care expenditure; 4) lead to personalized cancer therapy.

PO061

Laboratory strategy for the rapid confirmation of heterophilic antibodies interferences in immunometric assays

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Heterophilic antibodies are an important cause of interference in immunoassays that would account for up to 15-20% of interference in clinical laboratories. Suspicion of heterophilic antibody interference should always be taken into account when test results are inconsistent with clinical evidence or with other radiological and laboratory findings, and when other causes of interference have been excluded. This type of interference is particularly relevant for tumor biomarkers immunometric assays, where high levels of tumor biomarkers can adversely affect the patient in terms of diagnostic practices, such as unnecessary imaging or biopsy, as well as causing psychological problems. In this regard, it may be useful to have a procedure for the rapid confirmation of the suspected interference. Here, we describe the case of a 52-years-old woman with a history of remission for poorly differentiated adenocarcinoma of the sigmoid colon. During a routine check, her CA19.9 dosage value was 4909 U/ml (ref value < 34 U/ml). Two weeks later, the woman underwent a WB-CT and PET screening, which both excluded tumor recurrence. Based on the evidence of a high level of tumor biomarkers not supported by symptoms and imaging evidence, heterophilic antibodies interference was suspected. In order to confirm the presence of heterophilic antibodies, the serum sample was first incubated with immobilized A / G protein resin; then, the supernatant was subjected to an electrophoretic run to confirm the removal of immunoglobulins. Finally, CA19.9 was re-assayed both in pre-treated and post-treated sera. Serum pre-treatment reduced the immunoglobulins below the detection limit of electrophoresis. Following this pre-treatment, the CA19.9 value was reduced by 50%, supporting the hypothesis of possible interference by heterophilic antibodies in CA19.9 immunometric assays. We explained the persistence of levels of CA19.9 beyond the cut-off value, by the presence of non-removed antibodies, particularly IgM, towards which the resin of protein A / G shows low binding capacity. Based on this experience, we decided to optimize a laboratory strategy that allows us to reconcile the need for a quick confirmation of the suspected interference with an inexpensive and practical procedure. The procedure consists of the following: (a) rapid treatment of the sample (1h) with the protein A/ G; (b) Re-testing-testing of both pre- and post-treated samples; (c) Calculation of the percentage reduction of the analyte value in the post-treated sample as compared to the pre-treated sample. Using this procedure, we are currently carrying out tests on control serum samples to identify a percentage reduction threshold that can confirm the presence of heterophilic antibodies with statistical confidence

PO062
Reattività aspecifiche per HIV

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Premessa:Sono 2.847 le nuove diagnosi di infezione da HIV registrate in Italia nel 2018, pari a 4,7 nuovi casi per 100mila residenti, numeri in deciso calo rispetto ai 5,7 casi registrati nel 2017 (Istituto Superiore di Sanità). L'incidenza maggiore di infezione da HIV è nella fascia di età 25-29 anni. Scopo del seguente studio è stato quello di capire quanto i dati nazionali si rispecchiano nella nostra realtà territoriale e quindi misurare l'incidenza delle infezioni da HIV e la tendenza nel tempo, registrata presso la struttura trasfusionale di Aversa.
Metodi:sono stati analizzati i test di screening virologico nel triennio 2017-2019. I test di screening eseguiti in chemiluminescenza con lo strumento Vitros (OrthoClinicalDiagnostics) oltre a rilevare la presenza di anticorpi anti-HIV1/2 consentono il rilevamento dell'antigene p24 per una diagnosi precoce dell'infezione. La ricerca in biologia molecolare del virus HIV è stata fatta con l'apparecchio Cobas (Roche).I nuovi casi ripetutamente reattivi ai test di screening HIV1/2 Combo, sono stati processati per conferma con metodica immunoblotting su secondo prelievo attraverso ritorno presso il SIT del donatore entro 20 gg dalla donazione (alleg. 8 d.m. 2/11/2015).
Risultati: nel 2017 su un totale di 20.204 donatori, 24 sono risultati positivi ai test di screening, di cui solo 1 è stato refertato positivo (HIV-nat pos; test di conferma positivo;screening positivo). Nel 2018 su 19.063 donatori 15 erano falsi positivi, e di questi uno è risultato positivo. Nel 2019 sono risultati 18 falsi positivi su 19.863 donatori e ad essere confermato dalla biologia molecolare e dal test di immunoblotting solo uno.
Conclusioni:i dati analizzati mostrano un andamento costante nelle nuove infezioni conclamate dell'HIV nel periodo 2017-19. Si vuole focalizzare l'attenzione sul ruolo di grande importanza dei test di conferma per l'individuazione di possibili interferenze nei risultati.Non meno importanti risultano le campagne di informazione e prevenzione messe in atto dal Sit Asl Ce con le Avis di riferimento, per mantenere uno stato di attenzione riguardo l'andamento delle infezioni virali sul nostro territorio per mantenere sempre alto il livello di attenzione nel proprio modo di vivere.

PO063
Vitamin D and Interleukine 6 evaluation in Covid-19 patients: our experience

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Background and aim. Coronavirus disease 2019 (COVID-19) is a viral pandemic that emerged from East Asia and quickly spread to the rest of the world due to Coronavirus 2 SARS-CoV–2. The clinical manifestations are accompanied by the onset of an inflammatory storm (cytokine release syndrome CRS). The key role in the cytokine storm is played by interleukin 6 (IL-6) owing to its robust pro-inflammatory function. It induces a variety of acute-phase proteins, increasing body temperature. Vitamin D signaling has emerged as a key regulator of immunity in humans. Studies have shown that Vitamin D, acting in an intracrine fashion, is able to induce expression of antibacterial proteins. The net effect of these actions is to support increased bacterial killing in a variety of cell types. Aim of this study was to evaluate the correlation between Vitamin D and IL-6 in patients with laboratory-confirmed COVID-19. Methods. 160 patients with laboratory-confirmed COVID-19 (85 males- median age 62y, and 75 female- median age 66y) were recruited (between march and may 2020). Patients did not require intensive care admission and they aren't in treatment with drugs Vitamin D homologous before the access to the hospital. Vitamin D (ng/ml) and IL-6 (pg/ml) were measured using chemiluminescence method on TGSTS-Techno Genetics and Cobas e 8100-Roche respectively in three consecutive days. Statistical analysis was obtained using MedCalc software and p-value threshold of 5% was adopted. Results. Linear regression line: y=121,16 - 0,98 (x); intercept 121,16 (95% CI =99,42 to 151.15, P<0.0001); slope = -0,98 (95% CI = -3,29 to -0,35, P<0.0001). Pearson correlation coefficient: R= -0.62 (95% CI= -0.71 to -0.33, P <0.0001) show a statistically significant inverse correlation. Conclusions. In Covid-19 patients, the increase of Vitamin D concentration, mainly in the third day, correlated to IL-6 lowering may be considered a signal of better clinical outcome of these patients. Further studies are needed to confirm the Vitamin D utility in Covid-19 patient management and to better explain its role in immunity response.

PO064

A rapid screening test for cystinuria with Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR) coupled with chemometric analysis

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Introduction: Guidelines on Nephrolithiasis underline the importance of laboratory investigation in blood and urine for detecting major systemic causes of stone disease. The Brand's test, necessary for diagnosis of cystinuria, requires a dangerous substances, so in our laboratory has been replaced with the dosage of cystinuria by high-performance liquid chromatography with fluorimetric detection (HPLC-FL). However, this technique requires the use of complex and costly equipment. Infrared spectroscopy, universally used for stone analysis, recently was used to detect insoluble cystine in urine.The aim of this study was to evaluete Infrared Spectroscopy combined with chemometric analysis for a possible introduction in our laboratory as screening method to identify which patients really require confirmation by HPLC-FL analysis. Material and Methods: We examined 57 subject specimens (24h urine) from Division of Nephrology and Urology of Fondazione Policlinico Universitario A. Gemelli IRCCS. Cysteine were determined by HPLC-FL. The infrared spectroscopic analysis was performed with an ATR accessory (ATR-FTIR). Multivariate (PCA-LDA) statistical analysis was performed to identify significant results.Results and Discussion: The HPLC-FL quantitative determination of cysteine in the 57 samples of the patients showed a normal excretion of cystine in 49 samples (85.9%) and an abnormal excretion in the remaining 8 samples (14.1%). Qualitative inspection of the mid-IR spectra shows the presence of relevant differences between the two groups in the spectral range 1200-1400 cm-1. Based on these differences, we evaluated the possibility to automatically classify subjects using PCA combined with LDA. Our preliminary results show a large proportion of the variance explained by the first two principal components (92%) and a good classification ability for the PCA-LDA algorithm. The effectiveness of the classifier was further tested with the leave-one-out cross-validation approach. According to these preliminary results, the introduction of the ATR-FTIR technique in our clinical laboratory may reduce time and cost analysis for diagnosis of cystinuria.

PO065

INFEZIONE DA T.pallidum NEI DONATORI DI SANGUE DEL SIT ASL CE

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PREMESSA: L'allegato VIII del DM 02/11/2015 prevede lo screening sierologico di tutte le unità di sangue per la ricerca dell'agente eziologico della sifilide. Il donatore risultato ripetutamente reattivo (RR) al test di screening viene convocato entro 7 giorni dalla donazione mediante mezzo raccomandata per confermare o meno tale risultato su secondo campione. Scopo del lavoro è stato quello di valutare l'infezione da T.pallidum nei donatori di sangue afferiti presso il SIT ASL CE nell'anno 2019 e l'efficacia del sistema di notifica utilizzato.
MATERIALI E METODI: Il test di screening sierologico è stato eseguito con Vitros 3600-Ortho (CLIA) presso il CQB di Caserta. Su secondo prelievo è stato ripetuto il test di screening ed effettuata la ricerca IgM ed IgG anti treponema mediante tecnica dell'immunoblotting con Alifax.
RISULTATI: Nell'anno 2019 su 19863 donatori di sangue, 51 (0,25%) sono risultati RR reattivi per T.pallidum (62,5% maschi, 37,5% femmine; 39,2% stranieri, 60,8% italiani; età compresa tra 22 e 60 anni). Di questi, il 39% sono ritornati dopo convocazione presso il nostro SIT per il test di conferma con risultato: 75% positivi per IgG e negativi per IgM; 25% negativi sia IgM che IgG.
CONCLUSIONI: La tecnica mediante immunoblot ha permesso di confermare la positività nel 75% dei donatori e di individuare falsi positivi nei casi restanti. Quasi tutti i donatori positivi su secondo prelievo risultano aver donato per la prima volta presso il nostro SIT. In nessun donatore si è riscontrata infezione di sifilide in atto (IgM ed IgG positive); i donatori con reattività alle IgG come infezione pregressa rientrano per il 35% in una fascia di età compresa tra18-35 anni mentre il restante 65% nella fascia 36-60 anni che potrebbe indicare una bassa percezione del rischio di malattie sessualmente trasmesse anche da parte di adulti. Inoltre il 61% di donatori non è tornato per il test di conferma, con conseguente impossibiltà a definirne lo stato di positivà e di sospensione alla donazione. Ciò è probabilmente da imputare a condizioni socio culturali dei donatori che porta a sottovalutare il problema, oppure al mezzo di notifica utilizzato dal SIT che potrebbe essere implementato con altra tipologia (indirizzo email, comunicazione telefonica).

PO066

Metabolomic changes in COVID-19 patients

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The lipid structure of membranes regulates the penetration and replication of SARS-CoV-2 in the alveolar pneumocytes. Specific side-chain oxysterols generated by enzymatic activity such as 25-hydroxycholesterol (25OHC) and 27-hydroxycholesterol (27OHC) have inhibitory effect on viral replication. Reduction of poly-unsaturated fatty acids, such as arachidonic acid (AA) and decosahexaenoic acid (DHA) were related to increased viral replication. By isotope dilution mass spectrometry, we investigated the lipid profile (fatty acids, sterols and oxysterols) in plasma collected from 27 mild SARS-CoV-2 positive subjects and 117 COVID-19 patients with moderate (n=36) and severe stages (n=81) of the disease, compared with 123 age matched healthy volunteers. Cholesterol was reduced of 20% (P<0.001) in moderate and 25% (P<0.001) in severe patients. The cholesterol precursors desmosterol and lathosterol, marker of cholesterol synthesis, were reduced from 50 up to 70% in moderate and severe patients (P<0.001). While 25OHC was increased by 15% (P = 0.03) in the mild individuals and reduced of about 13% in the severe patients (P<0.001), the antiviral 27OHC was reduced by 17% in the group of pauci-asymptomatic (P<0.001), becoming 30% (P<0.001) in the moderate and 50% (P<0.001) in the severe COVID-19 patients. AA and DHA were significantly reduced in moderate and severe patients in which was observed an increase of the very long chain fatty acid (> 24C) which are markers of peroxisomal dysfunction. We found increased levels of 7□-hydroxycholesterol and 7-ketocholesterol, markers of oxidative stress. In patients affected by COVID-19 there are evidence of several metabolomic changes involving mitochondrial and peroxisomal functions and resulting into changes of the lipid profile. Metabolomic investigation would be useful for studies about the mechanism of disease, the biomarker discovery and to find possible therapeutic targets. Civra et al., J Steroid Biochem Mol Biol, 2019:193, 105424. doi: 10.1016/ j.jsbmb.2019.105424.

PO067

Automated classification of cancer cell-derived exosomes by Attenuated total reflection - Fourier Transform Infrared Spectroscopy

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Introduction: Exosomes possess great potential as cancer biomarkers in personalized medicine due to their easy accessibility and capability of representing their parental cells. In order to boost the translational process of exosomes in diagnostics, the development of novel and effective strategies for their label free and automated characterization is highly desirable. In this context, Fourier Transform Infrared Spectroscopy (FTIR) has a great potential as it provides direct access to specific biomolecular bands that give compositional information on exosomes in term of their protein, lipid and genetic content. We used attenuated total reflection - Fourier transform infrared spectroscopy (ATR-FTIR) to study exosomes released from HT-29 cancer cells cultured in different media. Material and Metods : In this study, we analyzed exosomes extracted from HT-29 cancer cells cultured in different media, with 10% of exosome-depleted FBS (EVd-FBS), and under serum starvation with 0.5% EVd-FBS, using FTIR-ATR spectroscopy and structural techniques, including dynamic light scattering (DLS), Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).Results and Discussion : Structural techniques did not reveal any significant difference between well-fed and starved cells, showing that FTIR spectroscopy is likely to outperform conventional structural methods for classification purposes. Our data show the presence of statistically significant differences in the shape of the Amide I and II bands in the two conditions. Based on these differences, we showed the possibility to automatically classify cancer cell-derived exosomes using PCA-LDA. Interestingly, the results point out the effectiveness of FTIR in discriminating different classes of exosomes, also showing its great potential in the search of novel quantitative and label-free exosome biomarkers of cancer. Acknowledgement: The authors acknowledge the financial support of “Progetto Giovani Ricercatori 2014–2015” grant number GR-2016-02363310.

PO068

Infra-Spectro-Genomic approach in High-Grade Serous Ovarian Cancer: a pilot study

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Introduction

High-grade serous ovarian cancer (HGSOC) is the deadliest form of epithelial ovarian cancer. Noteworthy improvements are been achieved in the management of HGSOC patients. BRCA mutational status is considered a predictive biomarker of response to platinum-based and i-PARP chemotherapy. A multidisciplinary approach is essential to clinical decision making and patient outcome. We evaluated the possibility of using Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) technique to detect a typical band in HGSOC samples related to cancer severity. Moreover, we investigated the potential correlation between ATR-FTIR profile differences and BRCA status.

Material and Methods

50 Fresh Frozen Tissue acquired from HGSOC patients were analyzed. Among these, n=20 patients showed a tumor dissemination. Two matched representative specimens were taken from each tissue sample: one for tumor BRCA testing and the other for ATR-FTIR measurement. BRCA Devyser Kit was used to analyze the entire coding sequences of BRCA1/2 genes. The sequencing was performed on Illumina platform. Sequence data were processed using Amplicon Suite Software v.1.0. The spectroscopic analysis was performed by Spectrum one-Perkin Elmer Spectrometer. All spectra were pre-processed by rubber band baseline correction and normalization to the amide I peak. Student t-test was performed to compare the difference in the relative intensities of specific bands.

Results and Discussion

The comparison of absorbance peaks between the two cohort of patients highlighted a significant difference in the region of spectrum related to vibrations of functional groups of proteins, lipids and nucleic acids (1535 cm-1 Amide II; 1400 cm-1 COO- symmetric stretch; 1084 cm-1 PO2- symmetric stretch and 973 cm-1 PO3- symmetric stretch). Clinically relevant BRCA variants were observed in n=11 subjects. Further investigations are needed to compare the genetic results and ATR-FTIR analysis. Our results showed the potential utility of ATR-FTIR to identify biochemical differences between the two groups. Based on our preliminary data, we hypothesize that chemometric analysis on a higher number of tissue samples will allow us to identify a spectral markers typical of BRCA-positive subjects. This interdisciplinary approach could improve the quality of care patient management.

PO069

Correlation between Interleukin 6 (IL-6) and Human Epididymis Protein 4 (HE4) in Covid-19 patients: two new potential biomarkers

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Background and aim: Coronavirus disease 2019 (COVID-19) is a systemic illness, that recognizes the throat and nose as a gateway, due to Coronavirus 2 (SARS-CoV-2). In the lower respiratory tract, it is responsible of interstitial pneumonia developing into a severe acute respiratory distress syndrome. Human Epididymis Protein 4 (HE4) is present in many normal human tissues but also in the affected tissue of the SARS-CoV-2 (oral cavity tissue, nasopharynx, respiratory tract). Many studies have shown the presence of HE4 in the lining fluid of the airway surface, secreted by the sub-mucous glands. Its function is not fully clarified but HE4 seems to play an important role in the immune defense. Instead, in the bloodstream of COVID-19 patients, Interleukin 6 (IL-6) is the main inflammatory cytokine with a key role in the inflammatory storm (cytokine release syndrome, CRS) that accompanies the clinical onset of COVID-19 and thus represents a valid biomarker of the acute phase. Aim of this study was to evaluate the correlation between HE4 and IL-6 concentrations in patients with laboratory-confirmed COVID-19. Methods: 190 patients with laboratory-confirmed COVID-19 (115 males- median age 65y, and 75 female- median age 67y) were recruited (between March and May 2020). Patients did not require intensive care admission and they had no diagnosis of ovaric tumor, lung tumor and renal and/or lung fibrosis. HE4 (pmol/L) and IL-6 (pg/mL) were measured using chemiluminescence method by Architect i1000SR (Abbott) and Cobas e8100 (Roche) respectively in three consecutive days. Statistical analysis was obtained using MedCalc software and a p-value threshold of 5% was adopted. Results: Linear regression line: y=11,87(x) + 78,16; intercept 11,87 (95% CI=7,22 to 16,21; P<0,05); slope=78,16 (95% CI=34,41 to 96,22; P<0,05). Pearson correlation coefficient: R=0.58 (95% CI=0,25 to 0,66; P<0,05) shows a statistically significant correlation. Conclusions: Based on our results, HE4 could play an important role in the inflammatory response in COVID-19 patients and it could be used as a potential biomarker with IL-6 in the management of patient COVID-19. Further studies are needed to confirm the usefulness of the HE4 assay and to better explain its possible role in the immune response.

PO070

Identification of protein corona around niosome nanoparticles in human plasma

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Nanoparticles (NPs), systemically injected in the blood stream mainly for drug delivery, interact with other biological fluids and tissues. Here, NP surface is responsible for the binding of biomolecules, mainly proteins, and therefore it is coated by the so-called Protein Corona (PrC). This represents a sort of biological barrier between NPs and biological systems, mediating their cytotoxicity, cellular targeting and internalization. PrC composition depends not only on biological environments interacting with NPs, but also on their physicochemical and biological properties. Unveiling the interaction at nano-bio interface between NPs and plasma proteins is instrumental to predict the pharmacokinetics and biodistribution of NPs and rationally design nanocarries for cancer drug delivery. To this aim, we generated different formulations of niosomes (NIOs), non-ionic surfactant/ non-phospholipid vesicles, using polyethylene glycol (PEG)-mimetic tween-polysorbates as biological safe components, which are also non-toxic, low immunogenic and cheap compared to PEG. By label-free quantitative proteomics, the PrC composition and its relative abundance in various designed NIOs were evaluated ex vivo in human plasma (HP). We also evaluated PrC-NIO cytotoxicity and correlated the relative abundance of identified proteins in the corona with the cellular uptake in healthy and cancer human cell lines.Our data showed that PrC composition qualitatively/quantitatively depends on NIO sizes and on their physicochemical properties. Our in silico analysis showed that HP proteins adsorbed on NIOs are immunoglobulins, lipoproteins and complement proteins. Herein, among the observed PrC compositions, we were able to select a specific set of proteins shared and also those differentially represented within each type of PrC.Moreover, PrC-NIOs show a very low cytotoxicity confirming the biological safety of the different tween-polysorbates we used. We also found that these derivatives are able to modulate the internalization of the various NIOs in human cancer cell lines.Our results highlight the effects of different PEG-mimetic polysorbate moieties on nano-bio interactions to define protein patterns virtually aimed to enhance the NIO targeting in vivo for better drug disposal and efficacy. Acknowledgments: This work was supported by Italian Ministry of Health [RF-2010–23183729 to F.S.] and Regione Campania ["Sviluppo di Approcci Terapeutici Innovativi per patologie neoplastiche resistenti ai trattamenti (SATIN)", POR Campania FESR 2014/2020 to F.S.].

PO071

HOXA2 down-regulation promotes breast tumorigenesis and is associated with unfavorable clinical outcome in Breast Cancer patients

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Breast cancer (BC) is a heterogeneous group of diseases, each one characterized by different biological, molecular and clinical features. Accumulating evidences indicated the currently promising role of genetic and epigenetic subtype-specific biomarkers for BC early detection and disease monitoring.HOXA2, a member of the HOX gene family, is a transcription factor involved in gene expression regulation during embryonic development. HOXA2 mRNA expression dysregulation has been associated with different cancers. However, limited information is available on HOXA2 functions in BC tumorigenesis. Here, we have demonstrated that HOXA2 is down-regulated in human BC tissues and cell lines, using a RNA-sequencing approach and validated by molecular and bioinformatics dataset analyses. In addition, we have also proven the HOXA2 deregulation in murine BC tissues from a model of progesterone induced mammary gland tumors, by quantitative real time PCR. To the best of our knowledge, for the first time we have investigated the prognostic and functional role of HOXA2 in BC. Immunohistochemistry and Kaplan-Meier survival curves analyses indicated that the deregulation of HOXA2 negatively correlates with tumor grade overall in BCs and predicts a shorter relapse-free survival in luminal BC patients, respectively. At functional level, we demonstrated that HOXA2-knockdown significantly enhances cell proliferation, S cell cycle phase, cell migration and invasion. In contrast, forced increased expression of HOXA2, induced by HOXA2-overespression, remarkably inhibits cell proliferation by suppressing G1/S cell cycle transition and promoting apoptosis. Moreover, mechanistically, we demonstrated that the decreased expression of HOXA2 is epigenetically regulated via DNA methylation at CpG islands in the promoter region. Particularly, DNA demethylating treatment in BC cells restored HOXA2 mRNA expression. In addition, cell cycle analysis performed on BC demethylated cells, which re-express HOXA2, revealed a block of cell proliferation that was characterized by an increase of G0/G1 phase. In conclusion, HOXA2 is a novel tumor suppressor gene, whose down-regulation is implicated in BC progression, and could represent a promising biomarker not only for the diagnosis of BC but also to predict a worse clinical outcome.

PO072

The abundance of long intergenic non-coding RNA 01087 differentiates luminal from triple-negative breast cancer patients and predicts their clinical outcome

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Background: The molecular complexity of human breast cancer (BC) is one of the current challenges for the clinical management of the disease. Thus, the identification of subtype-specific biomarkers, such as long non-coding RNAs (lncRNAs), is currently an increasing and promising area of study and may support BC patient's stratification, early detection and disease monitoring. Methods: High-throughput sequencing approach of rRNA-depleted RNA samples extracted from 61 laser-microdissected breast tissues, followed by in silico investigation based on The Cancer Genome Atlas (TCGA) data (n=813) were performed to define a specific signature of lncRNAs in the different BC subtypes. Focusing on the long intergenic non-coding RNA 01087 (LINC01087), we estimated its prognostic value through Kaplan Meier Plotter Database, while its association with common clinical parameters and the histological origin of the tumor within the cohort of BC patients available in the TCGA database. Data from cBioPortal were used to evaluate the expression level of LINC01087 according to the mutational status of TP53, BRCA1 and BRCA2 in BC patients. TCGA samples of triple-negative BC (TNBC) and luminal A and B BC have been segregated according to the level of expression of LINC01087 (intermediated versus low/high) for comparative analysis of the transcriptomic profiles in order to identify genes modulated upon variation of the level of LINC01087. In depth in silico experimentations were carried out on these differentially expressed genes to gain indirect insights into the molecular and cellular processes that LINC01087 may regulate in both TNBC and luminal BC subtypes.Results: We identified the involvement of LINC01087 in breast oncogenesis. LINC01087 appeared significantly downregulated in TNBCs and upregulated in the luminal BC subtypes in comparison to mammary samples from cancer-free woman and matched normal cancer pairs. Interestingly, deregulation of LINC01087 allowed to accurately distinguish luminal and TNBC specimens, independently of the clinicopathological parameters, and of the histological and TP53 or BRCA1/2 mutational status. Moreover, a higher level of expression of LINC01087 predicted a better prognosis in luminal BCs, while TNBC tumors that harbored a lower expression of LINC01087 were associated with a reduced relapse-free survival. Furthermore, bioinformatics analyses performed on TNBC and luminal BC samples suggested that putative tumor suppressor activity of LINC01087 may rely on interferences with pathways involved in cell survival, proliferation, adhesion, or again inflammation. Conclusions: The assessment of LINC01087 deregulation could represent a novel, specific and promising biomarker not only for the diagnosis of luminal BC subtypes and TNBCs, but also to predict their clinical outcome.

PO073

Design and validation analysis of CFTR and 28 CF modifiers' gene panel in one-step strategy

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Cystic fibrosis is one of the most common monogenic recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR). The incidence is about 1 in 3,000 in populations of northern Europe but it is a little lower in the rest of Europe. The cystic fibrosis is related to many features, mainly respiratory disease of varying severity; however, also liver and other organs dysfunctions, and alterations of the pancreas for the presence of many cysts and fibrosis in infants affected have been observed at autopsies, and also salty sweat and meconium ileus [1-3]. Other than the CFTR gene itself, phenotypic variations may be dependent on both the environment and the genetic background, and the potential role of some candidate gene modifiers that may ameliorate the phenotype or aggravate it [4]. In this study, we designed a multi-gene panel (n=29) to investigate possible alterations of some candidate modifiers genes, by using a web-tool already used by us for the construction of other multi-gene panels in Predictive Medicine. Besides CFTR, thirteen genes are related to lung disease severity, six genes are related to cystic fibrosis-related diabetes, five are related to meconium ileus and other four genes are known in the recent literature to be associated with CF-related diseases (1,4). The designed customized panel showed a number of target regions (382) and 8,488 probes, for a total size of 309.574 kbp. A total of 69 patients affected by cystic fibrosis and with different clinical features were enrolled for the panel tested. Libraries were prepared using an ad hoc procedure based on a probe enrichment strategy and 5 different sequencing runs were set up using the Illumina MiSeq Instrument. Data Analysis was carried out using Alissa double tool: Align & Call for the alignment of sequences and the subsequent production of the QC reports; Align &Call produces also a VCF file and lastly Alissa Interpret helps with variants' filtration and interpretation of the big data obtained. We found a total of 243 total exonic variants: of which, 131 were missense, 98 were synonimous and 14 were nonsense/frameshift variants. Furthermore, forty-seven variants were splice-site variants. Besides the pathogenic variants found in the CFTR gene, some pathogenic variants were found in MBL2, PRSS1 and SERPINA1 genes related to a greater susceptibility to infections and liver pathology, pancreatitis and pulmonary disease, respectively. The results of this study indicate the usefulness to analyze in one-shot-strategy a multi-gene panel of alterations that could make predictive analysis toward the possible presence of genes alterations able to modify the phenotype or/ and the severity of the CF diseases. Furthermore, this pilot methodological study on samples from a population of CF subjects support the possibility to study also the CFTR phenotype and its modifications, which could be extended and verified on larger multiple cohorts of these subjects. Acknowledgment: This work is supported by CIRO and SATIN projects (to FS) from Campania Region (Italy), Medicina Predittiva project (to FS) from Campania Region (Italy). References:[1] O'Neal WK, et al. Annu Rev Genomics Hum Genet. 2018 Aug 31;19:201-222.[2] Bartlett JR, … Castaldo G,… Salvatore F, et al. JAMA. 2009;302:1076-83.[3] Shanthikumar S, et al. Pediatr Pulmonol. 2019;54:1356-1366. [4] Scorza M, … Salvatore F, Castaldo G. Clin Chim Acta. 2015;451:78-81.

PO074

New dimension of cell culture in cancer progression physiopathology: from spheroids to organoids

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For many decades, the 2D in vitro cultures have been used as tool to evaluate the biological formation, function and eventually pathology of tissues and organs; however, this type of cultures cannot reproduce the real complexity of the anatomical structures existing in human body. For this reason, during the last few years new types of tools, the 3D in vitro cultures, have emerged and they are currently studied particularly due to their capacity to mimic some features of solid tumors [1]. Therefore, we focus our research in this contest since our project could be focused on the study at multicellular level of some molecular mechanisms that are intrinsic to the development of neoplasia. Given that human colorectal cancer is the third most frequent malignancy and the fourth leading cause of cancer death worldwide, with approximately 1.8 million new cases and an estimated 880,792 deaths (484,224 males; 396,568 females) in 2018, we are going to build, as a first approach, monocellular 3D spheroids [2] from immortalized established cell lines (NIH3T3) and then 3D organoids from tumoral primary tissue taken directly from patients in cooperation with clinical scientists of local structures. The principal aim of our research is to study the interactions between our 3D in vitro cultures and bacterial cells in collaboration with scientists from other laboratories. These interactions are one of the main focus of cancer research, since it is well known that in many tumors there are significant inflammatory components, due also to bacterial presence. First of all, we formed the spheroids from the NIH3T3 (fibroblast deriving from mouse embryo). The cells were seeded, in a coated agarose 48-wells, at various cell densities to verify the possibility of obtaining the spheroids at least 300-400 ▫m in diameter (from 1x103 to 7 x103 cells/well). That spheroids were analyzed under the microscope (Zeiss observer Z.1) and a statistical analysis of the sphericity and of the size were performed; moreover the NIH3T3 formed spheroids were infected, as first example, with Staphylococcus aureus (ATCC 6538P) and other types of bacteria and during the time of the observation the size, the sphericity and then the amount of the bacteria were evaluated. Moreover, to investigate and better understand the size of the necrotic area of the spheroids we performed fluorescence assay [2].The first phase of the project will also be useful for the subsequent stabilization of organoids [3, 4], more specifically tumoroids; indeed we are stabilizing tumoroids deriving from patients affected by adenocarcinoma and we will report some images of this phase. Therefore, the aim of this project is also the stabilization and the characterization of organoids from colon cancer biopsies. Thanks to this technology we expect to give our contribution to precision medicine with ad hoc drugs. Acknowledgment: This work is supported by CIRO and SATIN projects (to FS) from Campania Region (Italy), Medicina Predittiva project (to FS) from Campania Region (Italy). Bibliography: Kapa#czy#ska M, Kolenda T, Przyby#a W, et al. 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. Arch Med Sci. 2018; 14:910-919. Costa EC, Moreira AF, de Melo-Diogo D, Gaspar VM, Carvalho MP, Correia IJ. 3D tumor spheroids: an overview on the tools and techniques used for their analysis. Biotechnol Adv. 2016; 34:1427-1441. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011;141:1762-1772. Xu H, Lyu X, Yi M, Zhao W, Song Y, Wu K. Organoid technology and applications in cancer research. J Hematol Oncol. 2018; 11:116. Published 2018 Sep 15.

PO075

Comparison of BRAF testing techniques in patients with advanced malignant melanoma: a real-life study.

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Introduction. Malignant melanoma (MM) is one of the deadliest skin cancers. BRAF mutation testing plays a predominant role in the management of MM patients, because modern targeted therapies essentially consist of inhibitors of BRAF. BRAF V600 mutation must be detected using a FDA-approved (USA) or CE-IVD certified (Europe) test. The aim of this study was to compare BRAF mutational testing performed by conventional nucleotide sequencing approaches with either real-time PCR (rtPCR) or next-generation sequencing (NGS) assays in a real-life, hospital-based series of advanced MM patients. Materials and Methods. Formalin-fixed and paraffin-embedded tissues from consecutive patients with AJCC stage IIIC and IV MM from Sardinia, Italy, who were referred for molecular testing, were enrolled into the study. Initial screening was performed to assess the mutational status of the BRAF and NRAS genes, using the conventional techniques recognized by the nationwide guidelines at the time of the molecular testing: at the beginning, Sanger-based sequencing (SS) and, after, pyrosequencing. The present study subsequently focused on BRAF mutation detecting approaches only. BRAF wild-type cases with available tissue and adequate DNA were further tested with rtPCR (Idylla™) and NGS assays. The study was approved by the Committee for the Ethics of the Research and Bioethics of the National Research Council. Results. Globally, 319 patients were included in the study; pathogenic BRAF mutations were found in 144 (45.1%) cases examined with initial screening; BRAF mutations were significantly more frequent in individuals older than 55. The V600E variant was the most common BRAF mutation found (83.4%). The rtPCR detected 11 (16.2%) and 3 (4.8%) additional BRAF mutations after SS and pyrosequencing, respectively. NGS detected one additional BRAF-mutated case (2.1%) among 48 wild-type cases, previously tested with pyrosequencing and rtPCR. Conclusions. Our data evidenced that rtPCR and NGS are able to detect additional BRAF mutant cases in comparison with conventional sequencing methods; therefore, we argue for the preferential utilization of the former assays (NGS, rtPCR) in clinical practice to reduce false-negative cases and improve the global accuracy of BRAF detection.

PO076

Routine laboratory tests alterations in relation with the severity of coronavirus disease 2019 (COVID-19).

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Introduction. Coronavirus disease 19 (COVID-19) is the greatest pandemic in modern history. The aim of this study was to investigate the alteration and prognostic potential of routine blood tests in a series of consecutive Italian patients with COVID-19. Methods. Clinical data and routine laboratory tests of a consecutive series of 62 COVID-19 patients treated in the Units of Infectious and Respiratory Diseases of the University of Sassari from 15 March through 30 April 2020, have been retrospectively collected. Differences in laboratory tests performed at hospital admission between COVID-19 survivors and non survivors were statistically searched and analyzed. Results. Patients in non-survivors group had higher number of WBCs (median: 9.16 x109L; IQR: 6.29-13.07 x109L vs 6.37 x109L; IQR: 4.95-9.04 x109L, p=0.037), neutrophils (mean: 9.2±6.0 x109L vs 5.4±2.7 x109L, p=0.001), and lower lymphocytes number (median: 0.6 x109L; IQR: 0.6-0.85 x109L vs 1.0 x109L; IQR: 0.7-1.2 x109L, p=0.013). In addition, non-survivors showed lower albumin (median: 3.2 g/dL; IQR: 2.9-3.4 g/dL vs 3.5 g/dL; IQR: 3.0-3.9 g/dL, p=0.035), and increased PCR/albumin ratio (median: 3.65; IQR: 2.17-6.86 vs 1.56; IQR: 0.64-4.36, p=0.035) and De Ritis ratio (median: 1.14; IQR: 0.89-1.48 vs 1.73; IQR: 1.29-2.27, p=0.002). Increased levels of LDH (median: 359 IU/L; IQR: 259-504 vs 273 IU/L; IQR: 197-356 IU/L, p=0.017), procalcitonin (median: 0.28 ng/mL; IQR: 0.19-0.52 ng/mL vs 0.07 ng/mL; IQR: 0.03-0.17 ng/mL, p=0.0006) and troponin (median: 0.181 ng/mL; IQR: 0.068-0.193 ng/mL vs 0.004 ng/mL; IQR: 0.000-0.017 ng/mL, p=0.002) has been found in non-survivors. In ROC curve analysis the better performing indexes were troponin, with a threshold of 0.037 ng/mL, 86% sensitivity and 100% specificity (AUC=0.908, 95% CI 0.701 to 0.989, p<0.001) and procalcitonin with a threshold of 0.18 ng/mL, 79% sensitivity and 79% specificity (AUC=0.807, 95% CI 0.681 to 0.900, p<0.001). Conclusions. Differences in routine laboratory test alterations between COVID-19 survivors and non-survivors have been detected; troponin and procalcitonin were the biomarkers which showed the highest prognostic abilities in our study.

PO077

SARS-CoV-2 identification and IgA antibodies in saliva: one sample two tests approach for diagnosis

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Introduction. Saliva has been proposed as a valid alternative to naso-pharyngeal swabs for detecting viral SARS-CoV-2 RNA sequences. In addition salivary glands have been described as a potential SARS-CoV-2 virus reservoir, thus supporting the search for antibodies in saliva. Furthermore, the non-invasive nature of saliva collection is conducive to self-collection, patients' compliance for repeated testing, and reduction of risk to operators, thus making saliva an eligible matrix in the SARS-CoV-2 diagnostic process. Aim. The aim of this study was to verify whether standardized and safe saliva collection is suitable for SARS-CoV-2 molecular detection and IgA class antibody measurement. Methods. A total of 49 COVID-19 patients hospitalized at the University-Hospital of Padova (Italy) and 326 subjects who underwent screening underwent naso-pharyngeal (NP) swab and saliva collection using Salivette®. Repeat blood collections were performed to evaluate hematological and coagulation parameters, biochemical markers of inflammation, and renal, liver, heart and pancreatic involvement in hospitalized patients. In all patients and subjects, saliva SARS-CoV-2 (gene E) rRT-PCR was undertaken in parallel with NP swabs. Salivary IgA and serum IgA, IgG, IgM were measured on samples from hospitalized patients. Results. NP swabs were SARS-CoV-2 positive in 9/49 patients. The comparison with saliva testing was possible for 43/49 patients, 7 of whom shared positivity, and 35 negativity while in one, the saliva result, not NP-swab, was positive. Positive molecular testing results were significantly associated with disease duration (p=0.0049). All the 326 screened subjects were SARS-CoV-2 negative on both NP and saliva swabs. Among the 27 saliva samples tested for IgA, 18 were IgA positive. Salivary IgA positivity was significantly associated with pneumonia (p=0.002) and CRP values (p=0.0183), not with other clinical and molecular data, or with immunoglubulins in serum.Conclusions. The results reported in the present study demonstrate that a standardized and safe saliva collection method can be adopted to detect SARS-CoV-2 infection in alternative to NP-swabs. Preliminary data on salivary IgA also support the use of saliva in local adaptive immunity patient monitoring.

PO078

PLASMA FREE METANEPHRINE FOR THE IMPROVEMENT OF ADRENAL VENOUS SAMPLING OUTCOMES IN PRIMARY ALDOSTERONISM

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Introduction: According to all current clinical practice guidelines, adrenal vein sampling (AVS) is recommended in almost all patients with primary aldosteronism (PA) for the assessment of plasma aldosterone concentration (PAC) before undergoing adrenal surgery. Selective catheterization is usually verified by the determination of plasma cortisol concentration (PCC) in the adrenal vein (AV) compared with a peripheral vein (PV). Dekkers et al. demonstrated that metanephrine (MN) provides a superior analyte compared with cortisol in assessing the selectivity of AVS during procedures without cosyntropin stimulation [1]. Nevertheless, plasma free metanephrines (pMets) need an accurate determination using LC-MS/MS method [2].
Aim: The aim of this study was to investigate whether pMets determination in AVS by LC-MS/MS might improve the rate of success and the diagnostic accuracy of the procedure.
Methods: pMets levels were determined by a CE-IVD kit for LC-MS/MS. AVS blood samples were manually prepared following the manufacturer instructions. The separation and quantification were performed on LCMS Shimadzu 8060, with ESI source in MRM mode. The optimized MS conditions were: interface temperature 300°C, desolvation line temperature 300°C, heat block temperature 450°C, nebulizing gas flow 3L/min, heating gas flow 14L/min, drying gas flow 5L/min. Two positive ion selected transitions were monitored for MN: 180.20>148.20 as ion quantifier and 180.20>165.20 as ion qualifier.
Results: The MN imprecision monitored with three internal quality controls demonstrated a coefficient of variation of 6% at 267 pmol/L, 2% at 859 pmol/L and 2% at 5010 pmol/L. The pairwise within-patient comparison showed that the selectivity index based on MN was significantly higher than that based on PCC, both on the right and the left sides.
Conclusions: This study establishes novel use of MN as a more sensitive alternative to cortisol to assess the selectivity of AVS. Better assessment of selectivity by use of biomarkers superior than those currently used can be an important step in the improvement of patients outcome.
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PO079

Determination of serum hepcidin-25: comparison between a research use only immunoassay and a CE-IVD kit for LC-MS/MS

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Introduction. Hepcidin is a liver-produced 25- amino acid peptide hormone (Hep-25), which regulates systemic iron levels by counteracting the cellular iron exporter ferroportin. Therefore, Hep-25 is considered a powerful diagnostic tool for iron metabolism disorders [1]. The development of analytical strategies that allow its accurate quantification in body fluids has been growing exponentially. Two types of strategies have been applied for Hep-25 quantification: MS based methods (MSs) and immunoassays (IAs). MSs permit to discriminate between hepcidin forms, while most of the existing IAs measure the total hepcidin concentration [2].
Aim. The aim of this work was to compare the performances of a research use only IA with a CE-IVD kit for LC-MS/MS in order to evaluate the reliability for routine implementation.
Methods. Serum from healthy volunteers (n=23) and from patients (n=7) were collected. Sensibility, imprecision and stability were estimated. The comparability of the methods was studied (n=42). Correlations with ferritin levels were also evaluated.
Results: The functional sensitivity of IA was 5 □g/L and the limit of quantification of MS was 2.2 □g/L. Intra-assay imprecision was <20% and <10% for IA and MS, respectively. No significant differences in Hep-25 levels were found after 3 freezing and thawing cycles for both methods. The comparability of the methods was IA= 2.09xMS+5.90 with a correlation coefficient of 0.98. Hep-25 positively correlates with ferritin levels (r=0.92 for IA and r=0.87 for MS).
Conclusions: Both methods demonstrated limitations in the reliability of routine implementation. The microplate of IA must be processed in one batch, extending the report times or causing too high cost per sample. Simultaneously, the results demonstrated cross-reactivity with other forms of Hepcidin. At the same time, the CE-IVD kit for LC-MS/MS does not meet the requirements for MS validation guidelines in relation to the calibrators (level numbers and concentrations). On the basis of all these considerations, it seems appropriate to search for a third approach, that could be optimizing a home-made LC-MS/MS method.
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PO080

HAIR DRUG SCREENING TEST FOR ILLEGAL DRUGS IN LABORATORY MEDICINE: VERIFICATION ACCORDING TO ISO 15189 STANDARD

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Background: Hair testing is an alternative or complementary matrix for diagnosis and monitoring of drug misuse such as workplace drug testing, driving licence re-granting and drug rehabilitation programmes. Aim: The aim of the present study was to evaluate the imprecision and diagnostic accuracy of 7 semi-quantitative immunoassay methods using hair specimens according to the ISO 15189 standard's internal procedure, in order to introduce hair drug screening in our practice. Materials and methods: Homogeneous Enzyme Immunoassays: QuantILab DRI Cocaine Metabolite (COC), Opiate (OPI), Cannabinoid (CAN), Methadone (METH), Amphetamines (AMP), Esctasy (EXT) and Immunalysis Buprenorphine (BUP), where applied on Ilab Taurus. The imprecision verification study consisted of three parts: a) three replicates per run, for five runs using negative and positive quality controls (QC, TricoCheck®Screening); b) calculations of inter-assay laboratory imprecision (SWL); c) assessment of uniformity with manufacturer's inter-assay claims and acceptability of test results. A total of 25 EQAS and 29 real samples were tested for the verification of the diagnostic sensitivity (SElab) and specificity (SPlab). To confirm all obtained results from hair samples an HPLC-MS/MS method was used. Results: SWL obtained for COC, OPI, METH and BUP assays were lower than those declared by the manufacturer, while SWL for CAN, AMP and EXT assays were greater, thus Upper Verification Limit (UVLWL) were calculated by using Software R. SWL for CAN and AMP assays were lower than UVLWL, instead for EXT (8.40%) was greater than UVLWL (8.39%), but still lower than state-of-the-art imprecision (<15%). SElab were 100% for all assays, showing better results for COC (94%), CAN (90%) and METH (89%). SPlab were 100% for all assays except CAN (95,2%) and AMP (92,9%). In order to verify uniformity with CAN and AMP manufacturer's specificity claims, 95% confidence intervals of manufacturer diagnostic specificity (SPm) were calculated using Wilson method. SPlab for CAN and AMP are both included in the 95% CI of SPm. Conclusion: For all immunoassays the verifications were successful and exhibit good diagnostic efficiency for hair drug screening as they do not present any false negative results.

PO081

SCREENING DELL'INFEZIONE DA WEST NILE VIRUS SUI DONATORI DI SANGUE DELL'A.O.R.N. CARDARELLI –NAPOLI : STUDIO PRELIMINARE NELLA STAGIONE ESTIVO-AUTUNNALE 2019

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PREMESSA

La West Nile Disease (WND) è una malattia infettiva di origine virale non contagiosa, trasmessa da insetti vettori (varie specie di zanzare). La WND è una zoonosi e l'uomo viene contagiato attraverso la puntura di zanzare infette, che hanno assunto a loro volta il virus da uccelli (ospiti principali) in fase viremica . Il virus, quindi, si trasmette nelle popolazioni di uccelli selvatici sino a quando, in condizioni ecologiche favorevoli, può trasferirsi dalle zanzare agli uomini ed agli equidi che rappresentano gli ospiti a fondo cieco dell'infezione.In letteratura sono riportati casi di trasmissione dell'infezione attraverso meccanismi rari , ma comunque documentati quali: trapianto d'organo, trasmissione dalla madre al feto in gravidanza (transplacentare), trasfusioni di sangue.Nel 2018, in Italia e in altri Paesi dell'Europa centro-meridionale, è stato registrato un aumento della circolazione da virus West Nile (WNV). In Italia l'infezione ha provocato 595 casi umani distribuiti in sei regioni (Veneto, Emilia-Romagna, Lombardia, Piemonte, Sardegna, Friuli-Venezia Giulia).Nel 2019 il Ministero della Salute ha emanato la circolare "Piano nazionale integrato di sorveglianza e risposta ai virus West Nile e Usutu - 2019" invitando all'applicazione di tutte le misure di prevenzione, sorveglianza e controllo dell'infezione da WNV su tutto il territorio nazionale, dal mese di Maggio a tutto Novembre2019. Il D.M. del 2 novembre 2015 "Disposizioni relative ai requisiti di qualità e sicurezza del sangue e degli emocomponenti"sancisce, tra i criteri di esclusione temporanea alla donazione di sangue, una sospensione di 28 giorni per i donatori che abbiano soggiornato almeno una notte in un'area a rischio per infezione da WNV ma essa non si applica nel caso in cui sia eseguito il test NAT WNV su singolo campione con esito NEGATIVO.

MATERIALI E METODI

Sono stati esaminati 1023 campioni di plasma in EDTA appartenenti a Donatori considerati a rischio di trasmissione dell'infezione dal Luglio 2019 fino alla conclusione del periodo di sorveglianza fissato al 31 novembre 2019 .La ricerca dell'RNA WNV su campioni di plasma di Donatori è stata effettuata impiegando lo strumento analitico Cobas Roche 6800 – MPX Test .Dal momento che la trasmissione del WNV mediante trasfusione avviene nella fase di infezione acuta, quando cioè il donatore infetto è viremico ma asintomatico, durante la somministrazione del questionario il donatore non ha percezione del suo stato di infettività pertanto, l'assenza dei sintomi non è sufficiente ad escludere l'eventuale esposizione al virus. Da qui la necessità di utilizzare una metodica analitica che possa rilevare in modo specifico e con un' alta sensibilità diagnostica l'acido nucleico in esame.Il test Cobas NAT- WNV

si basa,infatti, su una tecnologia Real-Time PCR, un sistema totalmente automatizzato per la preparazione dei campioni (estrazione e purificazione degli acidi nucleici, amplificazione e rilevazione attraverso PCR).
RISULTATI e CONCLUSIONI
Dalle analisi effettuate sulle 1023 donazioni non sono stati evidenziati casi di positività.Nei mesi estivi si verifica un fisiologico aumento di bisogno di sangue;ne consegue che le scorte possono facilmente diminuire e rendere particolarmente difficile tutte le attività chirurgiche e non solo.La possibilità di evitare la sospensione per 28 giorni dei donatori che provenivano dalle zone a rischio ha permesso di aumentare l'autosufficienza trasfusionale nel periodo estivo limitando così il numero dei soggetti esclusi dalla donazione. La nostra esperienza,pertanto,ha dimostrato come l'adozione di adeguate e tempestive misure per la salvaguardia della salute pubblica sia mostrata efficace.

PO082
Validazione della metodica di NGS per la ricerca di mutazioni puntiformi e macro-riarrangiamenti in geni associati a malattie metaboliche ereditarie

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I riarrangiamenti del genoma (macro-delezioni, macro-duplicazioni, CNVs) rappresentano una causa significativa di mutazioni associate a malattie ereditarie, e possono essere evidenziate mediante tecniche specifiche quali MLPA e a-CGH. Le recenti tecnologie di Next-Generation-Sequencing (NGS) non sono, in genere, certificate per rilevare possibili riarrangiamenti. Scopo del lavoro è stato di verificare l'applicabilità del kit Nextera Flex for Enhrichment (Illumina), per identificare alterazioni sia qualitative che quantitative, in pazienti con malattie metaboliche ereditarie (MME). Abbiamo analizzato 16 neonati con sospetto diagnostico o diagnosi di MME e i genitori di due bambine affette da Acidemia Propionica (PA), decedute prima che si potesse eseguire il test genetico per PA. In 10 pazienti abbiamo rilevato la presenza di mutazioni causative di malattia, pervenendo così alla diagnosi definitiva. In 6 pazienti sono state identificate varianti classificabili come VUS (Variant of uncertain significance) secondo i recenti criteri dell'ACMG (American College of Medical Genetics). In particolare, un neonato con sospetta glicogenosi presentava una VUS (c.989G>C; p.Gly330Ala) nel gene GYS2, associato alla glicogenosi 0. Non sono state identificate mutazioni puntiformi nei due soggetti possibili portatori di PA.
Nei pazienti con risultati apparentemente negativi abbiamo calcolato l'indice diagnostico (ID), mediante normalizzazione delle reads appaiate alle regioni geniche di nostro interesse (geni PCCA, PCCB, codificanti per l'enzima propionil-CoA-carbossilasi, carente nella PA; GYS2) rispetto ad un controllo interno di doppia dose e a tre controlli. Abbiamo ritrovato un ID di 0,5 per l'esone 21 del gene PCCA, nei due possibili portatori di PA, e per l'esone 8 del gene GYS2, nel neonato con sospetta glicogenosi. Questi risultati hanno suggerito la presenza di delezioni in eterozigosi, confermate successivamente mediante Real Time-PCR. In conclusione, l'approccio di NGS sia di tipo qualitativo che quantitativo ha consentito di pervenire a diagnosi definitiva in 13 dei 18 pazienti analizzati. Il calcolo dell'ID utilizzando il numero delle reads può essere applicato anche per la ricerca di macro-delezioni, sebbene necessiti di conferma con metodiche quantitative mirate.

PO083

ANALISI DEL TAT COME RISPOSTA ALLA PANDEMIA DA COVID-19 PER LA RIORGANIZZAZIONE DELLE ATTIVITA' IN BANCA DEL SANGUE

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INTRODUZIONE

La pandemia da Coronavirus 2 (SARS-CoV-2) si è diffusa esponenzialmente in tutto il mondo ed è tutt'ora in corso. Le Autorità Sanitarie di diversi paesi, compresa l'Italia, hanno attuato misure draconiane per fronteggiarla limitando di fatto anche gli spostamenti negli ospedali se non per motivi gravi ed urgenti.

Nel nostro Servizio afferiscono principalmente due tipologie di pazienti (Pz): quelli che necessitano di terapia trasfusionale e quelli che devono sottoporsi a salasso. Per entrambi si rende necessario il controllo dell'esame emocromocitometrico (EMS) con rapidi tempi di risposta così da poter intraprendere l'appropriata decisione terapeutica evitando contemporaneamente inutili assembramenti in sala d'attesa.

SCOPO DEL LAVORO

Attraverso la mappatura delle attività suddivisa per fasce orarie con contestuale analisi del TAT dell'EMS è possibile ottenere evidenze per migliorare l'efficienza di erogazione del Servizio così da garantire un accesso contingentato ed ordinato dei Pz nella Struttura in accordo con quanto richiesto dalla Direzione Sanitaria così da ridurne al minimo i tempi di permanenza in Ospedale.

METODI

Contaglobuli: Sysmex XN 1000 SA-01 (utilizzato dalle 08:00 alle 16:00)

Periodo: dal 5 al 15 maggio 2020

Campioni analizzati: 186 (divisi in liste di lavoro giornaliere: mediamente 23/giorno, 9 nella prima fascia)

RISULTATI

- TAT in minuti:

media: 15; mediana: 14.

- Suddivisione dei campioni per fascia oraria:

7:50 - 08:59: 90, TAT medio 22'

9:00 - 09:59: 43, TAT medio 11'

10:00 - 10:59: 14, TAT medio 14'

11:00 - 12:59: 15, TAT medio 7'

13:00 - 14:30: 24, TAT medio 11'

- Wilcoxon test tra dati complessivi e dati prima fascia:

Rango medio 1° gruppo = 125,05 (n=186); Rango medio 2° gruppo = 166,29 (n=90);

Z test = 4,02 ; P a due code = 0.0001

CONCLUSIONI

Il lavoro ha messo in evidenza un allungamento a carico della prima fascia oraria legato al numero di campioni più elevato, all'esecuzione dei QC e all'orario di ingresso in servizio del personale.Con il monitoraggio del TAT si è potuto rimodulare l'accesso dei pazienti in Struttura, l'assetto organizzativo ed evitare inutili e pericolosi stazionamenti.

PO084

Radio-response biomarkers to Proton Therapy in Triple negative Breast cancer xenograft: molecular signature by immunohistochemical and gene expression profile analyses.

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Triple Negative Breast Cancer (TNBC with ER-/PR-/HER2- receptor status) is characterized by a more aggressive phenotype and a poorer prognosis than other breast cancer subtypes (BC), mainly due to fewer targeted medicines. Although radiation therapy (RT) represents one of the treatment options for BC, the RT administration to patients with TNBC is currently debated. Besides, the Proton Therapy (PT) provides various advantages compared to conventional RT such as the treatment of inaccessible area located or conventional RT resistant cancers, thus making it an effective option in TNBC treating. Here we explore the molecular response to PT showing how PT affects the gene expression of tumour. Immunohistochemical (IHC) and Gene expression profile (GEP) analysis were carried out on TNBC xenograft model exposed to 2, 6 and 9 Gy of PT to identify predictive biomarkers and potential targets for increasing radiosensitivity. We observed after 10 days post-PT a reduction in necrotic foci number and cleaved CASP3 expression (apoptotic marker) at higher doses, also associated with an increase in CD68 (macrophages marker) demonstrating as these cells were able to scavenge dead tumour cells and cell debris. Also, both the survival molecule Cyclin D1 and the stem cell marker CD133 were down regulated at 9Gy.Comparative differential gene expression analysis showed that all PT schedules affected the expression levels of many genes by 2-fold or greater compared to the untreated group. For instance, of 1279 significantly altered genes 407 were down-regulated and 872 were up regulated after 9 Gy exposure. The clustering analysis with DAVID tool allowed us to group them based on the involvement in specific biological pathways, including the cell-cell communication and/or the immune system activation (e.g. antigen processing and presentation; graft-versus-host disease; chemokine signaling pathway; focal adhesion), tumour progression, angiogenesis and invasiveness (e.g.

signaling pathway of VEGF, Ras, HIF-1 and pluripotency of stem cells). Some of them were validated by qRT-PCR, thus confirming the gene-expression trends of microarray. Finally, 290-genes, shared among all the configurations assayed, were identified as PT cell response molecular signature mainly demonstrating the regulation of immune response, cell cycle and stem cell proliferation.

PO085

WHEN THE HEMOGLOBINIC VARIANTS HAVE BEEN FOUND IN HPLC

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Purpose of the study: Always looking for hemoglobin variants during chromatographic analysis in HPLC for glycata Hemoglobin dosage, more attention has been paid to graphs and new accesses of carrier patients or not in relation to their origin from the Mediterranean basin or second ethnicity.
Material and methods: The instrumentation in use at our laboratory is the Variant II Biorad with Biorad standards and their two-tier controls. The EDTA whole blood samples come from wards or the clinic.

Results: Two cases of hemoglobinopathy S were found, one in a carrier not suffering from manifest disease and one in a foreign patient hospitalized with sickle cell anemia for pregnancy. With regard to hemoglobin E, D in thalassemic patient in paediatric age the association of variants was related to the thalassemic trait and in adult patient the same case occurred with variant E and D. In another patient of a control of the occupational medicine has been detected a highly elevated hemoglobin F (area's value 11.9 % towards to HbA1c 61 mmoli/moli).

Discussion and conclusion: Chromatographic spikes have been useful in framing variants and resolving clinical cases, although patients are often already aware of their genetic characteristics because they have already performed diagnostic tests.

PO086

Adherence to the gluten-free diet: a novel LC-MS/MS method to monitor urinary gluten exposure biomarkers

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The only treatment currently available for celiac disease (CD), an immune-mediated enteropathy of the small intestine caused by the ingestion of gluten in genetically predisposed individuals, is the gluten-free diet (GFD). A tight adherence to GFD is essential to reduce symptoms, avoid nutritional deficiencies and improve patient's quality of life. To monitor effective exposure to gluten, great interest has been devoted to the identification of specific biomarkers and development of analytical methods allowing their fast detection and quantization. A "33 mer" peptide, alkylresorcinols (ARs) and their main urinary metabolites (3,5-dihydroxybenzoic (DHBA) acid, 3-(3,5-dihydroxyphenyl)-propanoic (DHPPA) acid) were suggested as potential molecular biomarkers for short-term monitoring of the compliance to the GFD. Our aim has been to develop and validate analytical methods using ultra high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) for the monitoring of these biomarkers in human urinary samples. In order to guarantee a minimal interference from the pre-analytical procedure, urinary samples underwent a simple dilution protocol. UHPLC-MS/MS analyses were carried out using an Ultimate300 UHPLC coupled with a TSQ-Endura ESI-Q-q-Q mass spectrometer. Chromatographic separation of the compounds was achieved using a reverse-phase C18 column and a mixture of 0.1% formic acid in water and acetonitrile as mobile phase. Multiple reaction monitoring transitions were set for the "33 mer" peptide and the main alkylresorcinols urinary metabolites DHBA and DHPPA. The method was validated and some preliminary data were acquired on patient samples. Promising results were obtained for DHB and DHPPA, whereas the urinary levels of 33mer peptide were very low, also in the case of subjects not undergoing a GFD. These evidences suggested that monitoring urinary levels of DHBA and DHPPA by LC-MS/MS might represent a promising tool to help both CD patients to keep their diets under control and physicians to understand the causes of any adverse events potentially occurring. In this framework, the proposed approach provides good efficiency and wide applicability, even if further evaluations need to be performed by analysing a larger number of real samples.

PO087

Development and validation of a LC- MS/MS based method to monitor opioids plasma concentration in pain management

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Opioids are a class of drugs used to treat different forms of pain, which are characterized by a reduced therapeutic index. Opioids can be dispensed through different routes of administration, which are chosen based on patient's condition, type, intensity of pain and chemical characteristics of the drugs themselves. The use of these drugs in clinical practice is managed by the clinician based on the observed reduction or absence of pain and possible onset of adverse drug reactions. Therefore, there is a fundamental lack of studies addressing the individual pharmacokinetic response to these drugs, which hampers a rationalized fine-tuning of the therapy personalized to the specific patient. We developed a selective and sensitive LC-MS/MS assay to monitor a large panel of opioids and related metabolites in blood plasma including morphine, buprenorphine, fentanyl, meperidine, oxycodone, oxymorphone, dihydromorphine, norbuprenorphine, morphine 3- and 6-glucuronide and hydromorphone, hydrocodone. Molecules and internal standards were extracted from blood plasma using a single step protein precipitation in methanol. Moreover, the efficacy of this approach was also evaluated on volumetric adsoprtive microsampling devices (VAMS) and on saliva samples. The method was linear in the range from 0.1 to 100 ng/mL. The methodology developed may be easily implemented in clinical settings such as Palliative Care and Pain Medication Units where polytherapeutic treatments are often used and rapid and versatile methodologies such as those based on mass spectrometry can be informative and useful to help the clinician in decision making process.

PO088
qPCR, dPCR and targeted NGS for the detection of EGFR mutations in lung cancer patients

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Lung cancer is a leading cause of cancer death worldwide. Patients with EGFR oncogene driving mutations benefit from treatment with tyrosine kinase inhibitors but eventually progress developing resistance to therapy. The most common mechanism of resistance is the secondary acquired mutation EGFR p.T790M. As a consequence, it is important to assesses EGFR mutational status at the time of diagnosis and during treatment. Molecular testing guidelines for selection of lung cancer patients for tyrosine kinase inhibitors indicate cell-free DNA (cfDNA) as a surrogate for the determination of EGFR status in patients with limited and/or insufficient tissue.We performed a pilot study to compare different methods for the detection of EGFR mutations in cfDNA from a small cohort of lung cancer patients. In particular, we evaluated the performances of three approaches based on quantitative PCR (qPCR), digital PCR (dPCR) and targeted next generation sequencing (NGS). Results were mostly concordant, but some discrepancies were found for qPCR and dPCR. NGS in some cases allowed a more sensitive detection of variants, especially when a tag sequencing approach was adopted. The major advantage of NGS is represented by the possibility of a simultaneous detection of multiple targets. The pre-analytical phase, in particular cfDNA extraction methods, could also influence the results. From our preliminary data emerges that the combined use of multiple methods allows a more comprehensive and reliable assessment of the presence of mutations in cell free DNA (cfDNA).

PO089
THE HbA1c IN COVID 19 PATIENTS

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Purpose of the study: During the emergency period Covid -19 the glycosilate hemoglobin has been required for patients admitted to specialist wards suitable for metabolic control in diabetics and non-diabetics.

Material and methods: The test HbA1c has been performed on Variant II Biorad, calibrated with standard Biorad for glycate hemoglobin, using EDTA whole blood samples after running two-tier Biorad controls.

Results: The patients have been studied in the wave of coronavirus infection corresponding to the two months of loackdown. Thirty patients that have been hospitalized in covid area have been tested in our laboratory . In 12 females and 6 males with average age 50 years old the HbA1c has been resulted in the limits (up to 42 mmoli/ moles) and in 10 patients (6 females and 4 males average age 48 years old) higher values (between 51 and 79 mmoli/molioli) have been found. The patients have been so followed for the care of the case.

Discussion and conclusion: Thus it has been possible to discern among patients with comorbidity and patients with lower degree of exposure to risks because in return to metabolic improvement and it has been possible to collaborate for the health of the sicks.

PO090

Detecting Protein Kinase A in human blood and urine samples: a new frontier in laboratory diagnostic procedure?

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Protein Kinase A (PKA) is considered the main intracellular target for adenosine 3',5'-cyclic monophosphate (cAMP) in mammalian cells. Also known as cAMP-dependent protein kinase, PKA exists in two isoforms termed PKA I and II, which differ in regulatory subunits, cAMP binding affinity and cellular localization. Symmetrically arranged in pairs as a tetramer, regulatory subunits contain cAMP domains, whereas catalytic entities (PKA-C) phosphorylate serine and threonine residues on specific substrates modulating several cell processes. PKA has clearly been involved in cancer where, depending on spatial-temporal distribution and activation, it exerts both proliferative and inhibiting effects. Notably, overexpression of PKA-I isoform is associated with serious clinic-pathological outcomes in cancer. More recently, an extracellular PKA (sPKA) isoform has been identified in human serum and sPKA autoantibodies have been detected in cancer patients. However, sPKA serum functions, as well as its diagnostic significance, remain unknown. Even though several sPKA detection assays have been developed, no one refers to a laboratory diagnostic procedure. Among these, western blotting (WB) has been employed in detecting serum sPKA in different species, including human and canine. Since no data currently reports its presence in urine, we decided to investigate sPKA in human urine and serum specimens by WB, concurrently. Specifically, using two commercial endogenous PKA-C antibodies, we analyzed six blood and seven urine samples, respectively. Although patients-related diagnosis was unknown to us, samples differed for gender and age range. In agreement with previous findings, we detected a distinct band in all serum samples, albeit its molecular weight was slightly higher than the endogenous one. Surprisingly, four out of seven urine samples showed an analogue band appearance, thus identifying firstly the existence of sPKA in urine. Moreover, differences among sPKA positive urine samples, as well as in loading amount, were also observed. Despite quite preliminary, our findings recognize WB as a useful tool for sPKA detection in different body fluids and further emphasize the relevance of sPKA detection as an intriguing aspect in laboratory diagnostic, even in urine samples.

PO091

Esperienza di un anno di dosaggi di farmaci anticoagulanti orali diretti nel laboratorio HUB della Provincia di Modena

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INTRODUZIONE: In Italia a partire dal 2013 sono stati commercializzati i farmaci anticoagulanti orali diretti contro i fattori della coagulazione (DOAC) per i quali non era indicato alcun controllo di laboratorio. E'stato poi dimostrato che, in alcune condizioni, è auspicabile il dosaggio del farmaco. Se il gold standard è la spettrometria di massa, peraltro di difficile utilizzo nei laboratori clinici, sono stati implementati test di coagulazione, di più semplice e rapida esecuzione, dai quali ricavare la concentrazione del farmaco in particolare utile per le finalità cliniche dei DOAC utilizzati: Dabigatran, Rivaroxaban, Apixaban e Edoxaban. Si è verificata la possibile correlazione tra concentrazione dei DOAC e allungamento dei tempi di PT e APTT.MATERIALI E METODI: Nell'anno 2019 nel laboratorio dell'Ospedale di Baggiovara sono state eseguiti 275 dosaggi di DOAC con metodica IL Werfen: Dabigatran 28, Rivaroxaban 78, Apixaban 124 e Edoxaban 45. Sono stati studiati 173 pazienti (70 femmine e 103 maschi) di età compresa tra 31 e 92 anni.RISULTATI: Sono stati eseguiti 67 PT contemporaneamente al dosaggio dei DOAC. 46 PT sono risultati alterati con RATIO comprese da 1,22 a 3.08 (V.N. fino a 1.2). In 3 casi l'allungamento del PT era con dosaggio di Dabigatran inferiore a 30 ng/ml. In 4 casi il PT elevato era in presenza di concentrazioni di anticoagulanti orali antiXa inferiori 30 ng/ml e vi era anche insufficienza renale. Sono state eseguite anche 60 determinazioni di aPTT. 12 sono risultati alterati con RATIO tra 1.26 e 1.73 (V.N. fino a 1.25). 2 aPTT elevati erano in presenza di dosaggio di Edoxaban (159- 251 ng/ml), 1 di Dabigatran (178 ng/ml) e 9 di Rivaroxaban (valori compresi tra 25 a 444ng/ml). 8 di questi aPTT elevati erano associati a PT allungati.CONCLUSIONI: Se i DOAC interferiscono nei i test basali della coagulazione, l'allungamento dei tempi di coagulazione non è inequivocabilmente legato alla concentrazione del farmaco anticoagulante. Il Dabigatran allunga aPTT, mentre Rivaroxaban modifica, più degli altri antiXa, il PT. Alla luce dei nostri dati non è possibile individuare una correlazione utile clinicamente tra la concentrazione del farmaco e allungamento di PT e/o aPTT confermando la necessità del dosaggio di laboratorio dei DOAC.

PO092

IDONEITA' ALLA GUIDA: STUDIO DI UN PROTOCOLLO PER COMMISSIONE MEDICO LOCALE PER L'ACCERTAMENTO DEL CONSUMO DI ALTRE SOSTANZE D'ABUSO NEI CONDUCENTI SANZIONATI PER GUIDA IN STATO DI EBREZZA.

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Scopi ed obiettivi

Lo stato derivante dalla dipendenza da alcool e droghe 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 studiato dal nostro Laboratorio un Protocollo che vede oltre alla tradizionale quantificazione della CDT su siero e dell'Etilglucuronide (ETG) su matrice cheratinica, anche della cocaina e suoi metaboliti con particolare attenzione alla Coca-etilene.A tal fine, tramite uno studio retrospettivo andremo a dimostrare come l'introduzione di un Protocollo di questo tipo, possa rappresentare uno strumento efficace nel monitoraggio per l'idoneità alla guida, e come questo ci permetta di evidenziare un fenomeno misconosciuto perchè non valutato, quale l'uso di altre sostanze stupefacenti nei conducenti sanzionati per guida in stato di ebrezza.

Materiali e metodo

Le indagini su matrice cheratinica (ETG,COCAINA e METABOLITI) sono state eseguite in LC-MS/MS:Agilent Infinity 1260 LC-MS/MS 6470 Triple Quadrupole La determinazione della Transferrina Carboidrato Carente (CDT) è eseguita con :Ultimate 3000 Thermo Scientific HPLC UV/VIS

Risultati e Conclusioni

Dall'osservazione dei nostri dati, emerge che su 2215 utenti monitorati negli anni dal 2017 al 2019 per guida in stato di ebrezza a cui è stato applicato il nostro protocollo, circa il 11.1% presentava una positività alla Cocaina. Mentre il 7.1% di questi sarebbe risultato idoneo alla guida perché presentava una concomitante positività all'ETG ma con valori <30 pg/mg solo l'1%, presentando valori >30pg/ml, sarebbe comunque risultato inidoneo. Possiamo concludere che l'introduzione del nostro Protocollo dimostra che la ricerca di altre sostanze d'abuso potrebbe sicuramente essere di ausilio alle Commissioni Medico Locali fornendo nuovi elementi valutativi per dichiarare l'idoneità alla guida.

PO093

Cell-free circulating tumor DNA as molecular biomarker in thymic epithelial tumors

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Background: Thymic epithelial tumors (TETs) are rare thoracic malignancies. Widely recognized as different histopathological entities, thymoma (T) and thymic carcinoma (TC), show a different biological behavior with a higher tendency to hematogenous dissemination for TC and thoracic recurrence for T, sharing, however, a poor prognosis when characterized by high tumor burden. Up to date, there are no specific biomarkers for monitoring the biological course of these rare tumors. Analysis of circulating cell-free DNA (cfDNA) has potential applications throughout the natural course of cancer development, diagnosis and treatment, nevertheless several studies have suggested that cfDNA levels closely parallel overall tumor burden. For the first time the detection and the correlation of cfDNA levels with tumor burden and histological subtype of TET, has been carried on in this monocentric study.

Methods: Starting from July 2018, plasma samples from 11 patients with TC, 7 with completely resected TET (rTET) and 15 with T, were prospectively obtained before the initiation of therapy. Plasma samples from 13 healthy donors were used as control. Five ml of blood was collected and processed within one hour or less. Plasma was obtained, aliquoted and stored at #80 °C. The isolation of cfDNA from human plasma was performed with QIAamp MinElute ccfDNA Kits. cfDNA quantification was assessed by Real-time PCR. Clinical, and histo-pathological features of TET were assessed.

Results: ctDNA concentrations were significantly higher in patients with TETs compared to controls (14.8 versus 3.8; $p<0.001$). We found significantly higher ctDNA amount in patients with T and TC separately compared to controls with a median cfDNA level in healthy donors of 3.8 ng/µl , in T patients of 11.4 ng/µl ($p<0.001$) and in TC subjects of 25.6 ng/µl ($p<0.001$). No significant difference in ctDNA concentrations was found among thymomas according to clinical stage (IIIB/IVA versus IVB; $p= 0.662$). In addition, we demonstrated that ctDNA concentrations were higher in metastatic TETs (M1a and M1b) compared to non-metastatic (25.6 ng/ul versus 7.2 ng/ul; $p= 0.037$). Conversely, no significant difference was shown between advanced (T3/T4) and local (T0/T1/T2) TETs ($p= 0.862$). Furthermore, there was no significant difference between local (IIIB/IVA) and advanced (IVB) Masaoka-Koga stages ($p= 0.854$) and no significant correlation between ctDNA quantification and tumor burden, based on RECIST

criteria (r= 0.07, p= 0.725). Finally, we compared ctDNA circulating levels of patients with no evidence of disease after tumor resection with healthy subjects and we found no significant difference (p= 0.203).
Conclusions: To the best of our knowledge, this is the first study that explore detection and quantification of cfDNA in TET. Higher baseline levels than the control group have been registered for both advanced T and TC. Highest levels of cfDNA was associated with the presence of distant metastasis. We envision that further valuable information will be obtained with mutational analysis.

PO094

Il fattore di crescita placentare (PIGF) ed il fattore solubile anti-angiogenico1 (sFlt1) come predittori di preeclampsia in gestanti in emodialisi

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La gravidanza in emodialisi (HD) è un evento ad alto rischio. Inefficiente depurazione e scarso controllo dell'acqua corporea espongono a sofferenza placentare ed aumentano il rischio di pre-eclampsia. Il fattore di crescita placentare (PIGF) ed il fattore solubile anti-angiogenico1 (sFlt1) sono usati come predittori di preeclampsia in gestanti normo-funzione renale. Nella gestione di una gravidanza in HD abbiamo predisposto un algoritmo per la stima del peso secco integrando i dati della bioimpedenzometria multifrequenza con le stime ecografiche del peso fetale. Questo ci ha permesso di prevenire episodi ipotensivi intra- e peri- dialitici. Inoltre, abbiamo somministrato una dose dialitica incrementale da 12 a 24 ore/settimana dalla 13 alla 32 settimana di gestazione tale da garantire un'azotemia pre-dialitica <100 mg/dl. La paziente ha partorito a 35 settimane di gestazione un bambino sano di 2,05 Kg in assenza complicanze. Come end-points sperimentali a sostegno del benessere placentare abbiamo valutato i valori assoluti di PIGF e sFlt1, e le loro variazioni pre-post dialitiche settimanalmente. I valori di PIGF e sFlt1 non hanno mai raggiunto livelli di rischio pre-eclamptico, né hanno presentato brusche oscillazioni in corso di HD, possibile segno di sofferenza placentare. Questi dati preliminari supportano l'utilizzo della determinazione di PIGF e sFlt1 nel monitoraggio della gravidanza di pazienti in emodialisi.

PO095

European Biological Variation Study (EuBIVAS): Within and between-subject biological variation estimates obtained from 91 healthy subjects for serum thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), thyroglobulin (

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Background: The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group on Biological Variation (BV) designed the European Biological Variation Study (EuBIVAS) for obtaining reliable BV estimates for the measurands of greater clinical importance. In this study, BV estimates for thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), thyroglobulin (TG), and calcitonin (hCT) are delivered. Methods: Analysis were performed on serum samples obtained from the EuBIVAS population (91 healthy individuals from 6 European laboratories bled for 10 consecutive weeks, aged 21-69 years) and stored at -80 °C prior to analysis. Analysis were performed with Roche Cobas e801 at the San Raffaele Hospital (Milan, Italy). All samples from each individual were measured in duplicate within a single run. The data were subject to outlier analysis prior to CV-ANOVA, to determine the BV estimates with confidence intervals at 95% (CI). Homogeneity of analytical CV (CVA) and of intra-subject BV (CVI) were examined. Trend analysis was performed to verify the steady-state of the subjects. The normality assumption of the distributions were verified. Reference change values (RCV) and analytical performance specifications (APS) for imprecision (CVAPS) and bias (BAPS) were obtained using CVA and BV estimates, choosing the lowest value of CV when statistical differences between sexes were found.Results: CVI value obtained for TSH (17.7% (16.8-18.7)), FT3 (5.0% (4.8-5.3)), FT4 (4.8% (4.5-5.0)), TG (10.3% (9.8-10.9)) are comparable to the meta-analysis values reported on the EFLM BV database, calculated from 8, 5, 3 and 2 papers respectively. The CVI obtained for hCT, data not available in the EFLM database, resulted 13.0% (12.3-13.9). CVA calculated by ANOVA on sample's replicates, were below desirable CVAPS based on current BV data for each measurand. Differences between sexes in mean values, with the exception of TG, were found for all measurands, so that between-subjects BV estimates (CVG) were considered separately for males and females.Conclusions: Updated BV estimates EuBIVAS based for serum TSH, FT4, FT3, TG, and hCT allowed to obtain reliable APS and RCV that in the clinical practise may be key tools for the diagnosis and management of overt or subclinical thyroid diseases.

PO096

Deletion of ANKRD11 gene in a boy with autism spectrum disorder and epilepsy

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The array-Comparative Genomic Hybridization (a-CGH) is currently a fundamental technique used to identify genetic alterations associated or involved in disorders characterized by autism spectrum disorder, mental disabilities, language delay and behavior problems. Most severe forms have a single genetic cause such as chromosomal aberrations, monogenic defects, metabolic disorders and imprinting/epigenetic disorders, whereas mild forms are thought to be more commonly the result of the interplay of several genetic and environmental factors. In this study, we describe a male child with autism spectrum disorder and epilepsy. High resolution a-CGH analysis was performed on genomic DNA from the patient by using 170,334 60-mer oligonucleotide probes that cover the whole genome with an average spatial resolution of 13 Kb and an average 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).By using a-CGH we detected a deletion of around 31.97 Kb on the 16 chromosome at q24.3 region that includes Ankyrin Repeat Domain 11 (ANKRD11) gene. ANKRD11 gene encodes a nuclear protein, which acts as a transcriptional co-regulator. Studies shown that this protein plays an important role during neural development, regulating histone acetylation and gene expression, and in so doing determining precursor proliferation, neurogenesis, and neuronal positioning. Mutations in this gene have been associated with KBG syndrome, which is characterized by macrodontia, distinctive craniofacial features, short stature, skeletal anomalies, global developmental delay, seizures and intellectual disability. Moreover, individuals with ANKRD11 mutations display neurodevelopmental phenotypes, including aspects of ASD, cognitive disability and neuroanatomical perturbations. The role of Ankrd11 as a chromatin regulator that controls histone acetylation and gene expression during neural development provides a likely explanation for its association with cognitive dysfunction and ASD.

PO097

Oral and colon microbiome in colorectal cancer patients with obesity: a pilot study.

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Weight gain is associated with increased risk of metabolic disorders and of cancer, including colorectal cancer (CRC). Inflammation is a common feature of both obesity and cancer, but the origin of this inflammation is not yet clear. In the last years, growing evidence has shown that the gut microbiota composition and activity might be associated with both the onset of inflammation and cancer. Our aim was to evaluate the oral and colon microbiomes in two cohorts of CRC (n=10) and control subjects (n=10) from the Campania Region, to identify the CRC-associated microbial profile in the two gut niches, and also any difference between lean- and overweight-CRC associated microbiomes. Any similarity between oral and colon microbiome might allow to use oral samples, that are sample easier to obtain than the colon biopsies, for screening and diagnostic purposes of CRC-associated dysbiosis in at risk for CRC subjects. Colon biopsy and oral specimens from the enrolled subjects were obtained during colonoscopy for diagnostic purposes and by saliva collection, respectively. DNA extraction was performed using commercial kits and microbiome profiling using the Microbiota solution B kit and software (Arrow Diagnostics service, Genova, Italy), according to the manufacturer's specifications and the NGS system MiSeq (Illumina). We also tested as external Quality controls two microbial standards, the Gut and Oral Microbiome Genomic Mix (ATCC), with the same procedure as patient samples. Preliminary data showed that, among the five phyla present in the colon microbiome with a relative abundance >1%, Bacteroidetes and Proteobacteria were the most abundant in CRC patients and controls, respectively. Furthermore, oral and colon microbiomes similarities were observed in CRC patients regarding the relative abundances of Bacteroidetes and Firmicutes. Despite our data need to be confirmed in a larger than the present number of samples, they give hope that saliva could be used as first sample in the screening of CRC-associated dysbiosis. Grants: SATIN (Sviluppo di Approcci Terapeutici Innovativi per patologie neoplastiche resistenti ai trattamenti) – D.D. n. 355 del 5/06/2017– Fondo FESR 2014/2020.

PO098

Information Technology in Laboratory Medicine: development of a stand-alone application for the management of the traceability and outcomes of SARS-COV-2 swabs sent for analysis to external structures.

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During the emergency for SARS-COV-2 the high number of samples from different applicants related to the internal analytical capacity, initially limited and dedicated to inpatients, the emergency room and triage tents, has lead for the analysis of a large number of swabs to be entrusted to IZSLER, a structure with high skills and adequate technology for massive virological analyses. The need to trace these samples was immediately clear, given by the multiple applicants to the laboratory Service, for which complete traceability had to be guaranteed from check-in until delivery of the result to the correct subject. The initial table in Excel (Microsoft) used in the early days has been replaced by an application (TraceCOVID) conceived and developed by our laboratory. TraceCOVID was written in Visual Basic .NET (Microsoft) initially using a relational SQLlite database and, subsequently, SQL Oracle as the amount of data and the number of accesses to the application by different users required a more performing database. The application has an integration with the LIS (Dedalus) to receive samples' information. Actually it manages the sample check-in in the laboratory and the check-out in the preparation of shipment's list and the accompanying sheet (with barcode, name, surname and origin) plus a second excel file sent via SFTP allowing IZSLER to manage the samples with their systems. The results from the IZSLER are returned via SFTP in an excel file which TraceCOVID automatically import, associate the report with the patient and send an email for each patient containing the outcome. The application offers the possibility to print the report if necessary as well as the possibility of entering all the information (patient data and outcome) manually. There is also a statistics section that monitors the number of samples and outcomes generating different types of outputs. At the request of the Administrative Offices, a data export function consistent with the format requested by the Lombardy Region was also included. The emergency for COVID19 has highlighted how the presence of computer skills in the laboratory has made it possible to cope with a critical aspect regarding the swabs' management and allows efficient information flows to the competent health authorities.

PO099

Integrazione della preparazione in manuale su piastre 96 pozzetti con il LIS e creazione della sequenza per l'analisi in LC-MS/MS.

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Nonostante la sempre maggiore presenza di automazione e di integrazione con i sistemi informatici, persistono metodiche, in area specialistica, che prevedono una fase preparativa manuale. Nel caso specifico quella relativa all'analisi degli ormoni steroidei e la successiva analisi in LC-MS/MS (Chromsystems - Waters). La procedura prevede la preparazione dei campioni su piastra a 96 pozzetti con la creazione di una sequenza di lavoro sullo strumento utilizzando le "coordinate" della piastra. Questo passaggio è estremamente sensibile ad errori di inserimento ed identificazione in quanto è poco agevole trasporre una lista di campioni in una matrice bidimensionale. Inoltre è necessario anche distinguere i campioni analizzati per i differenti steroidi richiesti ed inserire i valori target per calibratori e controlli. La soluzione adottata dal nostro laboratorio è stata la realizzazione di un applicazione "Crea Sequenza", realizzata in Visual Basic .NET (Microsoft), interfacciata con il middleware del settore LC-MS/MS. In "Crea Sequenza" l'operatore indica il numero di campioni da analizzare, la prima posizione libera sulla piastra (predefinito il pozzetto A-1) e procede con la lettura dei barcode delle varie provette. Successivamente il software interroga il middleware per le analisi da effettuare e seleziona a quale pannello associare il campione oltre a completare l'anagrafica. A video compare quindi una rappresentazione grafica (stampabile) della piastra riportante la distribuzione dei campioni. Infine genera le sequenze pronte per essere importate sullo strumento. Il risultato viene in seguito esportato verso il middleware. Si ottiene in tal modo una notevole riduzione della necessità di inserimento manuale da parte dell'operatore e conseguentemente del rischio di errore essendo la sequenza dei campioni e delle analisi da eseguire, trasferita sulla mappa della piastra da un processo automatico, migliorando in tal modo l'efficienza e la sicurezza del processo analitico.

PO100

Analisi in emergenza: sviluppo di un "cruscotto" per il monitoraggio TAT e Valori Critici

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La gestione dei campioni e delle relative analisi nel laboratorio di emergenza presenta diverse criticità, tra cui la tempestiva identificazione dei campioni con valori critici e dei campioni in ritardo. Non sempre i LIS in dotazione sono ottimizzati per tali scopi o consentono l'accesso alle informazioni in modo veloce ed efficace. A tal proposito è stato sviluppato internamente al laboratorio il programma "Cruscotto Urgenze", realizzato in Visual Basic .NET (Microsoft). L'applicativo è interfacciato direttamente con il database del LIS (DNLab, Dedalus) per la lettura delle richieste presenti nel sistema in carico al laboratorio e, tramite un processo temporizzato configurabile dall'utente, ne monitora lo stato; l'applicazione è così in grado di fornire in modo sia aggregato sia dettagliato (come grafico e come lista) le informazioni sui campioni, indicando quelli in ritardo rispetto al tempo di consegna (TAT) previsto, su quelli che presentano valori critici, su campioni segnalati come non pervenuti e ovviamente quelli che necessitano della validazione di II livello. Tutto questo sia per il laboratorio centrale sia per i presidi esterni durante la validazione in telemedicina (i secondi attivabili tramite un'opzione con cui selezionare quali presidi monitorare). Il Cruscotto Urgenze presenta un'integrazione con l'applicativo di laboratorio (CriticoLab), creato in Access (Microsoft), per la registrazione dei valori critici e la loro comunicazione ai reparti. L'integrazione permette l'inserimento automatico delle informazioni necessarie rendendole subito disponibili per essere comunicate. È inoltre possibile registrare e tracciare le modifiche al contratto (inserimento di esami su campioni già ricevuti) sempre tramite la selezione da una lista dedicata. L'accurata valutazione del contesto, coniugato alle competenze informatiche del personale dirigente, ha portato alla realizzazione del "Cruscotto Urgenze" che fornisce in tempo reale un quadro completo ed aggiornato dello stato dei campioni che transitano nel laboratorio di emergenza. Oltre ad eliminare modulistica cartacea e una tediosa attività di trascrizione, Cruscotto Urgenze permette l'archiviazione e la tracciabilità delle informazioni ed una loro valutazione per revisioni operative.

PO101

May artificial intelligence improve the detection of Laboratory Medicine errors? The example of delta check.

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Background:
Delta check (DC) is used to detect discrepancies between sequential laboratory results of the same patient. Despite the high utility of DC for determining potential diagnostic error, limited discriminative power has been described for threshold-based rules. Artificial intelligence (AI) and machine learning (ML) might help in determining multiparametric algorithms for improving DC strategies for better distinguishing physiological changes from samples mix-up.
Methods:
Complete blood count (CBC) raw data from three XE 2100 hematological analyzers (Sysmex, Milan Italy) were obtained from the Department of Laboratory Medicine of Padova during the periods Apr-Maj and Oct-Dec 2019. The two closest sequential results were extracted for patients with repeated testing, while mixed-up samples were artificially generated by subsampling two different batches (each of 2000 test results), which were randomly matched. ML algorithms parameters were tuned [Classification Trees (CT), Support Vector Machine (SVM), Neural Network (NNET)] using 10-fold cross-validation. The performances on identifying sample mix-up were evaluated by accuracy, sensitivity, and specificity and compared against a univariate MCV threshold generated by ROC curves (URC). Training and testing (75% and 25% of whole data, respectively) were used to validate results. R for statistical computing (v 3.6.2) was used for the analyses.
Results: Within the study periods, a total of 41380 tests were collected, and these included 20 CBC parameters. Sequential repetitions of 2367 patients' tests were obtained from the extraction and merged with the artificially generated mixed-up tests (4367 total tests were examined). Analyses showed that almost all variables (18/20) are useful for defining ML algorithms for delta check multiparametric rules, the most important being the MCH. After training, validated ML performances were: accuracy (A) 91.1% , sensitivity (SE) 90.0% and specificity (SP) 91.9% for SVM; A 89.7%, SE 90.1% and SP 89.3% for CT; A 86.9%, SE 88.8% and SP 85.0% for NNET; A 78.6%, SE 65.7% and SP 89.7% for URC.
Conclusion: AI applications, such as ML, successfully allow the improvement of quality of Laboratory Medicine results, enhancing efficiency in detecting samples mix-up with respect to a fixed cut-off for MCV. Although results require caution and further validations, AI could be considered a promising strategy for the detection of diagnostic errors.

PO102

Clinical significance of Anti-Ro52 Antibody Detection in Autoimmune Diseases

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Introduction: Antibodies to Ro52 subunit are historically reported in association to SSA/Ro60 system and described as marker for systemic lupus erythematosus, and Sjögren's syndrome. Although isolated anti-Ro52 antibodies were detected in several autoimmune diseases, the clinical significance of these antibodies is still controversial. The aim of this study was to investigate the clinical significance of isolated anti-Ro52 antibodies.

Materials and Methods: We performed a retrospective study of patients with anti-Ro52 antibodies identified in our immunology laboratory between June 2018 and July 2020. Antibodies to Ro52 were detected by ANA Profile 3 plus DFS70 strips (Euroimmun), which include the following antigens: Sm, RNP/Sm, SSA/Ro60, Ro52, SSB/La, Scl-70, PM-Scl, Jo1, CENP-B, PCNA, dsDNA, Nucleosomes, Histones, Ribosomal P-Protein, DFS-70, and AMA-M2. The test was performed when specifically requested and if a positive antinuclear antibodies (ANA) result was present with a titre ▫1:160.

Results: Anti-Ro52 antibodies were detected in 117 patients (75% female, median age 47.5, IQR 39-58). Among them, 59 (50%) patients had antibodies for both Ro52 and SSA/Ro60 antigens. 24 out of the 59 Ro52-SSA/Ro60 positive samples were also positive for SSB/La antibodies. The majority of these patients had established connective tissue disease. Isolated anti-Ro52 antibodies were detected in 30 (27%) samples. For the remaining samples, other antibodies associated with anti-Ro52 were Sm and/or RNP/Sm (11%), AMA-M2 (9%), CENP-B (7%), histones and nucleosomes (6%), Jo1 (3%), DFS-70 (3%), and PCNA (2%). Speckled pattern was the most frequent ANA pattern found by indirect immunofluorescence.

Conclusion: Isolated anti-Ro52 antibodies are often not reported routinely. These antibodies are often indicated in association with anti-SSA/Ro60 and reported as anti-SSA/Ro. In our study we observed that isolated anti-Ro52 antibodies have frequently been detected in hospital population. The specific clinical associations are undergoing investigation, but we certainly suggest that, in a suspected autoimmune context, anti-Ro52 without anti-SSA/Ro60 antibodies should be reported for a clearer clinical designation.

Reference:Robbins A et al. Front Immunol. 2019 Mar 12

PO103

RISCONTRO "OCCASIONALE" DI EOSINOFILIA IN UN CASO DI MALATTIA DI FABRY

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Gli Eosinofili stanno riscuotendo un crescente interesse da parte della comunità scientifica a causa del loro ruolo patofisiologico complesso in una vasta gamma di malattie infiammatorie locali e sistemiche, nonché nel cancro e nella trombosi. L'Eosinofilia viene definita dalla presenza nel sangue periferico di eosinofili in quantità >0,5×109/l. Per l'esecuzione di tali esami utilizziamo l'analizzatore Siemens ADVIA 2120i, che si basa su principi citochimici per la produzione della formula leucocitaria e di un principio ottico per il conteggio e la determinazione degli eritrociti. Nel grafico di distribuzione PEROX gli eosinofili si posizionano in basso e all'estrema destra in quanto ricchi di granuli contenenti perossidasi (asse Y) e apparentemente di volume ridotto a causa dell'ipergranularità citoplasmatica (asse X), mentre nel grafico BASO si posizionano in basso, sotto i polimorfonucleati ("pancia del girino). Nel Laboratorio utilizziamo anche un'altra tipologia di analizzatore, il Sysmex XN3000, che utilizza una citometria a flusso in fluorescenza in cui il campione viene diluito e marcato con fluorocromo: la reazione avviene in due fasi, inizialmente avviene una perforazione delle membrana cellulare, seguita da un legame con un fluorocromo che si lega agli acidi nucleici, gli eosinofili sono staccati in base al volume e alla fluorescenza e si posizionano in basso a destra. L'esecuzione dell'esame in doppia strumentazione segue algoritmi specifici del laboratorio, per il caso che segnaliamo essa è avvenuta per caso permettendo il rilievo di una condizione di eosinofilia: a fronte di una pari condizione di leucocitosi, mentre la strumentazione Sysmex XN3000 dava una percentuale di eosinofili dello 0% con neutrofili presenti all'86,4%, l'ADVIA 2120i forniva una percentuale di eosinofili del 40% con una percentuale di neutrofili del 46%. Allo striscio periferico veniva confermata l'eosinofilia con note di displasia sia a carico degli eosinofili che dei neutrofili. L'esperienza ci ha indotto a rivedere i nostri livelli decisionali per l'esecuzione dell'esame emocromocitometrico su diversa tipologia di analizzatore rafforzando la convinzione della necessità di possedere una doppia tipologia e ci ha indotto ad approfondire un eventuale legame tra la patologia di base e l'eosinofilia.

PO104

PIASTRINOPENIE IN CORSO DI INFEZIONE DA COVID-19: MECCANISMO CENTRALE O PERIFERICO?

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L'esame emocromocitometrico in corso di infezione da COVID-19 presenta alterazioni che correlano con la fase e la gravità della malattia. Linfocitopenia, leucocitosi neutrofila, piastrinopenia all'esordio rappresentano un parametro di valutazione prognostica. Numerosi studi hanno identificato un legame tra pazienti con COVID-19 gravi e un alto livello di D-dimero, un tempo di protrombina prolungato e un basso numero di PLT con una correlazione con lo stato di ipercoagulabilità. Le piastrine reticolate rappresentano le piastrine giovani contenenti ancora tracce di acido ribonucleico. Esse possono essere utilizzate come marker di attività megacariocitopoietica del midollo osseo. Numerosi studi hanno riportato la loro utilità nella diagnostica differenziale fra trombocitopenie centrali e trombocitopenie periferiche. Allo scopo di indagare la patogenesi della piastrinopenia nell'infezione da Covid-19 e di avere un ulteriore marcatore di attività di malattia abbiamo effettuato in tutti i pazienti afferiti, alla diagnosi e durante tutto il ricovero in concomitanza con l'esecuzione dell'esame emocromocitometrico la conta delle piastrine reticolate. I nostri dati confermano l'incidenza della piastrinopenia nell'infezione da Covid-19, essa correla con la gravità della malattia, e con altri indici prognostici negativi, come linfocitopenia e neutrofilia e l'aumento del D-dimero. Nel 16% del totale dei pazienti affetti da Covid-19 si riscontrava un aumento percentuale delle piastrine reticolate, con una percentuale relativa ai pazienti piastrinopenici dell'80%. In particolare in una paziente che aveva contratto il virus in corso di protocollo chemioterapico per Leucemia Mieloide Acuta in presenza di piastrinopenia severa (<10x109/L) abbiamo riscontrato aumento delle piastrine reticolate alla diagnosi. La presenza di un aumento delle piastrine reticolate sembra escludere la tesi di un'inibizione dell'ematopoiesi midollare centrale, e confermare la tesi di una patologia da "consumo" periferico concomitante con l'innesco di una coagulazione intravascolare, supportando l'utilizzo di farmaci che agiscano sui meccanismi infiammatori che conducono a tale complicanza.

PO105

Quality assessment of 99mTc-HMPAO labeled leukocytes preparation to improve diagnostic accuracy of cell-based infection imaging

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Labeled leukocytes with 99mTc-HMPAO are routinely used for infection imaging. The diagnostic efficiency is largely influenced by cell manipulation and labelling procedure. Some critical issues during the preparation process may compromise imaging results generating detrimental effects on leukocytes viability related to DNA damage, maintenance and over time reproducibility of the method, stability of the labeling technique. Aim of the study was a comprehensive assessment of in vitro/ in vivo quality of all steps of the procedure. Methods: We evaluated radiochemical purity (RCP), pH and labeling efficiency (LE) of 320 procedures. White Cell Viability Factor (WVF) was determined in three different blood samples before and after labeling to validate the method. Effect of the labeling process on blood cells was assessed in vitro with the fluorescence flow cytometer CELL-DYN Sapphire. Radioactivity of blood aliquotes was measured with a dose calibrator and labelling efficiency (LE%) was determined. Scintigraphic images obtained with labelled leukocytes (490 studies) were scored using a 5-point scale (0=inadequate; 5=optimal). Training program was evaluated by operators using a Learning Questionnaire and a score system (range: 1 "nothing" - 6 "a lot"). Results: Pre/post-labelling WVF was 0.99% (max value 1%) in all blood samples. The observed morphological alterations in hematologic analyzer were exclusively due to the centrifugation process and not to the labeling reaction. LE (mean value 72%) and RCP (>80%) yielded considerably high values and remained unchanged until 55 minutes. The vast majority of assessed images (490) were scored as diagnostic by three independent observer (90% with score ¤4). Training/learning programmes were scored as effective by key objective areas (mean value 5).Conclusions: The WVF did not vary after cell manipulation and labelling, confirming the effectiveness and efficiency of the whole labeling process. The final product was stable during time allowing simply workflow management. Results were highly reproducible with elevated RCP and LE values in all the assessed preparations, demonstrating that the used method is easy to use in clinical routine, especially when standardized training and total QA system are implemented.

PO106

Human use of heat-damage red blood cells for imaging splenic tissue: validation of in vitro labeling method

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Selective imaging of the splenic tissue is obtained with heat-damaged, or heat-denatured, red blood cells (RBCs) of the patient labeled with 99mTc in a variety of clinical scenarios. Aim of the study was to validate the process used for labelling heat-damaged red blood cells “totally in vitro”, after blood sample collection, before re-inject labeled RBCs to the patient. Moreover, we assessed efficacy of the staff training programme in order to guarantee repeatibility and method standardization in the clinical routine. Methods: Validation of the labeling method was performed in three different diagnostic sessions during three consecutive days. After collection of a blood sample using a heparinized syringe, we isolated erythrocytes from other blood components by centrifugation and washing steps. Then, we added the stannous pyrophosphate (PYP) to the erythrocytes pellet, after pH control. The ‘pretinning’ of RBCs was necessary to reduce Tc-99m once pertechnetate was entered them. After the labeling reaction (130 MBq of Tc-99m) erythrocytes were denatured in a water bath at a temperature of 49°-50°C, for 10 min. Radioactivity of blood aliquotes was measured with a dose calibrator and labelling efficiency (LE%) was determined. Lbelling purity was measured using a gamma counter and calculated as pellet counts/pellet counts+(surnatant counts)*100. Training program was evaluated by operators using a score system (range: 1 “nothing“ - 6 “a lot”). Results: We didn’t observed presence of macroaggregates during the entire process, until the final sample. Labelling efficiency resulted at very high values in the three consecutive measured aliquotes (mean value 73.67%) as well as the labelling purity (>95.22%). In our institution, splenic imaging with labelled heat-damaged RBCs is used to detect ectopic spleen, splenosis, extramedullary hematopoiesis. Training and learning programmes were scored by key objective areas with a mean value of 5. Conclusions: Our in vitro labeling process of heat-damaged RBCs is simple and safe, providing a useful and reproducible technique easy to implement in clinical routine for splenic imaging. Learning outcome of the training programme was scored as effective by all the operators allowing high-efficiency procedure mantained over time.

PO107

Development and validation of a rapid and specific HPLC method to determine chemical and radiochemical purity of 68Ga-DOTATATE (PET) tracer

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Many neuroendocrine tumors (NETs) overexpress somatostatin receptors (SSTRs) on the cell surface. Six different SSTRs have been identified (SSTR1, 2A, 2B, 3, 4, 5). Radiolabeled peptides targeting SSTRs are used clinically for imaging NETs with positron emission tomography (PET). 68Ga-DOTATATE has the highest affinity for SSTR subtype 2 (SSTR 2), which tends to be most overexpressed in NETs. 68Ga is linked to the peptide by means of the chelator DOTA. Preparation conditions may influence the quality and in vivo behaviour of the radiopharmaceutical. So that, HPLC method is crucial for identification/characterization of the final product, demanding higher resolution than standard TLC method. Before the use in routine quality control (QC) workflow, analytical methods must be validated. Aim of this study was to develop and validate a rapid and specific HPLC method of analysis for the routine QCs of 68Ga-DOTATATE to guarantee the high quality of the radiopharmaceutical before release.Methods: A stepwise approach was used, based on the quality by design (QbD) concept of the ICH Q2 (R1) and Q8 (Pharmaceutical Development) guidelines in accordance with the regulations and requirements of EANM, SNM, IAEA and WHO. Chromatography was performed on a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific). Separation was achieved on AcclaimTM 120 C18 column (3.0mm × 150 mm, 3µm, 120Å) with mobile phases A) water +0.1% trifluoroacetic acid (TFA) and B) acetonitrile +0.1% TFA; flow rate 0.6 ml/min; UV detection at 280 nm.Results: The purity and quality of the radiopharmaceutical obtained according to the proposed method resulted high enough to safely administrate it to patients. The method was found to be specific for DOTATATE, no interfering peaks were observed with an overall analytical run time of 16 min.The calibration curve was found to be linear with the equation y=1.251x+0.075, with a correlation coefficient of 0.9995 (R2) over a concentration range of 0.5–40 ▫g/ml. The average coefficient of variation (CV%) was <2% (0.10%) and the average bias value 1.44%. The intra-day and inter-day bias were between -5.10 to 4.76%. The limit of quantification (LOQ) for DOTATATE was 0.5 µg/mL.Conclusions: The described HPLC method for the determination of chemical and radiochemical urity of 68Ga-DOTATATE is rapid, simple, accurate and reproducible. The assay was validated to meet the requirements for patient injection, allowing routinely use

of the PET tracer as a diagnostic tool for in vivo imaging of SSTR2 expressing tumors.

PO108

LA VALUTAZIONE SIEROLOGICA NELLA PANDEMIA SARS-CoV2: ESPERIENZA DELLA UOC DI MEDICINA DI LABORATORIO DEL P.O. "SAN PAOLO"

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Il coronavirus (SARS-CoV2), agente dell'attuale pandemia, appartiene ad una vasta famiglia di virus causa di malattie che vanno dal comune raffreddore a malattie più gravi come la sindrome respiratoria mediorientale (MERS-CoV) e la sindrome respiratoria acuta grave (SARS-CoV). I segni clinici di infezione includono sintomi respiratori come tosse, respiro corto, difficoltà respiratorie e febbre, che nei casi più gravi possono evolvere in una grave polmonite interstiziale e morte. I dati epidemiologici indicano che una certa percentuale della popolazione è venuta in contatto con il virus pur rimanendo asintomatica, ma mantenendo la capacità infettante. La necessità di adottare una strategia che individui priorità per l'esecuzione dei test diagnostici per il nuovo coronavirus al fine di assicurare un uso ottimale delle risorse ed alleviare la pressione sui laboratori individuati dalle regioni per l'esecuzione della diagnostica molecolare per SARS Cov2 ha indotto lo sviluppo di tests sierologi, sia rapidi (POCT) in immunocromatografia sia a scopo epidemiologico/diagnostico mediante test immunoenzimatici. La UOC dii Medicina di Laboratorio del P.O. "San Paolo" dell'ASL Napoli 1 Centro, unico laboratorio COVID per l'area metropolitana di Napoli, dal 16 Marzo ad oggi ha eseguito 3000 test sierologici con metodica in chemiluminescenza (Maglumi 2019-nCoV IgG/IgM) e 2500 test in elettrochemiluminescenza (Elecsys Anti-SARS-CoV-ROCHE IgA/M/G), e 3500 test rapidi in immunocromatografia (Techno Genetics-KHB). I test sierologici eseguiti sia con metodica Roche che con Maglumi, come screening del personale sanitario e dei pazienti asintomatici, hanno evidenziato una positività alle immunoglobuline circa nel 2% dei casi, di cui solo il 0,5% è risultato essere positivo anche al tampone. Suggerendo che molti pur sviluppando una risposta immunitaria non avevano, anche su tamponi analizzati a tempi diversi, una carica virale tale da sviluppare una positività al tampone. Il test sierologico è una tecnologia ancora non pienamente validata, inficiata dalla possibile cross-reattività con altri coronavirus, utile a fini epidemiologici ma a causa di tale limitazione l'analisi Real-time PCR rappresenta ancora il test di riferimento sia in fase di screening che di follow-up della popolazione.

PO109

ESPERIENZA DELLA UOC DI MEDICINA DI LABORATORIO DEL P.O. "SAN PAOLO" NELLA PANDEMIA SARS-C>oV2: EVOLUZIONE DEI TESTS MOLECOLARI

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L'attuale pandemia provocata dal ceppo di β-oronavirus SARS CoV-2 ha reso necessario misure volte ad identificare tempestivamente i casi positivi, sintomatici ed asintomatici, per attivare idonee misure di contenimento alla propagazione del virus. La ricerca dell'RNA virale mediante RT-PCR, da tampone rino-faringeo e/o da campioni delle basse vie respiratorie, è la procedura indicata dall'OMS per porre diagnosi di infezione di SARS CoV-2. Il genoma del -Coronavirus SARS CoV-2 codifica per 4 proteine strutturali fondamentali per l'infezione e replicazione nell'ospite: Proteina Spike (S), dell'Envelope (E), del Nucleocapside (N) e RNA-dependent RNA polymerase (RdrP). L'UOC di Medicina di Laboratorio del P.O. "San Paolo" dell'ASL Napoli1 Centro, unico laboratorio di riferimento COVID per l'area metropolitana di Napoli, dal 16 marzo ad oggi, ha esaminato circa 16000 campioni inizialmente mediante RT-PCR kit AllplexTM 2019-nCoV Seegene e Roche (Tib Molbiol), entrambi per l'individuazione dei 3 geni E, RdrP ed N (come da indicazioni OMS) e, successivamente, il kit Seegene SARS-COV2TM capace di individuare anche il gene S (n.1200) migliorando la specificità e le performance diagnostiche. L'RNA virale è stato estratto con sistemi automatizzati mediante biglie magnetiche. Dei campioni analizzati, mediante RT-PCR, il 7,6% è risultato positivo alla presenza dell'RNA virale, di questi il 56% era di sesso maschile con prevalenza nella fascia di età compresa tra i 50 e 60 anni. Nella fase iniziale dell'infezione, in circa il 30% dei soggetti in cui è stata rilevata la presenza virale, si è osservata una positività ai tre geni target E, N e RdrP. Tali soggetti durante la fase di follow-up hanno mostrato persistenza dell'infezione per più settimane, evidenziata, di contro, dalla sola positività dell'amplificazione del gene N. Nel mese di luglio, con l'aumento degli spostamenti internazionali, si è riscontrato un nuovo aumento della positività a 2 o più geni (compreso il gene S) dei tamponi analizzati. L'analisi Rt-PCR rappresenta il test di riferimento sia in fase di screening che di follow-up della popolazione e con l'implementazione di nuovi test in multiplex per l'individuazione contemporanea di più geni permette di fornire risultati sempre più accurati ed in tempi più brevi.

PO110

IL DOSAGGIO DELLA 25-OH VITAMINA D IN HPLC: VALUTAZIONE CRITICA

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Il dosaggio della Vitamina D è oggi molto richiesto. E' chiamata "vitamina" ma in realtà è definita un "ormone" a causa delle sue diverse azioni su molti tessuti. La vitamina D, infatti, è coinvolta in moltissimi processi fisiologici ed i suoi recettori (VDR) sono distribuiti in tutto l'organismo dopo il legame subisce un processo di modificazione biologica che le consente di essere attivata e di intervenire nella crescita ossea, nella modulazione della funzione neuromuscolare e immunitaria e nella riduzione dell'infiammazione. Da Gennaio a Settembre 2019 abbiamo analizzato in cromatografia liquida HPLC n.769 campioni ematici con età media di 62 anni. Abbiamo riscontrato una media del valore di vitamina D pari a 34,30 ng/ml, valore considerato sufficiente mentre si parla di carenza e/o deficit quando i livelli sono inferiori a 20 ng/ml. Nei mesi di Marzo, Maggio e Settembre sono stati analizzati un numero maggiore di campioni, in particolare a Marzo 120 campioni di cui 93 femmine e 27 maschi, a Maggio 113 campioni di cui 87 femmine e 26 maschi ed a Settembre 129 campioni di cui 82 femmine e 47 maschi. Da questi dati si nota una prevalenza di campioni di sesso femminile. Il valore della vitamina D è risultato più alto per la maggior parte dei soggetti analizzati nel mese di Settembre, valori superiori ai 40 ng/ml, mentre i valori più bassi sono stati evidenziati nel mese di Aprile, valore medio pari a 29,16 ng/ml su un totale di 91 soggetti. Sul totale dei campioni analizzati da Gennaio a Settembre, invece, 136 hanno presentato una concentrazione inferiore ai 20 ng/ml (media 13,18 ng/ml). Il dosaggio della 25-OH vitamina D è stato eseguito con sistema HPLC isocratico, rivelazione spettrofotometrica (λ:265 nm) con valutazione quantitativa. Il dosaggio della vitamina D non è raccomandato random nella popolazione generale, in quanto non è economicamente accettabile. Nei pazienti invece che presentano condizioni di rischio, la carenza di vitamina D deve essere accertata precocemente al fine di trattare adeguatamente i soggetti carenti, ripristinare i depositi evitando conseguenze anche gravi. Infatti la concentrazione sierica di vitamina D, in particolare della 25-OH-vitamina D, è considerata il miglior indicatore clinico delle riserve di vitamina D nell'organismo.

PO111

Diagnosi emometrica di laboratorio in un caso di Leucemia Mieloide Acuta

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INTRODUZIONE. Presentiamo il caso di una paziente donna (aa 87), giunta in Pronto Soccorso per forte astenia e lieve stato febbrile. L' esame emocromocitometrico mette in evidenza un quadro ematologico suggestivo e degno di approfondimento. WBC: 23.220/µL N: 18 L: 25 M: 57 HGB: 11.1 g/L PLT: 122.000 µL MCV: 102.3 fL MATERIALI E METODI. L'osservazione e l'analisi dei dati numerici e degli scattergram di distribuzione cellulare inducono a pensare ad un possibile disordine mieloproliferativo acuto. Difatti, sullo scattergram WDF (White differentation) si osservano dei clusters di distribuzione cellulare anomali, sia nell'area dei monociti che appare molto intensa ed estesa lungo l'asse della fluorescenza, che in quella dei neutrofili (presenza cellule mieloidi immature). Viene allestito uno striscio di sangue periferico: N 54 L 22 M 6E / B1 MC 2 MMC 5 PMC 3 BL 7 Il quadro che si va delineando diviene sempre più concreto e pertanto si consiglia al reparto di appartenenza di richiedere una consulenza ematologica. Vengono eseguiti gli approfondimenti del caso. Esame citofluorimetrico: aspirato midollare ipercellulare (406.000 cells/ml). L'analisi citometrica evidenzia la presenza di una popolazione blastica mieloide CD 33 ++ CD 56 + + CD 117++ CD 38 + CD 13+/- CD 11C+ / - DR- CD15- CD 14- CD 19- CD 64- CD 34- pari al 67% della cellularità globale. Quota linfoide residua normale pari al 2%. CONCLUSIONI. Il percorso logico seguito nel porre il sospetto diagnostico da prospettare al clinico vede alla base del medesimo l'esame di laboratorio, considerato "procedura diagnostica" fondamentale alla prognosi e all' intervento sanitario. L'esame di laboratorio, l'emocromo in questo caso, assume un significato più ampio nell'ottica della Medicina di Laboratorio in quanto ha un ruolo centrale non solo nel diagnosticare, ma anche nel monitorare e gestire la malattia. La leucemia mieloide acuta è una malattia per definizione sistemica, cioè diffusa a tutto l'organismo. La classificazione resta comunque fondamentale per la scelta del trattamento e per definire la prognosi che si basa su criteri specifici, definiti dall'OMS e aggiornati al 2016. È importante ricordare che la ricerca nel trattamento della LAM non si ferma e che grazie agli studi clinici condotti anche in Italia recentemente (2017) sono stati approvati dagli enti regolatori europei e americani nuove categorie di farmaci per la LAM. Questi sono ad esempio gli inibitori tirosin-chinasici (anti FLT3) e gli anticorpi monoclonali diretti contro molecola di superficie (CD33) il cui uso nella terapia di prima linea consente di migliorare la prognosi della malattia.

PO112

Diagnosi emometrica di laboratorio in un caso di Leucemia Mieloide Acuta: Il ruolo del laboratorio

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INTRODUZIONE. Presentiamo il caso di una paziente donna (aa 60), che esegue un prelievo ematico di routine. L' esame emocromocitometrico mette in evidenza un quadro ematologico suggestivo e degno di approfondimento. WBC: 4.030/µL N:54 L:40 M:6 HGB:9.0 g/L PLT:117.000 µL MCV: 96 fL NE-SSC:136 MATERIALI E METODI. L'osservazione dei dati mette in luce uno stato di anemia accompagnata da lieve piastrinopenia. La distribuzione cellulare lungo il sistema di assi cartesiani x,y pone in evidenza un cluster anomalo: i linfociti ed i monociti tendono lievemente a sovrapporsi lungo l'asse della fluorescenza mentre i neutrofili sono spostati verso l'asse delle ascisse, sinonimo di possibile displasia a carico della serie granulocitaria.Degno di nota, a tal proposito, è l'indice di posizionamento cellulare NE-SSC, dato numerico estratto dalla precisa posizione delle cellule lungo un sistema di assi cartesiani. In particolare, l'NE-SSC , sinonimo di granularità e maturità cellulare nel nostro caso risulta essere inferiore ai range di normalità. L'esame microscopico risulta a questo punto fondamentale. E' stata evidenziata, come da sospetto diagnostico, un'importante displasia a carico della serie granulocitaria neutrofila, marcata anisocitosi e presenza di blasti. A questo punto risulta necessario avvisare l'U.O.C. di Ematologia per gli approfondimenti del caso. Viene eseguito un puntato midollare e una biopsia ossea: displasia trilineare con percentuale di blasti pari al 15 %. Sindrome mielodisplastica con eccesso di blasti ad altissimo rischio. La paziente è stata avviata a procedura trapiantologica di cellule staminali. CONCLUSIONI. La medicina di laboratorio ed il linguaggio laboratoristico in particolare, si basano sulla capacità di trasformare semplici dati numerici in informazioni diagnostiche ed interpretative. Il ruolo della morfologia risulta ancora oggi fondamentale, in un'epoca di automazioni ed informatizzazione dell' attività di laboratorio. I suggerimenti che nascono dall'osservazione microscopica delle cellule e dalla conoscenza delle loro espressioni in fisiologia e in patologia risultano alla base degli step successivi diagnostici sia in caso di patologie ematologiche che in malattie non ematologiche, dove il corredo dei segni e sintomi possono orientare verso una corretta interpretazione del caso clinico in esame. In particolare in casi di sindromi mielodisplastiche, dove porre il sospetto diagnostico è molto delicato appare di tutta evidenza il ruolo di protagonista che occupa l'esame microscopio e la corretta interpretazione dei dati di laboratorio

PO113
DIAGNOSI DI LABORATORIO EMOMETRICA IN UN CASO DI SINDROME MIELODISPASTICA

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INTRODUZIONE.Maggio 2019. Paziente maschio, ricoverato presso l'Unità di Osservazione Breve Intensiva (OBI) della nostra Azienda esegue un prelievo ematico di controllo per riferita astenia e febbre ricorrente. MATERIALI E METODI. I dati ottenuti WBC 38.170 µL, HGB 7.0 g/L, PLT 124.000 µ/L, e una quota di Monociti di 6.750 µ/L, combinati con l'osservazione degli scattergram di distribuzione cellulare dei nostri analizzatori , suggerisce l'immediato allestimento di uno striscio periferico per l'imprescindibile valutazione morfologica: N63 L20 M1 E2 B1 MC 2 MMC5 PMC3 BL3 EBOC 5. Il quadro che si va delineando pone il sospetto diagnostico di disordine mieloproliferativo e pertanto si consiglia al reparto di Osservazione Breve Intensiva di richiedere una consulenza ematologica. Il paziente viene affidato all'U.O.C di Ematologia e sottoposto ad ulteriori esami di approfondimento per porre una diagnosi definitiva e stabilire i criteri di prognosi e monitoraggio. Viene eseguito un puntato midollare: Cell. 2. Ipoplasia della serie eritroblastica. La serie granulocitica è rappresentata in tutte le fasi di maturazione specie nelle forme più mature. Cellule blastiche 6%. La caratterizzazione immunologica suggerisce marcata disgranulo-diseritropoiesi con eccesso di blasti. Si esegue anche una biopsia osteomidollare che dopo caratterizzazione immunoistochimica conclude per: sindrome mielodisplastica con eccesso di blasti tipo I. CONCLUSIONI. L'esame di laboratorio, l'emocromo in questo caso, assume un significato ed un ruolo predominante nell'ottica della Medicina di Laboratorio in quanto non solo nel diagnosticare, ma anche nel monitorare e gestire la malattia. E' fondamentale ricordare come l'elaborazione dei dati dell'emocromo non possa prescindere dalla valutazione morfologica tutte le volte che i parametri lo suggeriscono. Ad oggi la performance del moderno laboratorio, in particolare del laboratorio di Ematologia, risulta molto incisivo in tutte le sue tappe di intervento, dal riconoscimento delle cellule del sangue alla loro refertazione, arricchito ed approfondito dall'imprescindibile esame citofluorimetrico, con la possibilità di offrire ai clinici un impatto ed un approccio più rapido ed incisivo con il paziente.

PO114
DIAGNOSI EMOMETRICA DI LINFOMA

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INTRODUZIONE. Presentiamo il caso di una paziente donna (65 aa) giunta in Pronto Soccorso per febbricola ed astenia persistente. L'attenta analisi dei dati di laboratorio ha posto il sospetto diagnostico di un possibile disordine ematologico di tipo linfoproliferativo che necessitava di urgente approfondimento microscopico. MATERIALI E METODI. La valutazione dei dati dell'esame emocromocitometrico e dei relativi scattergram di distribuzione cellulare pongono il sospetto diagnostico di disordine linfoproliferativo degno di approfondimento. WBC: 52.270/µL HGB: 11.5 g/L PLT: 157.000 µL. Gli scattergram di distribuzione cellulare mettono in evidenza un cluster anomalo nell'area dei linfociti e dei monociti. E' evidente una popolazione cellulare che si estende lungo l'asse della fluorescenza sinonimo della possibile presenza di linfociti molto probabilmente di natura patologica. La valutazione degli indici di posizionamento cellulare, che danno informazioni sulla precisa posizione delle cellule lungo il sistema di assi cartesiani, in base a forma dimensione e granulosità della cellula, in particolare LY-WX, WY, WZ, risultano illuminanti nel caso in esame. La nostra esperienza ci induce a valutare in forma critica tali parametri e a discriminare tra le diverse patologie linfoproliferative. Viene allestito uno striscio di sangue periferico: Sono evidenziabili cellule di natura linfomatosa e di grandi dimensioni, non chiaramante riconducibili a disordine linfoproliferativo tipo LLC e quindi in accordo con l'interpretazione dei dati degli analizzatori. N 10 L 60 M 17 E /B/CELLULE LINFOMATOSE 13. A questo punto viene eseguito un approfondimento citofluorimetrico con caratterizzazione linfocitaria: si conferma il sospetto diagnostico: 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 e delle Ig. CONCLUSIONI. La medicina di laboratorio ed il linguaggio laboratoristico in particolare, si basano sulla capacità di trasformare semplici dati numerici in informazioni diagnostiche ed interpretative. Il quadro che ne emerge è ricco di informazioni analitiche che saranno fondamentali nella fase post-analitica con commenti di refertazione, che opportunamente valutati offrono la chiave d'apertura alla diagnosi per il clinico. In particolare, il referto ematologico affianca e guida il clinico nello studio, nella definizione della patologia e nel trattamento della medesima, sia in fase di diagnostica che di follow up, attraverso criteri razionali e basati sulla medicina delle evidenze. A tal fine questo "atto" medico deve risultare corretto nella forma e nei contenuti tecnici, e fornire informazioni non ambigue, utili sul piano clinico e facilmente interpretabili. Degna di nota è la morfologia cellulare. Il ruolo della morfologia risulta ancora oggi fondamentale, in un'epoca di automazioni ed informatizzazione dell' attività di

laboratorio. I suggerimenti che nascono dall'osservazione microscopica delle cellule e dalla conoscenza delle loro espressioni in fisiologia e in patologia risultano alla base degli step successivi diagnostici sia in caso di patologie ematologiche che in malattie non ematologiche, dove il corredo dei segni e sintomi possono orientare verso una corretta interpretazione del caso clinico in esame.

PO115

Hematological Reference Intervals estimation in the LUM (Laboratorio Unico Metropolitano) Pediatric Population

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LUM, a complex Unit of AUSL Bologna, consists of a main laboratory and 11 territorial laboratories, which provide an appropriate response to the diagnostic needs of Bologna province population. LUM organizational model is based on homogeneity of the offer to the citizen, standardized procedures and the same equipment in all locations; these features allow standardizing the report throughout the territory. Complete Blood Count is one of the most requested laboratory tests in clinical practice. Its correct interpretation in all age groups, including the pediatric one, must necessarily take into account specific variations of the hematological parameters in the different age classes. Particularly, there is poor literature evidence on reference intervals for pediatric age, as it mainly refers to adult. Laboratories use literature data or the instrument producer to determine pediatric reference values in hematology. To estimate reference intervals, direct methods, based on reference subjects selection, and indirect methods, based on a statistically defined healthy population, can be used. This work aims at defining pediatric reference intervals by age group, analyzing LUM outpatients values of 2017/2018. From the LUM database, consisting of 1,380,000 CBC for 2017 and 1,410,000 CBC for 2018, we obtained, with indirect method and with specific selection criteria, a sample of about 10,000 pediatric results per CBC parameter, produced with the same instruments. We determined such criteria in order to get a healthy population. For each CBC parameter, we performed descriptive analyses by sex and age and then created age groups. Within each age class, iteratively, we identified and removed outliers with Tukey's rule. Non-parametrically, we estimated reference limits, corresponding to the reference distribution 2.5th and 97.5th percentiles. With bootstrap method, we calculated 90% Confidence Intervals, highlighting the precision of estimates. Such procedure define reference intervals for all CBC parameters for the LUM pediatric population. With periodic reviews, any changes in the population will be evaluated. Future developments include a comparison with the literature and clinicians validation, followed by the adoption of these intervals in the whole territory reports.

PO116

Una strana Componente Monoclonale IgD ▫. Integrazione tra ISE ed IFE.

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Lo scopo principale dell'elettroforesi siero proteica (ESP) è la ricerca delle Componenti Monoclonali (CM). La prevalenza delle CM è intorno al 6,5% con lieve aumento nelle classi di età avanzate. La tipizzazione delle CM è realizzata con l'Immunofissazione sierica in gel d'agarosio (IFE) oppure in Immunosottrazione in capillare (ISE). La scelta delle due tecniche per la tipizzazione delle CM è demandata agli operatori che disporranno in ISE quelle CM ben evidenti in elettroforesi , mentre opteranno per l'IFE tutti i casi con CM poco evidenti o con bande anomale presenti sul tracciato. Rimane l'IFE come gold standard per la tipizzazione delle CM. Le CM IgD K o ▫ e IgE K o ▫ vengono trovate o per esplicita richiesta del medico oppure per una attenzione del personale del laboratorio che ogni volta che evidenzia una CM con solo catene leggere K o ▫ estende la IFE inserendo anche gli anticorpi anti-D e anti-E. Il 16/07/2020 è stata eseguita una tipizzazione di CM in IFE che non evidenziava alcuna CM con l'IFE standard. Il paziente PA, di anni 80, maschio, era sotto cura per CM evidente in elettroforesi sia in gel che in CE fin dal 2018 ma mai tipizzata. Gli esami di routine evidenziavano: Hb 17.4 g/dl e 5.63 *10^6 RBC, prot. T. 7.1 g/dl, e-GFR 109.69, LDH 179 u/LB2-microglob. 1,97 mg/L, IgG 536 mg/dl, IgA 51 mg/dl, IgM 36 mg/dl. Nel luglio del 2020 gli è stata richiesta l'IFE, che ha mostrato: assenza di CM con lievissimo addensamento in ▫, ma risaltava nel tracciato guida, una chiara CM in zona beta-gamma. Il siero veniva sottoposto in ISE che evidenziava una chiara CM ▫. A questo punto si è eseguito una IFE per IgD ed IgE e il risultato è stato inequivocabile: presenza di CM IgD con banda ben evidente ma con ▫ poco apprezzabile quasi lieve addensamento. Le urine di PA, esaminate con elettroforesi urinaria, evidenziavano una chiara CM in zona beta-gamma e con IFE urinaria si evidenziava una CM ▫ ben colorata, la proteinuria risultava di 10 mg/dl. In conclusione dagli esami effettuati si è evinto che la CM di PA è una IgD, evidenziata da IFE e ▫ evidenziata da ISE. Non si è potuto esaminare in ISE IgD per assenza del reagente in CE. Si sottolinea la scarsa evidenza della CM ▫ in IFE, mentre è ben evidente in ISE.

PO117

Standardization and harmonization in hematology measurements: haematology analyzer alignment, quality control materials and commutability issue. An open question.

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Background: In the Hub and Spoke laboratory network organization, the number of hematology analyzers (HAs) within each core center has increased, and the control of HAs alignment is becoming necessary requirement to ensure analytical quality. In this scenario, HA alignment can be assessed by analyzing the same control material used for CQI on multiple HAs, assuming its commutability. The aim of the study was to verify the applicability of a protocol for the instrument alignment of HAs based on control material rather than on fresh whole blood samples.Methods: The alignment of 5 modules of XN-9000 was evaluated for red (RBC, Hb, MCV, RET), white (WBC, NE, LY, MO, EO, BA, IG) and platelet (PLT) series parameters, using human sample (HS) collected in K3EDTA, and a quality control material (QC), after the verification of commutability. The commutability assessment protocol was developed following the IFCC approach using 50 fresh human samples, chosen with values covering a reasonable range around the QC. Maximum biases were arbitrarily derived from the Total Error values obtained by the biological variation data. The alignment was performed according to the recent document published by the Italian Society of Clinical Biochemistry and Clinical Molecular Biology, and verified using the same maximum allowable biases chosen for the commutability evaluation.Results: A complete alignment between instruments was confirmed for the majority of the parameters (RBC, Hb, PLT, WBC, NE, LY and EO) investigated both for HS and QC material. Partial misalignments or inconcludent results, with differences between HS and QC, were instead evident for MCV, MO, EO, BA and IG. Among the different WBC subpopulations (NE, LY and MO), QC data always displayed wider confidence bands than those shown by HS. These differences may be due to sample composition and preparation. Interestingly, QC material was found to be not commutable LY, MO and BA.Conclusions: The alignment of HAs for main cell population parameters may be verified with both QC and HS, displaying consistent results and interpretation. The evaluation for some white series parameters (EO, BA and IG) is critical and particular attention must be paid to the values and to the characteristics of the material used for the alignment.

PO118

Evidence of significant difference in key COVID-19 biomarkers during the Italian lockdown strategy. A retrospective study on patients admitted to a hospital emergency department in Northern Italy.

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Background. The Lombardy region, Italy, has been severely affected by COVID-19. During the epidemic peak, in March 2020, patients needing intensive care unit treatments were approximately 10% of those infected. This fraction decreased to approximately 2% in the second part of April, and to 0.4% at the beginning of July. In this region, a lockdown strategy was rigidly enforced since March the 9th to May the 18th. COVID-19 is characterized by several biochemical abnormalities whose discrepancy from normal values was associated to the severity of the disease. The aim of this retrospective study was to compare the hematological and biochemical patterns of patients during and after the establishment of a lockdown strategy to verify whether later patients were experiencing a milder COVID-19 course, as observed by several clinicians of the same Hospital. Material and Methods. Two groups of 84 patients, admitted at the emergency department of the San Raffaele Hospital (Milan, Italy), in March and April respectively, homogeneous for distributions of age, gender, and severity of symptom were selected. The laboratory findings (Complete blood count, glucose, creatinine, C-reactive protein, total bilirubin, urea, electrolytes, enzyme activities, hemogasanalysis and CO-Oxymetry data) of the two controlled groups, were analyzed and compared using the two-sample univariate Kolmogorov–Smirnov test revised for family-wise errors using the Bonferroni correction. Results. White blood cell, platelets, lymphocytes and lactate dehydrogenase showed a statistically significant improvement (i.e. closer or within the normal clinical range) in the April group compared to March. Creatinine, C-reactive protein, Calcium and liver enzymes were also pointing in that direction, although the detected differences were not significant. Discussion. Different distribution of laboratory parameters between the two groups are consistent with an increasingly milder disease phenotype. Since comorbidities were similar in the two controlled groups, we can reasonably presume that the enforcement of a lockdown strategy, as well as the widespread use of respiratory protective devices and social distancing may support our findings.

PO119

SARS-CoV-2 seroprevalence in hospital employees in Italy

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The SARS-CoV-2 outbreak early in 2020 overwhelmed the Italian national health system, and hospitals were considered places at high risk of spreading the infection. We explored specific antibody seroprevalence of all employees at a single hospital in the epicenter of the outbreak, to identify risky pathways and to evaluate the usefulness of antibody testing. Methods: all hospital workers were invited to fill in a questionnaire and undergo a blood test for SARS-CoV-2 IgG, using two commercial tests (DiaSorin and Abbott). The SARS-CoV-2 S1/S2 IgG test (DiaSorin, Saluggia, Italy) is a chemiluminescence immunoassay for quantifying anti-spike 1 (S1) and anti-spike 2 (S2) IgG on the LIAISON XL automated analyzer; according to the manufacturer, a titer <12 AU/ml is negative, from 12 to 15 is equivocal, and >15 is positive; values below 3.8 are undetectable. The SARS-CoV-2 IgG assay (Abbott, Abbott Park, Illinois, USA) is a chemiluminescent microparticle immunoassay for quantifying anti-capsid IgG on the ARCHITECT i System analyzer; according to the manufacturer, a titer <1.4 is negative and >1.4 is positive. Subjects who tested positive for SARS-CoV-2 IgG underwent a confirmatory nasopharyngeal swab PCR test. Seropositivity was determined overall and according to demographic and occupations characteristics, for both tests singly and combined. Results: the study enrolled 1562 hospital workers. Overall, 153 participants (9.8%) were positive for SARS-CoV-2 IgG on DiaSorin test, and 150 (9.6%) were positive on Abbott test; both tests were positive in 123 cases (7.9%) and at least one was positive in 180 cases (11.5%). Factors associated with SARS-CoV-2 seropositivity included: being a smoker, working in Emergency or Medicine Departments, self-reporting a relative with COVID-19 or symptoms suggestive of COVID-19.Conclusion: Seroprevalence for SARS-CoV-2 in this population of hospital workers was overall about 10%, with an excess prevalence in roles and departments associated with contacts with COVID-19 patients.

PO120

IMPACT of SARS-CoV-2 INFECTION on LABORATORY WORKFLOW: EXPERIENCE of INT - IRCCS "FONDAZIONE G. PASCALE" in NAPLES

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Focusing attention on cancer patients' frailty, Cancer Institute - IRCCS "Fondazione Pascale" in Naples has planned a tightened program of health surveillance for patients (PTs) and healthcare providers (HPs), in order to early detect and promptly quarantine subjects with SARS-CoV-2 infection. This program requires a multidisciplinary asset, within which Laboratory plays a crucial role. In our experience, the attention has focused on application of biosafety conditions, as stated by World Health Organization's guidance. A validated internal protocol was derived from it. Laboratory diagnostic workflow provided 3 steps: 1) rapid immunochromatographic assays (ICAs) to detect SARS-CoV-2 IgM and IgG from plasma samples of PTs and HPs; 2) automated qualitative electrochemiluminescence immunoassays (ECLIAs) to detect SARS-CoV-2 antibodies from serum samples of HPs; 3) Real-time PCR detection of SARS-CoV-2 RNA from nasopharyngeal swabs (NS) of PTs and HPs. Health surveillance program planned an initial evaluation of PTs and HPs with rapid ICAs (automated tests being not available yet) and a further screening with molecular tests on NS sent to external COVID-19 reference Laboratories. Subsequently, our Laboratory was included in CORONET network, allowing us to perform molecular tests for SARS-CoV-2. At the same time, availability of automated ECLIAs allowed us to screen HPs periodically, continuing to perform ICAs to all PTs afferent to hospital triage. At the beginning of the surveillance, rapid ICAs allowed to screen 1920 PTs (89 of which resulted positive, 4.63%) and 1050 HPs (48 positive, 4.57%). Subsequently, HPs' screening with ECLIAs revealed 25 positive subjects out of 1018 tested (2.46%). Early detection and quarantine of positive cases allowed to find very low percentages of positive SARS-CoV-2 subjects to molecular tests from NS: 1 positive out 2215 NS from HPs (0.05%) and 1 positive out of 742 NS from PTs (0.13%). All Laboratory staff efforts have been directed to guarantee an adequate turnaround time. The element "time" has been crucial to subject promptly to quarantine personnel identified as positive and to isolate patients affected, thus allowing health surveillance program to ensure an adequate protection for cancer patients afferent to our Institute.

PO121

EVALUATION of URINARY 5-HYDROXYINDOLACETIC ACID (5-HIAA) and para-HYDROXYPHENYLACETIC ACID (pHPAA) LEVELS in TWENTY-FOUR PATIENTS with NEUROENDOCRINE NEOPLASMS (NENs)

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Neuroendocrine neoplasms (NENs) are rare tumors that originate from neuroendocrine system's cells. NEN-related functional syndromes secrete bioactive mediators, notably serotonin, resulting in carcinoid syndrome (CS) manifestations. Urinary 5-Hydroxyindolacetic acid (5-HIAA), the metabolic product of serotonin, is the most commonly used biochemical marker in the diagnosis and monitoring of NENs, particularly when a CS is present. 24 patients with a diagnosis of NENs afferent to our Cancer Institute from March 2019 to March 2020 were tested for baseline determinations of urinary 5-HIAA levels by high pressure liquid chromatography. Chromatograms thus obtained allowed to detect and measure urinary para-Hydroxyphenylacetic acid (pHPAA) levels simultaneously to 5-HIAA. pHPAA is an important metabolite of tyrosine and has been demonstrated to be related with cancer. After an initial evaluation, 9 out of 24 patients were candidates for surgery, 10 for somatostatin analogues' first-line treatment, 5 for a second-line treatment (after a first-line treatment failure). Baseline urinary 5-HIAA (reference range: 0.7-8.2 mg/24h) and pHPAA (reference range: $\leq$ 9.7 µmol/mmol creatinine) medians and interquartile ranges (IQRs) were: 3.5 (2.1-6.4) mg/24h and 11.0 (8.0-14.5) µmol/mmol creatinine, respectively, for 9 patients undergoing surgery; 4.1 (1.7-20.8) mg/24h and 10.0 (5.0-24.5) µmol/mmol creatinine, respectively, for 10 patients treated with somatostatin analogues; 4.4 (2.5-57.4) mg/24h and 19.0 (6.0-26.5) µmol/mmol creatinine, respectively, for 5 patients undergoing a second-line treatment. To date, 5 out of 24 patients have been tested after one year, showing abnormal levels of 5-HIAA in 2 out of 5 patients and high levels of pHPAA in 4 out of 5 patients. Differently from baseline levels of 5-HIAA (similar in all three groups), baseline levels of pHPAA resulted higher (although not statistically significant) in non-responder patients candidates for a second-line treatment and response prediction to treatment would seem better correlate with pHPAA rather than 5-HIAA levels. Currently, the study is still ongoing and aims to monitor a larger cohort of patients, in order to evaluate the possible role of pHPAA as a prognostic factor in NEN-related functional syndromes.

PO122

Development of a new class of oncolytic virotherapy expressing an immune checkpoint inhibitor.

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Oncolytic virotherapy is an emerging therapeutic approach, based on replication-competent viruses, to selectively infect and destroy cancer cells, causing the release of tumor-associated antigens (TAA), therefore stimulating an antitumoral immune response. Indeed, adenoviruses, particularly oncolytic adenoviruses (Onc.Ads), can kill cancer cells in different ways by inducing immunogenic cell death. In order to increase the anticancer activity of Onc. Ads, it is possible to combine them with a protein able to block tumor immune evasion. Programmed death ligand 1 (PD-L1) binding to its receptor PD-1 inhibits CD8+ T cell proliferation and increases antitumoral response; therefore, it isolated a single-chain variable antibody fragment (scFV) that binds to both human and murine PD-L1 with high affinity. The small size of scFvs, made up of variable heavy (VH) and light (VL) domains bound together via a flexible polypeptide linker, allows for Onc's expression. Ads. We, therefore, developed an Onc.Ad expressing a scFV against PD-L1, to combine blockage of PD-1/PD-L1 interaction with the antitumoral activity of Onc.Ads. To this aim, we generated Onc.Ad5◘24-scFV(PD-L1) uses standard adenovirus preparation techniques for purification and production of the recombinant adenovirus. We confirmed the expression of anti-PD-L1 scFv-in the supernatant of infected cells by western blot analysis.Moreover, we evaluated tumoral progression in three groups of mice inoculated with syngeneic B16-OVA melanoma cells and then treated with either Onc.Ad5◘24-scFV(PD-L1), Onc.Ad5◘24 or mock treatment. Comparing the melanoma growth rate between the three groups, we observed a slowdown of tumor growth in mice treated with Onc.Ad5◘24-scFV(PD-L1). We are further investigating the novel engineered virus in vitro co-cultures of tumor cells and lymphocytes.

PO123

Covid-19 and blood analysis: Vaio hospital experience

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INTRODUCTION: In March 2020 OMS declared the global outbreak of SARS-CoV-2. Vaio hospital has been intended only for covid patients since 23th march until 18th may 2020. AIM OF THE STUDY: we observe the trend of leucocyte and lymphocyte counts, IL-6, PCR, PCT, LDH, D-dimer, AST and ALT at entrance in emergency room and at the end of hospitalization. MATERIALS AND METHODS: we have examined 43 covid patients, 10 women and 33 men. 25 of 43 patients has received intensive care (58%) and 7 of these 25 (28%) have died. IL-6 and PCT detections were performed by Acces2 immunoassay (Beckman Coulter), leucocyte and lymphocyte counts were performed on XM-1080 flow cytometer (Sysmex), PCR was determined with AU5800 (Beckman Coulter) immunoassay, AST, ALT and LDH with immunoenzymatic test were performed on AU5800, PT and D-dimer test on ACL TOP 550 (Werfen) with clot detection and immunoassay rispectively. For data statistical analysis we calculate mean, frequencies and t test (excel, Windows 10, Microsoft). RESULTS: The mean measure at the early stage of hospitalization were: IL-6 105.33 pg/mL, leucocyte count 6,79 *103/µL, lymphocyte count 0.994*103/µL, PCR 92,73 mg/L, D-dimer 2745 µg/L, LDH 758 U/L, PCT 0,195 ng/mL, AST 58,71 U/L and ALT 61 U/L. The IL-6 alive patients mean value (112,46 pg/mL) was statistically different (t test p=0,04) from IL-6 dead patients mean value (274,33 pg/mL) and the alive patients mean age (60,6 years) wasn't statistically different (t test p=0,15) from dead patients mean age (66,9 years). At the end of hospitalization we observe an increase of lymphocyte and a decrease of PCR for alive patients and an increase of PCT only for dead patients. There is a significative difference (t test p<0,01) for the lymphocyte count between alive patients (mean values 2,11*103/µL) and dead patients (mean values 0.57*103/µL), and between PCR (t test p<0,01) alive patients (mean values 45 mg/L) and dead patients (mean value 243,42 mg/L) and also between PCT (t test p<0,01) alive patients (mean value 0,24 ng/mL) and dead patients (mean value 2,45 ng/mL). DISCUSSION: The increase of number of lymphocyte is a favorable prognostic indicator, as well as low values of inflammatory markers (IL-6 and PCR).

PO124

Il Tecnico Sanitario di Laboratorio Biomedico in Toscana: stato dell'arte e nuovi orizzonti della professione.

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La costituzione dell'Ordine multialbo dei Tecnici Sanitari di Radiologia Medica e delle Professioni Sanitarie Tecniche, Riabilitative e della Prevenzione con Legge 11 gennaio 2018, n.3, a cui afferiscono 19 delle 22 professioni sanitarie italiane, ha fatto emergere la necessità di ridefinire i tratti di ogni professione, che negli anni è cresciuta nelle competenze per stare al passo con l'evoluzione scientifica e tecnologica. Lo scopo della presente indagine è mappare e contestualizzare il profilo professionale del Tecnico Sanitario di Laboratorio Biomedico (TSLB) in Toscana, per poter creare uno stato dell'arte della professione e valutare i suoi possibili sviluppi. Lo studio si è svolto nei mesi di febbraio e marzo 2020, mediante la somministrazione di un questionario on line, realizzato tramite l'editor Moduli di Google Suite, ai Tecnici Sanitari di Laboratorio Biomedico regolarmente iscritti ai tre Ordini della Regione Toscana. Il questionario si compone di 58 domande, articolate in 6 sezioni: dati personali, contesto organizzativo, dimensione lavorativa, focus qualità, formazione continua e sviluppo professionale. Hanno aderito all'indagine 221 TSLB, il 15.2% degli iscritti ai tre Ordini. Possiamo affermare che il 77% del campione in studio è costituito da TSLB di genere femminile con contratto di lavoro a tempo indeterminato in aziende pubbliche, di età compresa tra 41 e 60 anni, per la maggior parte in possesso della Laurea (D.M. 509/99). Il 21% ha portato a compimento i suoi studi con la Laurea specialistica o magistrale e il 61% non è in possesso di titoli post-laurea. È emerso che i TSLB nel 40% dei casi sono poco coinvolti nell'attività di programmazione e nel 58% dei casi sono a conoscenza solo in parte degli obiettivi di budget e nel 65% dei casi hanno scarsa conoscenza dei risultati delle unità organizzative di appartenenza. Per quanto riguarda la formazione continua, si è osservato un incremento nell'acquisizione dei crediti ECM nel triennio 2017-2019 rispetto al precedente e la tipologia formativa più frequentemente utilizzata, 81% dei casi, è risultata quella a distanza in virtù della sua elevata fruibilità. I professionisti vedono come possibili ambiti di sviluppo: la formazione, la ricerca, le tecnologie dell'informazione e della comunicazione (ICT), l'acquisizione di ruoli di gestione e governo aziendali (management). Si evince la necessità di incentivare la partnership con le Università, le Società Scientifiche e gli Ordini, principali attori nel contesto delle innovazioni e promotori del cambiamento, allo scopo di rendere possibile la presa di consapevolezza dell'importanza del ruolo e il riconoscimento da parte della comunità scientifica e civile.

PO125

Time course of the antibodies response in COVID-19

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Background The role of the immune response to SARS-CoV-2 infection is not yet well known, in particular about the persistence of circulating antibodies. The aim of the study is to assess the time course of the IgM and IgG response. Methods We conducted a study on 123 specimens of 62 subjects with disease confirmed by laboratory tests (time from the onset of symptoms from 3 to 110 days). Both IgM and IgG were measured with a chemiluminescence immunoassay (MAGLUMI 2019-nCoV IgM and MAGLUMI 2019-nCoV IgG respectively) on the Maglumi 800 Analyzer (Snibe, Shenzen, China) according to the manufacturer's instructions. Results The case study was subdivided into 8 groups (□10 days from the onset of symptoms, 11-13 days, 14-17, 18-21, 22-28, 30-43, 46-71 and 72-110). The clinical sensitivity of IgG rose from 52.9% to 100% after 18 days, and then remained constant. The sensitivity of IgM rose from 35.3% up to about 78.6% at 20 days, then fell to about 27% after 80 days. Considering the concentrations in the samples, after a rapid increase up to about 20 days, we can see a subsequent decrease of the mean concentrations of both IgM and IgG levels. The quantitative comparison of the different classes showed significantly higher IgG concentrations in the days from 14 to 28 compared to both the first two groups and the groups after 46 days (Kruskall-Wallis test p=0.000001). As for the IgM, the trend is similar to that found for IgG, but the differences between groups were lower (Kruskall-Wallis test p=0.0022) and the absolute concentrations were about one order of magnitude smaller. Discussion The persistence of antibodies against SARS-CoV-2 is not known. Studies on the immune response to other coronaviruses showed that the concentrations of IgG decline after few months from the onset of symptoms, although the positivity rate remained relatively stable over a longer period. In the present study, the results seems to confirm this trend. In fact, although the positivity rates did not fall below 100%, in the class with specimens from 72 to 110 days after the onset of symptoms the median IgG concentrations were less than 10% compared to the levels found after 15-20 days. Conclusion The antibodies titre in patients exposed to COVID-19 may decrease already after 6-7 weeks after the symptoms' onset.

PO126

QUADRO CLINICO ED INDAGINI DI LABORATORIO IN UN PAZIENTE AFFETTO DA COVID-19.

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ABSTRACT.
CLINICAL PICTURE AND LABORATORY INVESTIGATION IN A COVID-19 PATIENT.
In a 72-year-old male patient, laboratory data related to interleukin values, inflammatory indexes, coagulation parameters, during the period of hospitalization for COVID-19 infection, follow a trend associated with clinical data perfectly comparable with what reported in the literature.PCR and IgG and IgM serological tests provide a great contribution to assess the antibody pattern of the disease.ELISA-based IgM and IgG antibody tests have a specificity greater than 95% for the diagnosis of COVID-19. Testing the serum sample coupled with the initial PCR and the second 2 weeks later can further increase diagnostic accuracy. Typically, most antibodies are produced against the virus' most abundant protein, NC. Therefore, tests that detect antibodies against NC would be the most sensitive. However, the receptor-binding domain of the S protein (RBD_S) is the host attachment protein, and antibodies to RBD-S would be more specific and expected to be neutralizing. Therefore, using one or both antigens for IgG and IgM detection would result in high sensitivity. Antibodies may, however, have cross-reactivity with SARS-CoV-2 and possibly other coronaviruses.Rapid tests for the detection of antibodies have been widely developed and marketed and are of variable quality. Many manufacturers do not reveal the nature of the antigens used. These tests are of a purely qualitative nature and can only indicate the presence or absence of SARS-CoV-2 antibodies. The presence of neutralizing antibodies can only be confirmed by a neutralization test. However, high IgG antibody titers detected by the ELISA have been shown to correlate positively with neutralizing antibodies. The long-term persistence and duration of the protection provided by neutralising antibodies remains unknown.Many questions remain, in particular how long the potential immunity lasts in both asymptomatic and symptomatic subjects who are infected with SARS-CoV-2.

PO127

LABORATORY ROLE IN SELECTING POINT-OF-CARE TESTS OUTSIDE HOSPITAL SETTING: ANALYTICAL PERFORMANCE ASSESSMENT OF ABAXIS PICCOLO XPRESS DEVICE AND COMPARISON WITH ROCHE COBAS c702 ANALYZER.

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INTRODUCTION. Point-of-Care Tests (POCTs) are progressively expanding their scope of application outside hospital setting, such as in pharmacies involved in the "Farmacia dei Servizi" project, in compliance with Legislative Decree 153/2009. ISO standard 22870:2016, while primarily focusing on implementation and management of hospital POCT systems, can suggest Laboratory Medicine professionals providing support activities in implementing POCT even in non-traditional contexts. Therefore, ASST Spedali Civili of Brescia's Central Laboratory, in cooperation with Health Telematic Network (HTN), assessed and compared the analytical performance of a candidate POCT system (Abaxis Piccolo Xpress, De Mori) versus Roche Cobas c702, an analyzer routinely used by the Laboratory.
METHODS AND RESULTS. We performed the following verification tests on 16 clinical chemistry measurands: 1) Imprecision assessment of Piccolo Xpress as compared with the manufacturer stated specifications: by applying 3x5 experimental design from CLSI EP15-A3 Standard on 1 human plasma pool, we showed that all analytes but AST meet the stated goals. 2) Comparison of the two analytical systems through Passing-Bablok regression on 25 human samples: 4 analytes (albumin, alkaline phosphatase, bilirubin, amylase) showed a statistically significant proportional and/or constant bias. 3) Clinical acceptance assessment of bias between the POCT and laboratory devices through Equivalence Test (TOST): comparing systematic error with maximum acceptable bias goal obtained by the new EFLM database on Biological Variation, all analytes (except calcium, uric acid and the 4 aforementioned) complied with quality specifications.
CONCLUSIONS. Enrolling Laboratory specialists to select a POCT device for application outside the hospital ensured a more appropriate data collection to assess the compliance of analytical performance with the intended use. In this study, we demonstrated that POCT Piccolo Xpress device is a good candidate for pharmacy use; in fact, most of the biochemical parameters showed a good agreement with results obtained on automated instrumentation by the institutional Laboratory.

PO128

REVERSE TRANSCRIPTION REAL TIME PCR E COVID 19: LA NOSTRA ESPERIENZA

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La rapida diffusione a livello mondiale di SARS-CoV-2, un nuovo coronavirus scoperto a Dicembre 2019 in Cina, ha portato l'OMS a dichiarare una pandemia. L'infezione COVID-19, trasmessa principalmente attraverso goccioline respiratorie emesse da persone infette, si traduce in segni e sintomi non specifici, che vanno dall'infezione asintomatica a insufficienza multiorgano. Il genoma di questo virus è costituito da un singolo filamento di RNA a polarità positiva, di circa 30 kb di lunghezza, di cui due o tre regioni diverse vengono ricercate con la reverse Real Time PCR (qPCR), eseguita su tamponi nasofaringei. Abbiamo analizzato l'esito di tali tamponi, eseguiti a pazienti pervenuti presso il Pronto Soccorso dell'Ospedale di Cernusco sul Naviglio, dal 01 Marzo al 30 Aprile 2020. Il risultato anche di una sola regione positiva, veniva refertato come 'debolmente positivo'. In totale i tamponi analizzati sono stati 1229, di cui 628 relativi a soggetti di sesso maschile (51%) e 601 a soggetti di sesso femminile (49%). Sono risultati negativi 690 tamponi (56%), positivi 433 (35%) e debolmente positivi 106 (9%).Numerosi campioni negativi (82, circa 7%) appartenevano a persone che mostravano quadri clinici di Covid19 ma, ripetuti a distanza, risultavano ripetutamente negativi. Al contrario, 12 (1% circa) si sono positivizzati. Dei "debolmente positivi" ritestati a distanza di giorni, 18 sono diventati negativi mentre 11 si sono invece confermati positivi. La diagnosi dei casi confermati di COVID-19 semplicemente mediante test degli acidi nucleici non è, ovviamente, abbastanza accurataLa carica virale potrebbe non essere sufficientemente elevata per il rilevamento, con conseguenti falsi negativi. I risultati debolmente positivi potrebbero essere l'esito di tamponi malfatti, oppure eseguiti su soggetti in via di guarigione con affievolimento o addirittura scomparsa dell'infezione ma, con tracce di RNA virale ormai residuale ed inattivo. Potrebbero essere anche dovuti a tamponi eseguiti su infetti asintomatici. I deboli positivi risultano quindi insidiosi in quanto portano ad una sovradiagnosi che, caricando inutilmente il sistema di falsi positivi, costringerebbe questi ultimi a lunghissime e inutili quarantene.

PO129

SARS-CoV-2 infection serology validation of different methods: usefulness of IgA in the early phase of infection.

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In the late December 2019, an outbreak of pneumonia of unknown origin was reported in Wuhan, Hubei province, China. A novel coronavirus was isolated from the respiratory samples of patients with pneumonia as showed by the sequence analysis of the virus genomes obtained; The novel coronavirus was named SARS-CoV-2. Reverse-transcriptase real-time PCR (rRT-PCR) is the method of choice for detecting SARS-CoV-2 infection. Despite the high sensitive of the real-time PCR tests, sometimes samples from the upper respiratory tract may result negative even in the presence of radiological findings of pneumonia probably due to the viral load in the upper respiratory tract is low compared to the lower respiratory tract; low quality of the collected sample or technical reasons linked to the assay used. The use of serological assays may help in making diagnosis. The antibody response to SARS-CoV-2 is not well understood yet, but the availability of sensitive and specific serological assays will be crucial for the early diagnosis of infection, for epidemiological studies, for defining the presence of neutralizing antibodies in response to a possible vaccine. In this work, we tested and compared the performances of one chemiluminescent immunoassay, two ELISA assays and an ECLIA assay. Among the platforms assessed in this study, the ECLIA serological assay performed best, and may be a valid screening method for SARS-COV-2 infection. The IgA detected by the ELISA assay might be a more reliable and stable early serological marker than IgM. Instead, IgGs, as expected, showed stable level after 10 days from symptoms onset. Taken together, if a reflex test could be set in the laboratory, the ECLIA method could be used as screening test, considering both the excellent performance and the cost per single test; while ELISA assay for IgG and IgA, which are present at a higher level than IgM and last longer, might be used as confirmatory test.

PO130

SARS-2-Cov 2 induced coagulopathy: an antiphospholipid cohort study

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Introduction

Various coagulation alterations have been reported in COVID-19 patients, especially in those with the most severe forms: a) elevated d-dimer (x6 time normal value) correlates with poor prognosis; b) low-molecular weight heparins have been reported as an effective drug to support intensive care; c) thrombosis of the pulmonary vessels has often been found at the autoptic investigation of COVID-19 patients. It is well-known that bacterial and viral infections can induce antiphospholipid antibodies (aPL), which is often not associated to thrombotic events. A recent study reported the presence of aPL mainly of IgA isotype (not part of laboratory work-out for APS), in COVID-19 positive patients, they did not specify the aPL titres and LAC testing was not available. Most importantly from a clinical point of view, all the reported patients have suffered in the past for vascular manifestations. In order to better understand the SARS-2-Cov 2 coagulopathy, we performed an observational study aiming to investigate the status of haemostasis in these patients, performing a panel of coagulation markers.

Patients and methods

101 consecutive PCR-confirmed COVID-19 infected patients admitted at the AO Ordine Mauriziano Hospital, Torino, Italy, were included in this study. Patients were tested for aPL profile, D-Dimer, von Willebrand factor, and IL-6 levels. Patients defined as critically ill were those hospitalized in an intensive care unit at the time of the blood withdrawal.

Results

Our data showed positivity for any aPL in about half of the patients (48.5%). In detail, we found that a positivity for Lupus Anticoagulant (LAC) can be detected in up to 1 out of 3 symptomatic COVID-19 patients when tested according to the ISTH. However, the so called triple aPL positivity, the profile most strongly associated with a thrombotic event in patients with APS (the concomitant presence of LAC, anticardiolipin IgG and anti-beta2GPI IgG antibodies) has been observed in only one patient (1%). When including any triple positivity (for LAC plus anticardiolipin and anti-beta2-GPI either IgG/IgM) we were able to identify 3 patients (3%). More importantly, most of aPL positivity was detected at the low-medium titre; aPL positivity are known to be detectable during infections, to include viral diseases such as HIV and hepatitis C. The presence of aPL in these contexts is often transient and almost always non-specific (non-thrombosis-related).

Discussion

In conclusion, although isolated, LAC positivity is present in about a third of patients. Their clinical role in the pathogenesis of a coagulopathy and pulmonary thrombosis during COVID-19 infection still need to be demonstrated.

PO131

High resilience of an advanced POCT system during the Covid-19 crisis.

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During the peak of the SARS-CoV-2 pandemic, the San Gerardo Hospital in Monza made an essential contribution to the Regional Health Service by supporting, 3rd for number of Covid beds, the other regional health facilities. In the period of the epidemic peak (March-April), 1250 Covid patients were hospitalized, with 72% of the dedicated beds (416 out of 578). The number of beds in the Intensive Care Unit increased from 31 to 97. In many cases respiratory assistance with CPAP, mechanical ventilation, ECMO was required. In this context, the monitoring of the Acid-Base balance and respiratory exchanges by means of POCT bloodgas analyzers (BGA) was fundamental in the management of patient ventilatory support. For more than 15 years the Laboratory has managed an advanced POCT system based on the management of: connectivity, competence and quality control. All BGA are interconnected to the middleware (Rapid Point 500 and RapidComm 7.0, Siemens Healthineers), to the LIS (DnLab, Dedalus) and to the computerized medical records. Only trained and enabled operators access the system. The IQC is automatically and remotely monitored by dedicated software (URT, Biorad Laboratories). During the epidemic peak phase, the number of BGA increased from 19 to 31 (average activation time <24h). The average daily number of bloodgas analysis increased from 350 to 1300. The number of trained operators increased from 824 to 933, with 10 training courses in locations appropriate to the required safety standards. Main process indicators (KPIs) monitored compared with the same period of the previous year: % of sample identification error: 2.4 vs 1.8; average monthly number of out of control situation of the IQC/instrument: 16 vs 11; average monthly number of obstructions/instrument: 27 vs 11; number of technical assistance interventions 3 vs 1. The continuous 24h/24 remote monitoring by the Laboratory has allowed to safely manage out of control situations and most of the machine downtime. The presence of an advanced and monitored intra-hospital POCT system allowed to respond to the epidemic crisis in a rapid and timely manner, guaranteeing the high safety standards already present in the pre-crisis phase.

PO132

Seroprevalence of SARS-COV-2 antibodies in Southern Italy's hospital staff

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the first pandemic caused by a coronavirus. Respiratory/aerial droplets transmission and the high number of "hidden" asymptomatic cases play a critical role in the rapid spread of the virus across countries. The seroprevalence of SARS-CoV-2 antibodies in the general population is currently unknown. It has been estimated that undocumented infections were the source for #80% of the documented cases before traveling restriction policies took place. Serological evaluation is essential for investigating the extent of SARS-CoV-2. Even more, assessing the prevalence of anti-SARS-CoV-2 in hospital staff offers a unique opportunity to study the correlation between seroconversion and immunization because of their occupational exposure and at higher risk of contagious.

Methods: The study enrolled a total of 3242 employees of our hospital, "Policlinico Riuniti" of Foggia. The employees' group was stratified in 3 subgroups according to their relative exposure to SARS-CoV-2 (high, intermediate, and low-risk groups). We used a chemiluminescent immunoassay (CLIA, Shenzhen YHLO Biotech) to study the seroprevalence of SARS-COV-2 specific antibodies (IgG and IgM against nucleocapsid and spike proteins). The cut-off was set to 8 AU/mL for both IgG and IgM (specificity of 98,8% and 100%, respectively). A control group of 83 samples sera collected before the Italian COVID-19 outbreak (2018-19) was also tested. Healthcare workers with IgG or IgM concentration above 6.0 and below 8.0 AU/mL were considered borderline. Nevertheless, all of them were tested for the SARS-CoV-2 viral RNA presence (Allplex™ 2019 n-CoV Assay, Seegene).

Results: Sixty-two individuals (1.9%, 1.4-2.3%, 95% CI) tested positive for at least one antibody anti-SARS-CoV-2. Five individuals (8.0% of the positive) had IgG and IgM positive test results, while 32 and 25 had only IgG and only IgM positive results. Instead, viral RNA was detected in only nine individuals (13.8% of Ig positive) by RT-PCR. The cumulative proportion of individuals who tested positive (IgG and/or IgM) varied between 1-2.4%. The seroprevalence was lower in the high-risk group 1.4% (6/428,0.5-2.6%, 95% CI) vs. intermediate-risk group 2.0 % (55/2736, 1.5-2.5%, 95% CI). Only one participant (1.3%, 0-3.8%, 95% CI) of the low-risk group tested positive for SARS-CoV-2 IgM antibodies.

Conclusions: The low level of seropositivity (1.9%) shows that the COVID-19 containment measures adopted were

adequate and effective. Moreover, the combination of both serological and molecular tests can improve the likelihood of identifying asymptomatic subjects.

PO133

Immune-inflammatory biomarkers' panel for severe COVID-19 patients.

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Background: Severe acute respiratory syndrome caused by novel coronavirus 2 (SARS-CoV-2) emerged in Wuhan (China) in December 2019. This study aimed to evaluate a panel of biomarkers in order to better phenotype population and to define the role of mediators as biomarkers of severity.Materials and methods: Serum samples were obtained from 24 COVID-19 patients at the hospital admission before any treatments and infusion of intravenous steroids or invasive ventilation. KL-6 IL-6 and C peptide were measured with CLEIA methods. IL-6 assays were validated for accuracy and precision. The validity of variables used to distinguish severe from mild-to-moderate group was assessed by areas under curves (AUC) in the receiver operating characteristic (ROC) and a logistic regression was performed to combine parameters between the two groups.Results: In severe group, IL-6 CRP and KL-6 concentrations were significantly increased than mild-to-moderate patients. Moreover KL-6, IL-6 and CRP biomarkers resulted directly correlated. In the logistic regression, ROC curve analysis of the model comprise IL-6, KL-6 and CRP reported the best performance with an AUC 0,95. Conclusions: On corroborating previous reports on IL-6 over-expression in severe COVID 19 patients requiring mechanical ventilation, the analytical determination of other mediators showed that IL-6 concentrations are correlated with those of KL-6 and CRP. The combination of three prognostic bioindicators allowed to discriminate between severe COVID-19 patients with poor prognosis from mild-to-moderate patients.

PO134

La combinazione di differenti tecniche analitiche nella determinazione e individuazione di una variante emoglobinica rara durante la quantificazione dell'emoglobina glicata: Case report

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Introduzione: Alcune varianti della catena beta o alfa dell'emoglobina possono interferire con il dosaggio della HbA1C. L'utilizzo combinato di due metodiche (HPLC,Elettroforesi Capillare) ha permesso di evidenziare una variante emoglobinica e di fornire il risultato. Formulazione caso: Un uomo di 76 anni di origini maltesi, giunge al Laboratorio del Bambino Gesù di Palidoro per il controllo della HbA1C. La misurazione di HbA1C, in HPLC (VARIANT II Analyzer, Biorad) su un campione raccolto in provetta contenete K3EDTA, non ha prodotto alcun risultato. Lo stesso campione analizzato in elettroforesi capillare (Capyllarys HbA1C Tera 3, SEBIA), ha fornito il risultato, evidenziando una variante emoglobinica (2.2%) in prossimità della HbA0. L'esame emocromocitometrico (ADVIA 2120, Siemens) non mostra anomalie, così come gli esami biochimici (COBAS 501, Roche). Il sospetto della presenza di una variante anomala ha indotto il laboratorio ad approfondire le indagini inviando il campione presso un centro specializzato per la diagnosi molecolare delle emoglobinopatie (CEINGE Biotecnologie Avanzate-AOU Federico II). Trattamento: Sul campione, previo consenso informato del paziente, sono state eseguite le indagini molecolari. È stata eseguita l'amplificazione e analisi di sequenza del gene beta globinico per le regioni comprese tra i nucleotidi HBB:c.-158 e HBB:c.315+100 e tra i nucleotidi HBB:c.316-206 e HBB:c.*+177. I risultati ottenuti mostrano la presenza in eterozigosi della mutazione causa della sintesi della Hb Long Island-Marseille (b2,CAC>CCC, His>Pro) (HBB:c.[8 A>C]). Il genotipo è compatibile con lo stato di portatore di variante emoglobinica non patologica. L'Hb Long Island-Marseille è una variante che coeluisce con la frazione HbA1C in HPLC, ma che migra ad un tempo leggermente diverso dall'HbA1C in elettroforesi capillare, configurandosi sul tracciato come una variante a sé. Conclusioni: La valutazione del cromatogramma o del tracciato elettroforetico e la combinazione delle differenti tecniche analitiche (HPLC ed elettroforesi capillare) può risultare utile nella identificazione di varianti emoglobiniche nuove o rare, che anche se non clinicamente rilevanti, potrebbero dare luogo a fenotipi ematologici importanti se associati ad altri difetti emoglobinici.

PO135

Correlazione della catepsina S, cistatina C e metalloproteinasi di matrice-9 con il rischio di rottura dell'aneurisma dell'aorta addominale

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INTRODUZIONE: Ricerche precedenti hanno mostrato significativi aumenti dei livelli sierici della catepsina S (CatS) e della metalloproteinasi di matrice-9 (MMP-9) nei pazienti con aneurisma dell'aorta addominale (AAA). Inoltre, i livelli della cistatina C (CysC) erano più bassi che nelle persone sane. Lo scopo di questa ricerca era di comparare i loro livelli, i fattori geometrici e biomeccanici predittivi del rischio di rottura, tra i pazienti con AAA asintomatiche e sintomatiche. METODI: I campioni di siero sono stati prelevati da 80 pazienti con AAA, 64 uomini e 16 donne, di media età di 72 anni, ricoverati a causa della necessità di intervento chirurgico sulla AAA asintomatica (N=67) o sintomatica, rotte incluse (N=13). I livelli di CatS e CysC sono stati determinati usando ELISA, e di MMP-9 usando immunodosaggio fluorescente (R&D Systems, Minneapolis, USA). Sono stati determinati i valori delle attività endogene e totali (dopo l'attivazione di MMP-9 con p-aminofenilmercurio acetato). Nell'ambito della valutazione clinica, sono stati determinati anche il massimo diametro esterno centrale di AAA, il picco di stress della parete (peak wall stress, PWS), e il picco di rischio di rottura della parete (peak wall rupture risk, PWRR) dalle scansioni della tomografia computerizzata. RISULTATI: Per il campione completo, i coefficienti della correlazione di Spearman sono stati significativi ($p<0.05$) tra la CatS e il tipo di AAA (asintomatica o sintomatica), il fumo e la MMP-9 totale; tra entrambe le MMP-9, endogena e totale; tra il diametro della AAA e il PWS e il PWRR; tra il PWS e il PWRR. Il Mann-Whitney U test ha mostrato livelli significativamente più alti solo per la CatS e il diametro di AAA nel gruppo di pazienti con AAA sintomatica rispetto al gruppo con AAA asintomatica. La regressione lineare multivariata ha mostrato che solo la CatS e il PWS si sono correlate positivamente con le AAA tendenti a rottura. Tuttavia, la regressione logistica non ha confermato il potenziale predittivo di questi parametri come fattori di rischio indipendenti per l'instabilità e la rottura dell'AAA. CONCLUSIONE: Nessun parametro tra quelli presi in esame ha mostrato significativo valore predittivo per differenziare le AAA presenti tra asintomatiche e tendenti a rottura.

PO136

Extracellular vesicles as new circulating biomarkers for anti-thrombin (AT) deficiencies

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Biomarkers in both basic, clinical research and clinical practice are necessary to monitor physiology and disease onset. Indeed, parameters with high predictive and prognostic values are mandatory to define therapy effectiveness and clinical outcomes. Extracellular vesicles (EVs) and other biogenic nanoparticles represent an immense, easily accessible reservoir of such information. Those nanoparticles are multiplexed and very sensible to changes occurring during disease onset and evolution. It has been documented that EVs in plasma support fibrinolysis hence EVs might play an important role in the diagnostic process of coagulation disorders such as AT deficiencies. Antithrombin (AT) is a glycoprotein involved in the regulation of blood coagulation. It belongs to the family of serine-protease inhibitors and acts as the most important antagonist of the clotting factors IIa (thrombin), IXa, Xa, and XIa. Two types of inherited AT deficiency can be distinguished: Type I (quantitative deficit), and Type II (qualitative deficit). The latter is characterized by an impaired inhibitory activity related to dysfunctional domains of the protein. Three Type II subtypes can be defined: Type IIa (reactive site defect), Type IIb (heparin binding site defect) and Type IIc (pleiotropic defect). This classification has clinical importance since these subtypes have a different thrombotic risk. No functional routine diagnostic assay, however, can be assumed to detect all forms of Type II deficiencies since false-negative results may hamper the diagnosis.Our laboratory has recently evidenced that AT is in part associated to the external leaflet of EVs. We also found that specific AT isoforms are enriched in EV preparations in respect to total plasma and that those isoforms are selectively associated to EVs when comparing healthy or AT type II deficient patients. AT selective association pattern to EVs might be related either to mutations in the primary sequence of the protein or alterations in the glycosylation process, hence experiments are ongoing to reveal the nature of this phenomenon. Our findings suggest that analysis of AT enriched in EV preparations might useful to gain insights into the pathogenesis and be of support in the diagnostic algorithm of AT deficiencies.

PO137

Evaluation of biomarker KL-6 (Krebs von den Lungen-6 protein) in a healthy population and in a cohort of patients with idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic fibrosing, progressive devastating lung limited disease. An increasing attention has been played in identifying molecular biomarkers to support the diagnosis. KL-6, a substance deriving from lung epithelial cells showed promising results especially in identifying acute IPF exacerbations. We evaluated KL-6 concentration in healthy subjects and investigated its clinical significance in patients with IPF to identify the reference values and concentration changes in function of age. KL-6 serum levels have been measured on blood samples from a population of 130 healthy well-known anamnesis donor subjects (18-92 years). Also 92 patients with a diagnosis of IPF defined according to 2013 guidelines criteria, were examined in a retrospective study. Serum KL-6 levels were evaluated using the Lumipulse® G KL-6 kit, results were measured using an automated immunoassay system (LUMIPULSE G1200; Fujirebio, Tokyo, Japan). KL-6 values were correlated to the functional test in order to search for a possible prognostic and predictive value in response to therapy in follow up. KL-6 levels were (U/ml, mean ± sd): in controls 315.0 ± 104.8, in IPF 1805.3 ±1108.62, being KL-6 levels in IPF higher and more variable than in healthy controls. Populations show significant difference ($p = 0.002$) and a non-overlap between them: in healthy subjects the KL6 does not exceed 540, in affected it reaches 5000. Correlation with age was observed in healthy population (Pearson 0.233). In follow-up patients treated with Nintedanib or Pirfenidone biomarker trends were compared with the variations of instrumental parameters recorded at the same times. When drug therapy had positive results, with an improvement in living conditions, KL-6 concentration has dropped significantly. KL-6 blood concentrations analysis can be useful in order to support the diagnosis. It is advantageous in terms of execution times and minimal invasively for the patient. It is possible to define a preliminary cut-off value: 540 U/mL. However, to establish one definitive it will be necessary to proceed to a further study. Drugs are able to provide mild improvements to affected and biomarker reflects both patient stabilization and progress towards a bad outcome.

PO138
Warnings in NGS analysis, how to handle them?

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The interpretation of molecular genetic results is often challenging in cystic fibrosis (CF) diagnostic process. With this abstract, based on a case report, we want to focus on how to interpret and manage some warnings in the genetic analysis of the CFTR gene. Sequencing was performed on MiSeq (Illumina), and the FASTQ files were processed using the Amplicon Suite software, which proceeds with both the alignment, call of the SNVs found, and evaluation of the CNVs in the CFTR gene. All pathogenic variants, and/or rare variants not known and/or of uncertain significance, are confirmed by Sanger sequencing. In our case, no SNVs of relevant clinical significance emerged, but, the CNV analysis showed a warning at amplicon 2 level of exon 15 of the gene, suggesting a likely deletion event. However, when only one of the 3 amplicons that cover each exon of the gene is "deleted", the deletion signal is usually a technical artifact, maybe due to a starting low-quality DNA. The MLPA analysis, the gold standard to confirm this type of NGS results, was carried out to exclude any deletions or duplications. In this case, the result of the MLPA analysis was negative, showing no deletion or duplication at the level of exon 15 or at the level of the other gene exons. At this point, the patient's diagnosis would be closed as negative. However, a deep analysis by Sanger sequencing of the two PCR bands obtained from the entire exon 15, showed a wild-type sequence for the smaller band and the insertion of an ALU sequence in the larger band. Therefore, the presence of the ALU sequence caused the amplification of one of the three amplicons of exon 15 to fail, resulting in a deletion signal. With these data, we want to emphasize the need to pay maximum attention in the interpretation of the results from NGS methodologies, and to try to use different approaches to obtain a correct genetic analysis and a multidisciplinary comparison for the interpretation of genetic tests. Amato F, et al. Two CFTR mutations within codon 970 differently impact on the chloride channel functionality. Hum Mutat. 2019;40(6):742-748.Di Lullo AM, et al. An "ex vivo model" contributing to the diagnosis and evaluation of new drugs in cystic fibrosis. Acta Otorhinolaryngol Ital. 2017;37(3):207-213.

PO140
Vitamin D status modulates inflammatory response in HIV+ subjects

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Many studies show that vitamin D deficiency can be associated with the inflammatory and immune response in HIV+ subjects (1) and may be related to side effects of antiretroviral therapy (ART) or other conditions associated with HIV infection (2-5). Inflammation is an early response that can lead to various pathological conditions in HIV+ subjects and early markers of inflammation can be useful for monitoring HIV-induced degenerative processes. Therefore, we evaluated in peripheral blood mononuclear cells (PBMC) the effects of vitamin D status in the regulation of genes characteristic of inflammation. HIV+ subjects (n=57) under ART and healthy controls (n=40) were enrolled in this study. The 25(OH)D3 plasma levels were assayed by HPLC. The mRNA levels of CYP27B1, which encodes the enzyme that catalyzes the conversion of 25(OH)D3 to active 1,25(OH)2D3, and of the pro-inflammatory cytokines TNF-α and IFN-γ, were quantified by Real-Time PCR. In HIV+ subjects the serum concentrations of 25(OH)D3 were 59.9 ± 3.6 nmol/L, that were significantly lower than those in healthy subjects (87.4 ± 4.9 nmol/L, $p<0.001$). Both healthy and HIV + subjects included in our study were divided into 3 groups as follows: 25(OH)D3 deficiency (50 nmol/L), insufficiency (51-74 nmol/L) and sufficiency (75 nmol/L). Among the HIV+ subjects only 15 had sufficient 25(OH)D3 plasma levels (96.9 ± 3.7 nmol/L), while 17 subjects had insufficient (63.4 ± 1.3 nmol/L) and 25 subjects deficient (35.4 ± 1.8 nmol/L) 25(OH)D3 plasma levels. The 25(OH)D3 levels were negatively correlated with time since HIV diagnosis, while positively correlated with CD4+ number. In PBMC from HIV+ subjects, we found decreased CYP27B1 mRNA levels and increases in gene expression of both TNF-α and IFN-γ when compared to controls. The mRNA changes observed in PBMC from HIV+ subjects were dependent on vitamin D status. Our study found an association between 25(OH)D3 levels and markers of cell modification induced by HIV. Further evidence is needed to characterize the role of vitamin D supplementation and ART in the progression of disease.

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PO141

Comparison between five different assays to detect the levels of antibodies against Sars-CoV-2 in human popolation

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of Coronavirus Disease 2019 (COVID-19), rapidly spread in the current pandemic. The diagnosis of Covid 19 is based on the detection of viral RNA by molecular amplification (NAAT) in nasopharingeal swabs and virus-specific antibodies of different isotypes in the serum. IgM and IgG anti-Sars CoV-2 appear 4-7 days after the onset of symptoms but the highest level of IgM and IgG are detected after 2-3 weeks and 3-6 weeks, respectively. Serological assays are useful for the diagnosis and for epidemiological studies. To compare different methods, we measured serum antibody levels with three automated assays including Elecsys®Roche anti-SarsCov2, Abbott Sars-CoV-2 IgG and Diasorin Liason®SARS-CoV-2 S1/S2 IgG in a goup of 50 selected subjects (negative or positive for nasopharingeal swab, initial screening with Abbott serological test or previuos clinical suspect of COVID-19). A good concordance of the results (68%) was found between Roche and Diasorin assays, and between Roche and Abbott tests while the concordance between Abbott and Diasorin is lower (38%). The samples were also analyzed with other 2 non automated assays: Euroimmun Sars-CoV-2 ELISA (IgG) and Euroimmun Sars-CoV-2 ELISA (IgA). The percentage of positivity is 58% with Diasorin Liason®SARS-CoV-2 S1/S2 IgG, 42% for Abbott Sars-CoV-2 IgG, 32% for Euroimmun Sars-CoV-2 ELISA (IgA), 30% for Euroimmun Sars-CoV-2 ELISA (IgG) and 28% for Elecsys® Roche anti-SarsCov2. The concordance of ~88% and 86% was revealed between Roche test and Euroimmun (IgG), and between Roche and Euroimmun (IgA), respectively, while it reduced to 66% among Diasorin and Euroimmun (IgG) and 62% between Abbott and Euroimmune (IgG).The results in agreement with all five tests were the 34% of total selected cases. The percentage of positive specimens tested with Roche method confirmed with at least 2 other assays was 100%; this value was 93% for Euroimmun (IgG), 81% for Euroimmun (IgA), 52% for Diasorin assay and 47% for Abbott test.

PO142

Physical activity and natriuretic peptides in children with hypoplastic left heart syndrome and univentricular heart

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Objective: Hypoplastic left heart syndrome (HLHS) describes a heterogeneous group of pathologies characterized by underdevelopment of the structures of the left side of the heart. HLHS occurs in 0.16 - 0.36 per 1000 live births and represents 1.4 - 3.8% of congenital heart diseases. In the past, the syndrome was fatal within the first few weeks of life. Today, following the surgical and medical advances of the past four decades, many of these children undergo three surgical steps that enable them to survive childhood and reach adulthood with a univentricular heart. Recent studies have investigated the possible beneficial effects of physical activity in these patients, however this topic is not yet fully clarified. The aim of this study was to evaluate the effects of exercise on NT-proBNP in patients with HLHS.Methods: We enrolled 14 (9 male) patients with HLHS and univentricular heart. Each patient underwent a cardiological examination, echocardiogram and blood tests. Physical activity was assessed by submitting the International Physical Activity Questionnaire (IPAQ) to the children together with their parents. Based on the score obtained, the patients were divided into two groups. In group I, children who carried out regular daily physical activity. In group II, on the other hand, the children who, according to the questionnaire scores, led a sedentary life were considered. NT-proBNP (pg/L) was measured for each patient.Results: Among the echocardiographic parameters in the study, fractional area change (RVFAC) was considered to evaluate the function of the systemic right ventricle. Statistical analysis did not show a significant difference in right ventricle function between the two groups: RVFAC 36.29 ± 4.8% vs 35.14 ± 5.3% ($p > 0.05$) in group I and group II respectively . We then assessed whether there was a difference between the NT-proBNP levels between the two groups and the data analysis showed a significant difference between group I and group II, 65.98 ± 20.03 ng/L vs 102.95 ± 23.65 ng/L ($p < 0.01$) respectively.Conclusions: Our data showed a reduction of NT-proBNP levels in the group of children who perform physical activity compared to the group of sedentary children. To the best of our knowledge this is the first study that correlated NT-proBNP with physical activity in patients with HLHS and univentricular heart

PO143

PROTEINA C REATTIVA E SEVERITÀ CLINICA DELLA MALLATTIA DA SARS-COV-2, ANALISI RETROSPETTIVA

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SCOPO:Analisi retrospettiva dell'andamento dei valori ematici della Proteina C Reattiva (PCR) nei pazienti ricoverati per SARS-COV-2 presso il Presidio Ospedaliero SS. Annunziata di Chieti durante il mese di Aprile 2020. MATERIALI E METODI: sono stati presi in esame 50 pazienti giunti al Pronto Soccorso del presidio Ospedaliero SS Annunziata di Chieti, con sintomatologia sospetta per SARS COV-2. Tutti i pazienti sono stati sottoposti ad esami strumentali, al tampone rino-faringeo e ad esami ematochimici di laboratorio previsti dal protocollo diagnostico SARS-COV-2. Dei pazienti esaminati, 30 sono stati ricoverati direttamente nel reparto di rianimazione e terapia intensiva dal PS, o dopo un breve periodo di degenza presso i reparti internistici Covid, a causa di una maggiore severità clinica. Invece, gli altri 20 pazienti sono stati ricoverati nel reparto di Malattie Infettive. Il primo gruppo di pazienti presentava un'età media di 70 anni, diverse comorbilità e recenti accessi ospedalieri. Il secondo gruppo, invece, presentava un' età media intorno ai 50 anni e minor presenza di comorbilità. In entrambi i gruppi c'era una netta prevalenza del sesso maschile. L'analisi ematochimiche hanno evidenziato valori significativamente elevati della Proteina C Reattiva nei pazienti ricoverati nel reparto di terapia intensiva e rianimazione, sin dai primi giorni di degenza, a fronte dei valori più bassi nei pazienti ricoverati nel reparto di malattie infettive. Inoltre, l'aumento dei valori della PCR persisteva per un lungo periodo di tempo nel I gruppo esaminato rispetto al II gruppo di pazienti, così come la linfocitopenia riscontrata all'esame emocromocitometrico. CONCLUSIONE: I valori ematici della Proteina C Reattiva risultano statisticamente significativi come indice predittivo dell'outcome clinico dei pazienti affetti da SARS Cov-2, trovando conferma nel peggior esito clinico dei pazienti ricoverati in rianimazione, i quali, nella maggior parte dei casi sono andati incontro ad exitus, rispetto ad un outcome favorevole nella maggior parte dei pazienti ricoverati nel reparto di Malattie Infettive. Biobliografia: Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med 2020. doi: 10.1515/cclm-2020-0198. Epub ahead of print.

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Indice degli Autori

STAT PROFILE Prime+®

L'Emogasanalizzatore Stat Profile® Prime Plus aiuta la gestione dei pazienti critici COVID-19 grazie all'esclusivo menu di test di point-of-care

L'analisi EmoGas puo' aiutare la gestione dei pazienti critici COVID-19. L' Emogasanalizzatore Nova Stat Profile Prime Plus è la strumentazione ideale per i reparti di terapia intensiva e semi-intensiva in quanto, oltre ai parametri standard, permette con un unico campionamento, la possibilità di eseguire altri esami aggiuntivi utili alla lotta al Covid-19 quali:

- Urea (Bun),
- Creatinina (Creat),
- Magnesio Ionizzato (iMg)

Menù:

pH, PCO_2, PO_2, Na^+, K^+, Cl^-, iCa, Glu, Lac, Hct, TCO_2, SO_2%, O_2Hb, COHb, MetHb, HHb, tBil, tHb, HbF

…più Urea (BUN), Creat e iMg

Complicanze del paziente critico COVID-19	Parametri Point of Care
Sindrome da distress respiratorio acuto	PO_2, PCO_2, pH, HCO_3^-, SaO_2, PO_2/FiO_2, OI
Insufficienza respiratoria di tipo I.	PO_2, PCO_2, pH, HCO_3^-, SaO_2, PO_2/FiO_2, OI
Lesioni cardiache acute: • Con anamnesi di ipertensione o malattie cardiovascolari • Senza anamnesi di ipertensione o malattie cardiovascolari	iCa, K^+, iMg
Insufficienza cardiaca • Con anamnesi di ipertensione o malattie cardiovascolari • Senza anamnesi di ipertensione o malattie cardiovascolari	iCa, K^+, iMg
Sepsi	Lattato, Glucosio, BUN/Creatinina, iCa, K^+, iMg
Acidosi	PCO_2, pH, HCO_3^-
Alcalosi	PCO_2, pH, HCO_3^-
Lesione renale acuta	BUN/Creatinina, iCa, K^+, iMg
Equilibrio elettrolitico	Na^+, K^+, iCa, iMg, Cl^-, Gap Anionico
Shock	Lattato
Lesione epatica acuta	Bilirubina

Adattato da Chen T et al. BMJ 2020;368:m1091 | doi: 10.1136/bmj.m1091

Iscriviti al Webinar On Demand e scopri come *l'Emogasanalizzatore Nova Stat Profile Prime Plus aiuta la gestione dei pazienti critici COVID-19*

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